# 27 Biological inorganic chemistry


**The organization of cells**

27.1 The physical structure of cells

27.2 The inorganic composition of cells

**Transport, transfer, and transcription**

27.3 Sodium and potassium transport

27.4 Calcium signalling proteins

27.5 Zinc in transcription

27.6 Selective transport and storage of iron

27.7 Oxygen transport and storage

27.8 Electron transfer

**Catalytic processes**

27.9 Acid–base catalysis

27.10 Enzymes dealing with $H_2O_2$ and $O_2$

27.11 The reactions of cobalt-containing enzymes

27.12 Oxygen atom transfer by molybdenum and tungsten enzymes

**Biological cycles**

27.13 The nitrogen cycle

27.14 The hydrogen cycle

**Sensors**

27.15 Iron proteins as sensors

27.16 Proteins that sense Cu and Zn levels

**Biomineralization**

**The chemistry of elements in medicine**

27.17 Chelation therapy

27.18 Cancer treatment

27.19 Anti-arthritis drugs

27.20 Imaging agents

**Perspectives**

27.21 The contributions of individual elements

27.22 Future directions

FURTHER READING
EXERCISES
PROBLEMS



**Organisms have exploited the chemical properties of the elements in remarkable ways, providing examples of coordination specificities that are far higher than observed in simple compounds. This chapter describes how different elements are taken up selectively by different cells and intracellular compartments and the various ways they are exploited. We discuss the structures and functions of complexes and materials that are formed in the biological environment in the context of the chemistry covered earlier in the text.**


Biological inorganic chemistry ('bioinorganic chemistry') is the study of the 'inorganic' elements as they are utilized in biology. The main focus is on metal ions, where we are interested in their interaction with biological ligands and the important chemical properties they are able to exhibit and impart to an organism. These properties include ligand binding, catalysis, signalling, regulation, sensing, defence, and structural support.

## The organization of cells

To appreciate the role of the elements (other than C, H, O, and N) in the structure and function of organisms we need to know a little about the organization of the 'atom' of biology, the cell, and its 'fundamental particles', the cell's constituent organelles.

### 27.1 The physical structure of cells

**Key points:** Living cells and organelles are enclosed by membranes; the concentrations of specific elements may vary greatly between different compartments due to the actions of ion pumps and gated channels.

Cells, the basic unit of any living organism, range in complexity from the simplest types found in prokaryotes (bacteria and bacteria-like organisms now classified as archaea) and the much larger and more complex examples found in eukaryotes (which include animals and plants). The main features of these cells are illustrated in the generic model shown in Fig. 27.1. Crucial to all cells are membranes, which act as barriers to water and ions and make possible the management of all mobile species and of electrical currents. Membranes are lipid bilayers, approximately 4 nm thick, in which are embedded protein molecules and other components. Bilayer membranes have great lateral strength but they are easy to bend. The long hydrocarbon chains of lipids make the membrane interior very hydrophobic and impermeable to ions, which must instead travel through specific channels, pumps, and other receptors provided by special membrane proteins. The structure of a cell also depends on osmotic pressure, which is maintained by high concentrations of solutes, including ions, imported during active transport by pumps.

Prokaryotic cells consist of an enclosed aqueous phase, the **cytoplasm**, which contains the DNA and most of the materials used and transformed in the biochemical reactions. Bacteria are classified according to whether they are enclosed by a single membrane or have an additional intermediate aqueous space, the **periplasm**, between the outer membrane and the cytoplasmic membrane, and are known as 'Gram-positive' or 'Gram-negative', respectively, depending on their response to a staining test with the dye crystal violet. The much

more extensive cytoplasm of eukaryotic cells contains subcompartments (also enclosed within lipid bilayers) known as **organelles**, which have highly specialized functions. Organelles include the **nucleus** (which houses DNA), **mitochondria** (the 'fuel cells' that carry out respiration), **chloroplasts** (the 'photocells' that harness light energy), the **endoplasmic reticulum** (for protein synthesis), **Golgi** (vesicles containing proteins for export), **lysosomes** (which contain degradative enzymes and help rid the cell of waste), **peroxisomes** (which remove harmful hydrogen peroxide), and other specialized processing zones.

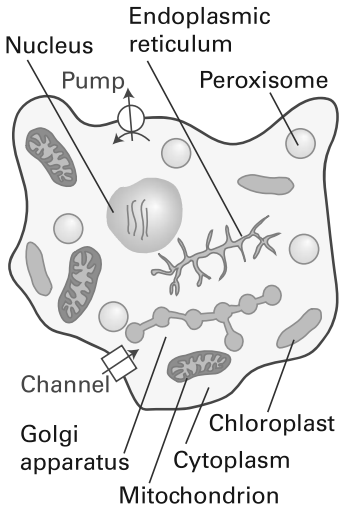



**Figure 27.1** The layout of a generic eukaryotic cell showing the cell membrane, various kinds of compartments (organelles), and the membrane-bound pumps and channels that control the flow of ions between compartments.

## 27.2 The inorganic composition of cells

**Key points:** The major biological elements are oxygen, hydrogen, carbon, nitrogen, phosphorus, sulfur, sodium, magnesium, calcium, and potassium. The trace elements include many d metals, as well as selenium, iodine, silicon, and boron.

Table 27.1 lists many of the elements known to be used in living systems, although not necessarily by higher life forms. All the second- and third-period elements except Be, Al, and the noble gases are used, as are most of the 3d elements, whereas Cd, Br, I, Mo, and W are the only heavier elements so far confirmed to have a biological function. Several others, such as Li, Ga, Tc, Ru, Gd, Pt, and Au, have important and increasingly well-understood applications in medicine.

The biologically essential elements can be classified as either 'major' or 'trace'. Although a good idea of the biological abundances of different elements is given in Table 27.1, the levels vary considerably among organisms and different components of organisms. For example, Ca has little role in microorganisms but is abundant in higher life forms, whereas the use of Co by higher organisms depends on it being incorporated into a special cofactor (cobalamin) by microorganisms. There is probably a universal requirement for K, Mg, Fe, and Mo. Vanadium is used by lower animals and plants as well as some bacteria. Nickel is essential for most microorganisms, and is used by plants, but there is no evidence for any direct role in animals. Nature's use of different elements is largely based on their availability. For example, Zn has widespread use (and, together with Fe, ranks among the

**Table 27.1** The approximate concentrations, log ([J]/mol dm$^{-3}$), where known, of elements (apart from C, H, O, N, P, S, and Se) in different biological zones

| Element | External fluids (sea water) | Free ions in external fluids (blood plasma) | Cytoplasm (free ions) | Comments on status in cell |
|---|---|---|---|---|
| Na | $>10^{-1}$ | $10^{-1}$ | $<10^{-2}$ | Not bound |
| K | $10^{-2}$ | $4 \times 10^{-3}$ | $\leq 3 \times 10^{-1}$ | Not bound |
| Mg | $>10^{-2}$ | $10^{-3}$ | *c.* $10^{-3}$ | Weakly bound as ATP complex |
| Ca | $>10^{-3}$ | $10^{-3}$ | *c.* $10^{-7}$ | Concentrated in some vesicles |
| Cl | $10^{-1}$ | $10^{-1}$ | $10^{-2}$ | Not bound |
| Fe | $10^{-17}$ (Fe(III)) | $10^{-16}$ (Fe(III)) | $<10^{-7}$ (Fe(II)) | Too much unbound Fe is toxic (Fenton chemistry) in and out of cells |
| Zn | $<10^{-8}$ | $10^{-9}$ | $<10^{-11}$ | Totally bound, but may be exchangeable |
| Cu | $<10^{-10}$ (Cu(II)) | $10^{-12}$ | $<10^{-15}$ (Cu(I)) | Totally bound, not mobile. Mostly outside cytoplasm |
| Mn | $10^{-9}$ | | *c.* $10^{-6}$ | Higher in chloroplasts and vesicles |
| Co | $10^{-11}$ | | $<10^{-9}$ | Totally bound (cobalamin) |
| Ni | $10^{-9}$ | | $<10^{-10}$ | Totally bound |
| Mo | $10^{-7}$ | | $<10^{-7}$ | Mostly bound |

most abundant biological trace elements) whereas Co (a comparatively rare element) is essentially restricted to cobalamin. The early atmosphere (over 2.3 Ga ago[1]), being highly reducing, enabled Fe to be freely available as soluble Fe(II) salts, whereas Cu was trapped as insoluble sulfides (as was Zn). Indeed, Cu is not found in the archaea (which are believed to have evolved in pre-oxygenic times), including the hyperthermophiles, organisms that are able to survive at temperatures in excess of 100°C. These organisms are found in deep sea hydrothermal vents and terrestrial hot springs and are good sources of enzymes that contain W, the heaviest element known to be essential to life. The finding that W, Co, and for the most part Ni are used only by more primitive life forms probably reflects their special role in the early stages of evolution.

### (a) Compartmentalization

**Key point:** Different elements are strongly segregated inside and outside a cell and among different internal compartments.

**Compartmentalization** is the distribution of elements inside and outside a cell and between different internal compartments. The maintenance of constant ion levels in different biological zones is an example of 'homeostasis' and it is achieved as a result of membranes being barriers to passive ion flow. An example is the large difference in concentration of $K^+$ and $Na^+$ ions across cell membranes. In the cytoplasm, the $K^+$ concentration may be as high as 0.3 M whereas outside it is usually less than $5 \times 10^{-3}$ M. By contrast, $Na^+$ is abundant outside a cell but scarce inside; indeed, the low intracellular concentration of $Na^+$, which has characteristically weak binding to ligands, means that it has few specific roles in biochemistry. Another important example is $Ca^{2+}$, which is almost absent from the cytoplasm (its free concentration is below $1 \times 10^{-7}$ M) yet is a common cation in the extracellular environment and is concentrated in certain organelles, such as mitochondria. That pH may also vary greatly between different compartments has particularly important implications because sustaining a transmembrane proton gradient is a key feature in photosynthesis and respiration.

The distributions of Cu and Fe provide another example: Cu enzymes are often **extracellular**, that is they are synthesized in the cell and then secreted outside the cell, where they catalyse reactions involving $O_2$. By contrast, Fe enzymes are contained inside the cell. This difference can be rationalized on the basis that the inactive trapped states of these elements are Fe(III) and Cu(I) (or even metallic Cu) and organisms have stumbled on the expediency of keeping Fe in a relatively reducing environment and Cu in a relatively oxidizing environment.

The selective uptake of metal ions has potential industrial applications, for many organisms and organs are known to concentrate particular elements. Thus, liver cells are a good source of cobalamin[2] (Co) and milk is rich in Ca. Certain bacteria accumulate Au and thus provide an unusual way for procuring this precious metal. Compartmentalization is an important factor in the design of metal complexes that are used in medicine (Sections 27.17–20).

The very small size of bacteria and organelles raises an interesting point about scale, as species present at very low concentrations in very small regions may be represented by only a few individual atoms or molecules. For example, the cytoplasm in a bacterial cell of volume $10^{-15}$ $dm^3$ at pH = 6 will contain less than 1000 'free' $H^+$ ions. Indeed, any element nominally present at less than 1 nmol $dm^{-3}$ may be completely absent in individual cases. The word 'free' is significant, particularly for metal ions such as $Zn^{2+}$ that are high in the Irving–Williams series; even a eukaryotic cell with a total Zn concentration of 0.1 mmol $dm^{-3}$ may contain very few uncomplexed $Zn^{2+}$ ions.

Two important issues arise in the context of compartmentalization. First, the process requires energy because ions must be pumped against an adverse gradient of chemical potential. However, once a concentration difference has been established, there is a difference in electrical potential across the membrane dividing the two regions. For instance, if the concentrations of $K^+$ ions on either side of a membrane are $[K^+]_{in}$ and $[K^+]_{out}$, then the

[1] Current geological and geochemical evidence date the advent of atmospheric $O_2$ at between 2.2 and 2.4 Ga ago (1 Ga = $10^9$ a). It is likely that this gas arose by the earliest catalytic actions of the photosynthetic Mn cluster described in Section 27.10.

[2] In nutrition, the common complexes of cobalamin that are ingested are known as vitamin $B_{12}$.

contribution to the potential difference $\Delta\phi$ across the membrane is

$$\Delta\phi = \frac{RT}{F} \ln \frac{[K^+]_{in}}{[K^+]_{out}} \qquad (27.1)$$

This difference in electrical potential is a way of storing energy, which is released when the ions flood back to their natural concentrations. Second, the selective transport of ions must occur through ion channels built from membrane-spanning proteins, some of which release ions on receipt of an electrical or chemical signal whereas others, the **transporters** and **pumps**, transfer ions against the concentration gradient by using energy provided by adenosine triphosphate (ATP) hydrolysis. The selectivity of these channels is exemplified by the highly discriminatory transport of $K^+$ as distinct from $Na^+$ (Section 27.3).

Proteins, the most important sites for metal ion coordination, are not permanent species but are ceaselessly degraded by enzymes (proteases), releasing both amino acids and metal ions to provide materials for new molecules.

**EXAMPLE 27.1 Assessing the role of phosphate ions**

Phosphate is the most abundant small anion in the cytoplasm. What implications does this abundance have for the biochemistry of $Ca^{2+}$?

***Answer*** We can approach this problem by considering how $Ca^{2+}$ is compartmentalized. In a eukaryotic cell $Ca^{2+}$ is pumped out of the cytoplasm (to the exterior or into organelles such as mitochondria) using energy derived from ATP hydrolysis. Spontaneous influx of $Ca^{2+}$ occurs under the action of special channels or if the cell boundary is damaged. The solubility product of $Ca_3(PO_4)_2$ is very low and it could precipitate inside the cell if the $Ca^{2+}$ concentration rises above a critical value.

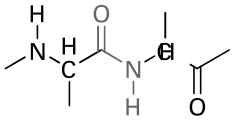
***Self-test 27.1*** Is Fe(II) expected to be present in the cell as uncomplexed ions?

### (b) Biological metal-coordination sites

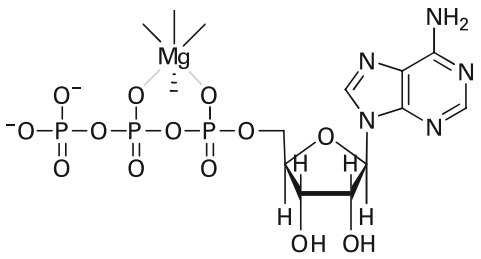
**Key points: The major binding sites for metal ions are provided by the amino acids that make up protein molecules; the ligation sites range from backbone peptide carbonyls to the side chains that provide more specific complexation; nucleic acids and lipid head groups are usually coordinated to major metal ions.**

Metal ions coordinate to proteins, nucleic acids, lipids, and a variety of other molecules. For instance, ATP is a tetraprotic acid and is always found as its $Mg^{2+}$ complex (**1**); DNA is stabilized by weak coordination of $K^+$ and $Mg^{2+}$ to its phosphate groups but destabilized by binding of soft metal ions such as Cu(I) to the bases. Macromolecules known as ribozymes may represent an important stage in the early evolution of life forms and are catalytic molecules composed of RNA and $Mg^{2+}$. The binding of $Mg^{2+}$ to phospholipid head groups is important for stabilizing membranes. There are a number of important small ligands, apart from water and free amino acids, which include sulfide, sulfate, carbonate, cyanide, carbon monoxide, and nitrogen monoxide, as well as organic acids such as citrate that form reasonably strong polydentate complexes with Fe(III).

As will be familiar from introductory chemistry, a protein is a polymer with a specific sequence of amino acids linked by peptide bonds (**2**). A 'small' protein is generally regarded as one with molar mass below 20 kg mol$^{-1}$, whereas a 'large' protein is one having a molar mass above 100 kg mol$^{-1}$. The principal amino acids are listed in Table 27.2. Proteins are



**2** Peptide bond

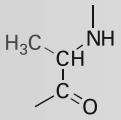
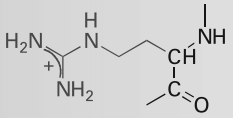
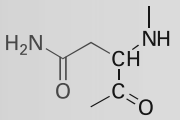
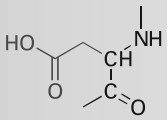
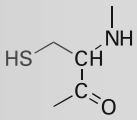
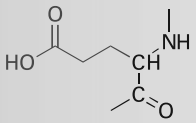
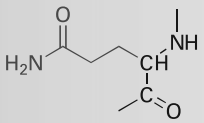
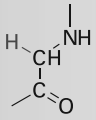
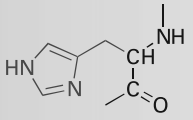
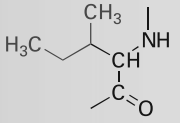


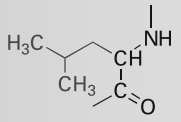
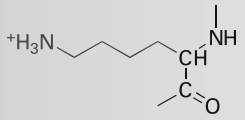
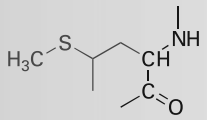
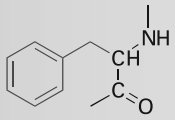
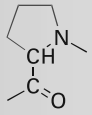
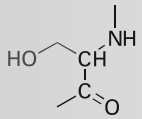
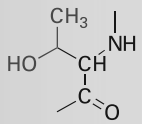
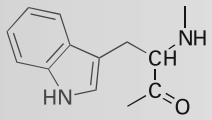
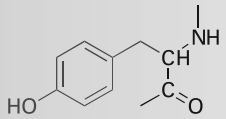
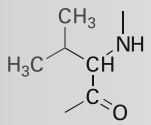
**1** Mg–ATP complex

synthesized, a process called **translation** (of the genetic code carried by DNA), on a special assembly called a *ribosome*. A protein may be processed further by **post-translational modification**, a change made to the protein structure, which includes the binding of **cofactors** such as metal ions.

**Metalloproteins**, proteins containing one or more metal ions, perform a wide range of specific functions. These functions include oxidation and reduction (for which the most

**Table 27.2** The amino acids and their codes

| Amino acid | Structure in peptide chain (side chain shown in blue) | Three-letter abbreviation | One-letter abbreviation |
|---|---|---|---|
| Alanine |  | Ala | A |
| Arginine |  | Arg | R |
| Asparagine |  | Asn | N |
| Aspartic acid |  | Asp | D |
| Cysteine |  | Cys | C |
| Glutamic acid |  | Glu | E |
| Glutamine |  | Gln | Q |
| Glycine |  | Gly | G |
| Histidine |  | His | H |
| Isoleucine |  | Ile | I |

| | | | |
|---|---|---|---|
| Leucine |  | Leu | L |
| Lysine |  | Lys | K |
| Methionine |  | Met | M |
| Phenylalanine |  | Phe | F |
| Proline |  | Pro | P |
| Serine |  | Ser | S |
| Threonine |  | Thr | T |
| Tryptophan |  | Trp | W |
| Tyrosine |  | Tyr | Y |
| Valine |  | Val | V |

important elements are Fe, Mn, Cu, and Mo), radical-based rearrangement reactions and methyl-group transfer (Co), hydrolysis (Zn, Fe, Mg, Mn, and Ni), and DNA processing (Zn). Special proteins are required for transporting and storing different metal atoms. The action of $Ca^{2+}$ is to alter the conformation of a protein (its shape) as a step in cell signalling (a term used to describe the transfer of information between and within cells). Such proteins are often known as **metal ion-activated proteins.** Hydrogen bonding between main-chain –NH and CO groups of different amino acids results in **secondary structure** (Fig. 27.2). The -**helix** regions of a polypeptide provide flexible mobility

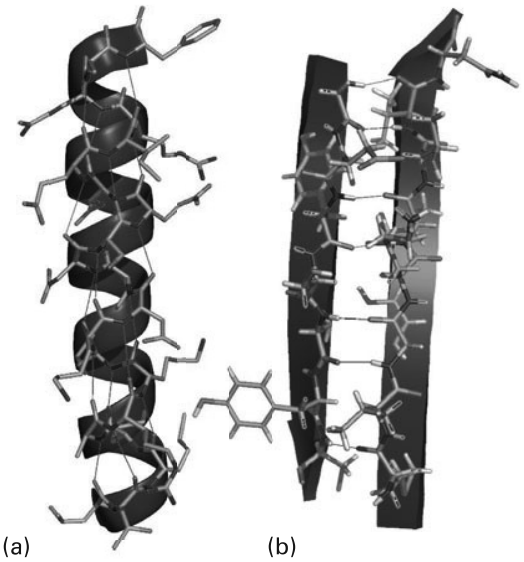
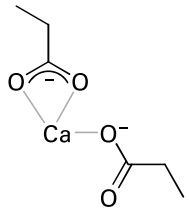
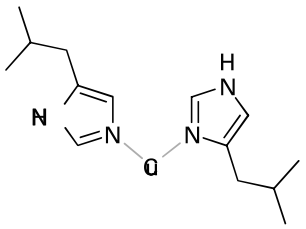


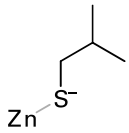

**Figure 27.2** The most important regions of secondary structure, (a) α helix, (b) β sheet, showing hydrogen bonding between main-chain amide and carbonyl groups and their corresponding representations.



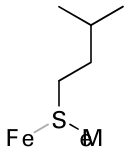

**3** $Ca^{2+}$ coordination



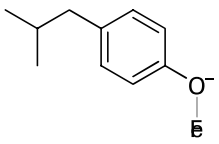

**4** Cu–imidazole coordination




**5** Zn–cysteine coordination




**6** Fe–methionine coordination




**7** Fe–tyrosine coordination

(like springs) and are important in converting processes that occur at the metal site into conformational changes; by contrast, a **χ-sheet** region confers rigidity to support a pre-organized coordination sphere suited to a particular metal ion (Sections 7.14 and 11.16). The secondary structure is largely determined by the sequence of amino acids: thus the α helix is favoured by chains containing alanine and lysine but is destabilized by glycine and proline. A protein that lacks its cofactor (such as the metal ions required for normal activity) is called an **apoprotein**; an enzyme with a complete complement of cofactors is known as a **holoenzyme**.

An important factor influencing metal-ion coordination in proteins is the energy required to locate an electrical charge inside a medium of low permittivity. To a first approximation, protein molecules may be regarded as oil drops in which the interior has a much lower relative permittivity (about 4) than water (about 78). This difference leads to a strong tendency to preserve electrical neutrality at the metal site, and hence influence the redox chemistry and Brønsted acidity of its ligands.

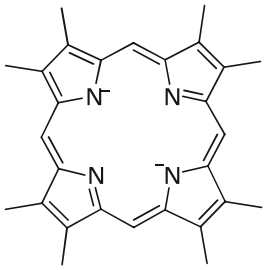
All amino acid residues can use their peptide carbonyl (or amide-N) as a donor group, but it is the side chain that usually provides more selective coordination. By referring to Table 27.2 and from the discussion in Section 4.12, we can recognize donor groups that are either chemically hard or soft and that therefore confer a particular affinity for specific metal ions. Aspartate and glutamate each provide a hard carboxylate group, and may use one or both O atoms as donors (**3**). The ability of $Ca^{2+}$ to have a high coordination number and its preference for hard donors are such that certain $Ca^{2+}$-binding proteins also contain the unusual amino acids γ-carboxyglutamate and hydroxyaspartate (generated by post-translational modification), which provide additional functionalities to enhance binding. Histidine, which has an imidazole group with two coordination sites, the ε-N atom (more common) and the δ-N atom, is an important ligand for Fe, Cu(**4**), and Zn. Cysteine has a thiol S atom that is expected to be unprotonated (thiolate) when involved in metal coordination. It is a good ligand for Fe, Cu, and Zn (**5**), as well as for toxic metals such as Cd and Hg. Methionine contains a soft thioether S donor that stabilizes Fe(II)(**6**) and Cu(I). Tyrosine can be deprotonated to provide a phenolate O donor atom that is a good ligand for Fe(III) (**7**). Selenocysteine (a specially coded amino acid in which Se replaces S) has also been identified as a ligand, for example it is found as a ligand to Ni in some hydrogenases (Section 27.14). A modified form of lysine, in which the side-chain $-NH_2$ has reacted with a molecule of $CO_2$ to produce a carbamate, is found as a ligand to Mg in the crucial photosynthetic enzyme known as rubisco (Section 27.9) and in other enzymes such as urease, where it is a ligand for Ni(II).

The primary and secondary structures of a polypeptide molecule can enforce unusual metal coordination geometries that are rarely encountered in small complexes. Protein-induced strain is an important possibility, for example the protein may impose a coordination geometry on the metal ion that resembles the transition state for the particular process being executed.

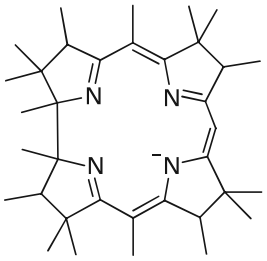
### (c) Special ligands

**Key point:** Metal ions may be bound in proteins by special organic ligands such as porphyrins and pterin-dithiolenes.

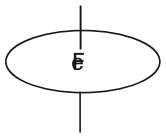
The porphyrin group (**8**) was first identified in haemoglobin (Fe) and a similar macrocycle is found in chlorophyll (Mg). There are several classes of this hydrophobic macrocycle, each differing in the nature of the side chains. The corrin ligand (**9**) has a slightly smaller ring size and coordinates Co in cobalamin (Section 27.11). Rather than show these macrocycles in full, we shall use shorthand symbols such as (**10**) to show the complexes they form with metals. Almost all Mo and W enzymes have the metal coordinated by a special ligand known as *molybdopterin* (**11**). The donors to the metal are a pair of S atoms from a dithiolene group that is covalently attached to a pterin. The phosphate group is often joined to a nucleoside base X, such as guanosine 5′-phosphate (GMP), resulting in the formation of a diphosphate bond. Why Mo and W are coordinated by this complex ligand is unknown, but the pterin group could provide a good electron conduit and facilitate redox reactions.



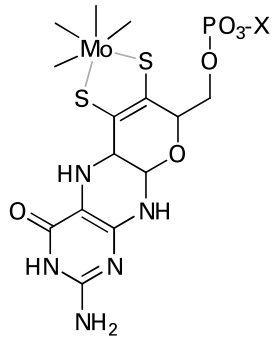

**8** Porphyrin$^{2-}$




**9** Corrin$^{-}$




**10**




**11** Molybdopterin as ligand

### (d) The structures of metal coordination sites

**Key point:** The likelihood that a protein will coordinate a particular kind of metal centre can be inferred from the amino acid sequence and ultimately from the gene itself.

The structures of metal coordination sites have been determined mainly by X-ray diffraction (now mostly by using a synchrotron, Section 8.1) and sometimes by NMR spectroscopy.[3] The basic structure of the protein can be determined even if the resolution is too low to reveal details of the coordination at the metal site. The packing of amino acids in a protein is far denser than is commonly conveyed by simple representations, as may be seen by comparing the representations of the structure of the $K^+$ channel in Fig. 27.3. Thus, even the substitution of an amino acid that is far from a metal centre may result in significant structural changes to its coordination shell and immediate environment. Of special interest are channels or clefts that allow a substrate selective access to the active site, pathways for long-range electron transfer (metal centres positioned less than 1.5 nm apart), pathways for long-range proton transfer (comprising chains of basic groups such as carboxylates and water molecules in close proximity, usually less than 0.3 nm apart), and tunnels for small gaseous molecules (which can be revealed by placing the crystal under Xe, an electron-rich gas).

**EXAMPLE 27.2** Interpreting the coordination environments of metal ions

Simple Cu(II) complexes have four to six ligands with trigonal-bipyramidal or tetragonal geometries, whereas simple complexes of Cu(I) have four or fewer ligands, and geometries that range between tetrahedral and linear. Predict how a Cu-binding protein will have evolved so that the Cu can act as an efficient electron-transfer site.

***Answer*** Here we are guided by Marcus theory (Section 21.12). An efficient electron-transfer reaction is one that is fast despite having a small driving force. The Marcus equation tells us that an efficient electron-transfer site is one for which the reorganization energy is small. The protein enforces on the Cu atom a coordination sphere that is unable to alter much between Cu(II) and Cu(I) states (see Section 27.8).

***Self-test 27.2*** In certain chlorophyll cofactors the Mg is axially coordinated by a methionine-S ligand. Why is this unusual coordination choice achieved in a protein?

[3]The atomic coordinates of proteins and other large biological molecules are stored in a public repository known as the Protein Data Bank located at http://www.rcsb.org/pdb/home/home.do. Each set of coordinates corresponding to a particular structure determination is identified by its 'pdb code'. A variety of software packages are available to construct and examine protein structures generated from these coordinates.

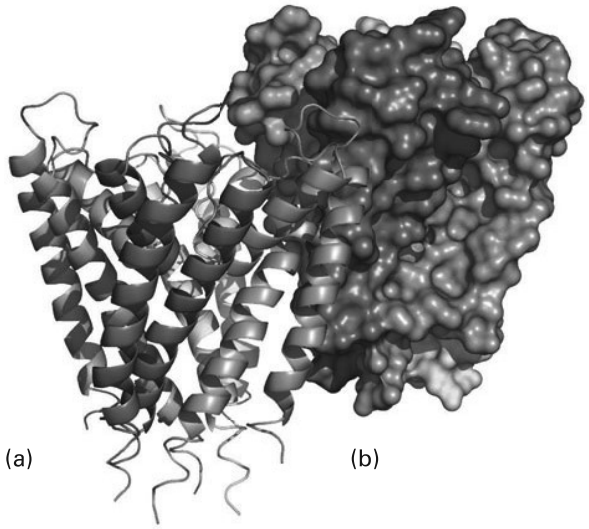



**Figure 27.3** Illustrations of how protein structures are represented to reveal either (a) secondary structure or (b) the filling of space by nonhydrogen atoms. The example shows the four subunits of the $K^+$ channel, which is found mainly embedded in the cell membrane.

Other physical methods described in Chapter 8 provide less information on the overall structure but are useful for identifying ligands. Thus, EPR spectroscopy is very important for studying d-block metals, especially those engaged in redox chemistry, because at least one oxidation state usually has an unpaired electron. The use of NMR is restricted to proteins smaller than 20–30 kg $mol^{-1}$ because tumbling rates for larger proteins are too slow and $^1H$ resonances are too broad to observe unless shifted away from the normal region ($\delta \approx 1-10$) by a paramagnetic metal centre. Extended X-ray absorption fine-structure spectroscopy (EXAFS, Section 8.9) can provide structural information on metal sites in amorphous solid samples, including frozen solutions. Vibrational spectroscopy (Section 8.4) is increasingly being used: IR spectroscopy is particularly useful for ligands such as CO and $CN^-$, and resonance Raman spectroscopy is very helpful when the metal centre has strong electronic transitions, such as occur with Fe porphyrins. Mössbauer spectroscopy (Section 8.7) plays a special role in studies of Fe sites. Perhaps the greatest challenge is presented by $Zn^{2+}$, which has a $d^{10}$ configuration that provides no useful magnetic or electronic signatures.

Metal ion binding sites can often be predicted from a gene sequence. **Bioinformatics**, the development and use of software to analyse and compare DNA sequences, is a powerful tool because many proteins that bind metal ions or have a metal-containing cofactor occur at cellular levels below that normally detectable directly by analysis and isolation. A particularly common sequence of the human genome encodes the so-called **Zn finger domain**, thereby identifying proteins that are involved in DNA binding (Section 27.5). Likewise, it can be predicted whether the protein that is encoded is likely to bind Cu, Ca, an Fe-porphyrin, or different types of Fe–S clusters. The gene can be cloned and the protein for which it encodes can be produced in sufficiently large quantities by 'overexpression' in suitable hosts, such as the common gut bacterium *Escherichia coli* or yeast, to enable it to be characterized. Furthermore, the use of genetic engineering to alter the amino acids in a protein, the technique of **site-directed mutagenesis**, is a powerful principle in biological inorganic chemistry. This technique often permits identification of the ligands to particular metal ions and the participation of other residues essential to functions such as substrate binding or proton transfer.

Although structural and spectroscopic studies give a good idea of the basic coordination environment of a metal centre, it is by no means certain that the same structure is retained in key stages of a catalytic cycle, in which unstable states are formed as intermediates. The most stable state of an enzyme, in which form it is usually isolated, is called the 'resting state'. Many enzymes are catalytically inactive on isolation and must be subjected to an

activation procedure that may involve reinsertion of a metal ion or other cofactor or removal of an inhibitory ligand.

Intense efforts have been made to model the active sites of metalloproteins by synthesizing analogues. The models may be divided into two classes: those designed to mimic the structure and spectroscopic properties of the real site, and those synthesized with the intention of mimicking a functional activity, most obviously catalysis. Synthetic models not only illuminate the chemical principles underlying biological activity but also generate new directions for coordination chemistry. As we shall see throughout this chapter, the difficulty is that an enzyme not only imposes some strain on the coordination sphere of a metal atom (even a porphyrin ring is puckered in most cases) but also provides, at fixed distances, functional groups that provide additional coulombic and hydrogen-bonding interactions essential for binding and activating substrates. Indeed, the active site of a metalloenzyme is arguably the ultimate example of supramolecular chemistry.

## Transport, transfer, and transcription

In this section we turn to three related aspects of the function of biological molecules containing metal ions, and see their role in the transport of ions through membranes, the transport and distribution of molecules through organisms, and the transfer of electrons. Metal ions also play an important role in the transcription of genes.

### 27.3 Sodium and potassium transport

**Key points:** Transport across a membrane is active (energized) or passive (spontaneous); the flow of ions is achieved by proteins known as ion pumps (active) and channels (passive).

In Chapter 11 we saw that differentiating between $Na^+$ and $K^+$, two ions that are very similar except for their radii (*Resource section 1*), is achieved through their selective complexation by special ligands, such as crown ethers and cryptands, with dimensions appropriate for coordination to one particular kind of ion. Organisms use this principle in the molecules known as **ionophores**, which have hydrophobic exteriors that make them soluble in lipids. The antibiotic valinomycin (Section 11.16) is an ionophore that has a high selectivity for $K^+$, which is coordinated by six carbonyl groups. It enables $K^+$ to pass through a bacterial cell membrane and thereby dissipate the electrical potential difference, so causing the bacterium's death.

At the higher end of the complexity scale are the ion channels, which are large membrane-spanning proteins that allow selective transport of $K^+$ and $Na^+$ (as well as $Ca^{2+}$ and $Cl^-$) and are responsible for electrical conduction in nervous systems as well as in coupled transport of solutes.[4] Figure 27.4 shows the important structural aspects of the potential-gated $K^+$ channel. Moving from the inside surface of the membrane, the enzyme has a pore (which can open and close on receipt of a signal) leading into a central cavity about 1 nm in diameter; up to this stage $K^+$ ions can remain hydrated. Polypeptide helices pointing at this cavity have their partial charges directed in such a way as to favour population by cations, resulting in a local $K^+$ concentration of approximately 2 M. Above the central cavity the tunnel contracts into a **selectivity filter** consisting of helical ladders of closely spaced peptide carbonyl-O donors that form a sequence of four cubic eightfold coordination sites. During operation of the channel these sites are occupied, at any one time, by a queue of two $K^+$ ions and two $H_2O$ molecules in alternate fashion, as in $\cdots K^+ \cdots H_2O \cdots K^+ \cdots H_2O \cdots$. The rate of passage of $K^+$ ions through the selectivity filter is close to the limit for diffusion control. A plausible mechanism for selective $K^+$ transport (Fig. 27.5) involves concerted displacement of $K^+$ ions between adjacent cubic carbonyl-O sites through intermediate, unstable octahedral states in which the $K^+$ ions are coordinated equatorially by four carbonyl-O donors and axially by the two intervening $H_2O$ molecules. This mechanism is not

[4] Roderick MacKinnon shared the 2003 Nobel Prize for Chemistry for his elucidation of the structures and mechanisms of ion channels.

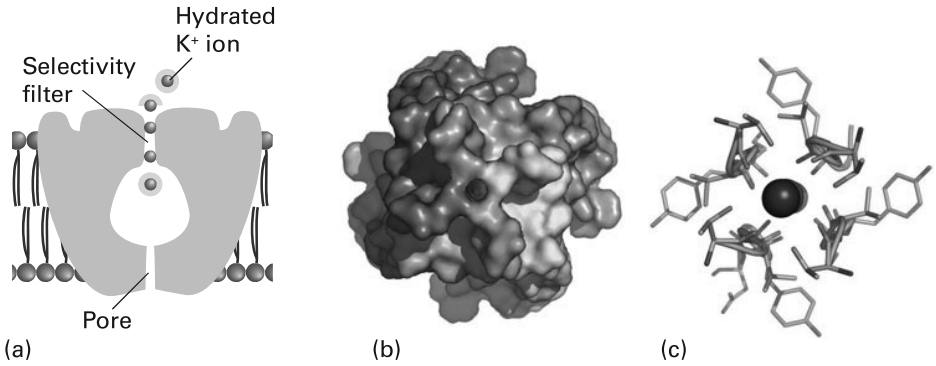



**Figure 27.4** (a) Schematic structure of the $K^+$ channel showing the different components and the transport of $K^+$ ions: the blue halo represents hydration. (b) View of the enzyme from inside the cell showing the entrance pore that admits hydrated ions. (c) View looking up the selectivity filter showing how mobile dehydrated $K^+$ ions are coordinated by peptide carbonyl-O atoms provided by each of the four subunits. Note the almost fourfold symmetry axis.

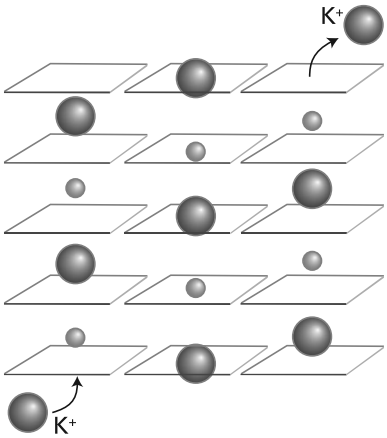



**Figure 27.5** Mechanism of transport of $K^+$ ions through the selectivity filter of the $K^+$ channel. Green spheres represent water molecules.

effective for $Na^+$ because the cavity is too large, which accounts for the $10^4$-fold selectivity of the channel for $K^+$ over $Na^+$. The binding is weak and fast because it is important to convey but not to trap $K^+$.

Overall, the $K^+$ channel acts by the mechanism illustrated in Fig. 27.6. Charged groups on the molecule move in response to a change in the membrane potential and cause the intracellular pore to open, so allowing entry of hydrated $K^+$ ions. Selective binding of dehydrated $K^+$ ions occurs in the filter region, a potential drop across the membrane is sensed, and the cavity closes. At this point the filter opens up to the external surface, where the $K^+$ concentration is low and the $K^+$ ions are hydrated and released. This release causes the protein to switch back to the original conformation and $K^+$ ions again enter the filter.

The $Na^+/K^+$ pump ($Na^+/K^+$-ATPase), the enzyme that maintains the concentration differential of $Na^+$ and $K^+$ inside and outside a cell, is another example of the high discrimination between alkali metal ions that has evolved with biological ligands. The ions are pumped against their concentration gradients by coupling the process to ATP hydrolysis. The mechanism, which is outlined in Fig. 27.7, involves conformational changes induced by ATP-driven protein phosphorylation.

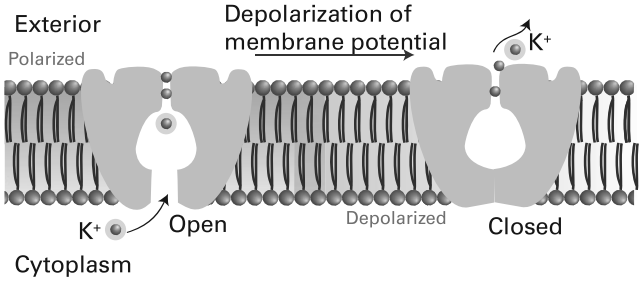



**Figure 27.6** Proposed mechanism of action of the $K^+$ channel. The potential difference across the membrane is sensed by the protein, which causes the pore to open, allowing hydrated ions to enter the cavity. After shedding their hydration sphere, $K^+$ ions pass up the selectivity filter at rates close to diffusion control.

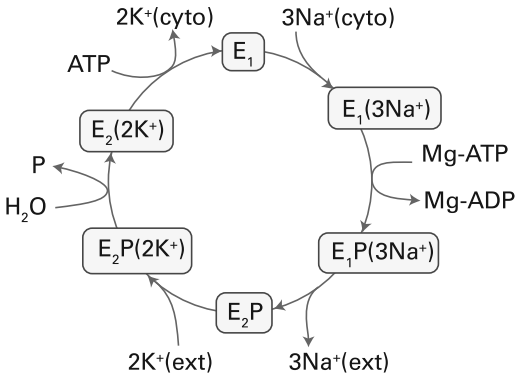



**Figure 27.7** General principle of the $Na^+,K^+$-ATPase (the Na pump). Release of two $K^+$ ions into the cytoplasm is accompanied by binding of ATP (from the cytoplasm) and conversion of the enzyme into state 1, which binds three $Na^+$ ions from the cytoplasm. A phosphate group (P) is transferred to the enzyme, which opens to the external side, expels three $Na^+$ ions, then binds two $K^+$ ions. Release of the phosphate group causes release of $K^+$ into the cytoplasm and the cycle begins again.

**EXAMPLE 27.3 Assessing the role of ions in active and passive transport**

The toxic species $Tl^+$ (radius 150 pm) is used as an NMR probe for $K^+$ binding in proteins. Explain why $Tl^+$ is suited for this purpose and account for its high toxicity.

***Answer*** To address this question we need to recall from Chapter 13 that Tl, in common with other heavy, post-d-block elements, displays the inert-pair effect, a preference for forming compounds in which its oxidation number is 2 less than the group oxidation number. Thallium (Group 13) thus resembles the heavy Group 1 elements (in fact, TlOH is a strong base) and $Tl^+$ can replace $K^+$ in complexes, with the advantage that it can be studied by NMR spectroscopy ($^{203}Tl$ and $^{205}Tl$ have $I = \frac{1}{2}$). The similarity with $K^+$ allows $Tl^+$, a toxic element, free entry into a cell because it is 'recognized' by the $Na^+/K^+$-ATPase. But once inside, more subtle differences in chemical properties, such as the tendency of Tl to form more stable complexes with soft ligands, are manifested and become lethal.

***Self-test 27.3*** Explain why the intravenous fluid used in hospital procedures contains NaCl, not KCl.

## 27.4 Calcium signalling proteins

**Key point: Calcium ions are suitable for signalling because they exhibit fast ligand exchange and a large, flexible coordination geometry.**

Calcium ions play a crucial role in higher organisms as an intracellular messenger, providing a remarkable demonstration of how organisms have exploited the otherwise rather limited chemistry of this element. Fluxes of $Ca^{2+}$ trigger enzyme action in cells in response to receiving a hormonal or electrical signal from elsewhere in the organism. Calcium is particularly suited for signalling because it has fast ligand-exchange rates, intermediate binding constants, and a large, flexible coordination sphere.

Calcium signalling proteins are small proteins that change their conformation depending on the binding of $Ca^{2+}$ at one or more sites; they are thus examples of the metal ion-activated proteins mentioned earlier. Every muscle movement we make is stimulated by $Ca^{2+}$ binding to a protein known as troponin C. The best-studied $Ca^{2+}$-regulatory protein is calmodulin (17 kg $mol^{-1}$, Fig. 27.8): its roles include activating protein kinases that catalyse phosphorylation of proteins and activating NO-synthase, a Fe-containing enzyme responsible for generating the intercellular signalling molecule nitric oxide. Calmodulin has four $Ca^{2+}$-binding sites (one is shown as **12**) with dissociation constants lying close to $10^{-6}$. The binding of $Ca^{2+}$ to the four sites alters the protein conformation and it is then recognized by a target enzyme.[5]

Calcium signalling requires special $Ca^{2+}$ pumps, which are large, membrane-spanning enzymes that pump $Ca^{2+}$ out of the cytoplasm, either out of the cell altogether or into Ca-storing organelles such as the endoplasmic reticulum or the mitochondria. As with $Na^+/K^+$-ATPases, the energy for $Ca^{2+}$ pumping comes from ATP hydrolysis. Hormones or electrical stimuli open specific channels (analogous to $K^+$ channels) that release $Ca^{2+}$ into the cell. Because the level in the cytoplasm before the pulse is low, the influx easily raises the $Ca^{2+}$ concentration above that needed for $Ca^{2+}$-binding proteins such as calmodulin (or troponin C in muscle). The action can be short-lived, so that after a pulse of $Ca^{2+}$ the cell is quickly evacuated by the calcium pump.

Although $Ca^{2+}$ is invisible to most spectroscopic methods, some Ca proteins, such as calmodulin or troponin C, are small enough to be studied by NMR. Because of their preference for large multicarboxylate ligands, the lanthanoid ions (Section 23.7) have been used as probes for Ca binding, exploiting their properties of paramagnetism (as chemical shift reagents in NMR spectroscopy) and fluorescence. Intracellular concentrations of Ca are monitored by using special fluorescent polycarboxylate ligands (**13**) that are introduced to the cell as their esters, which are hydrophobic and able to cross the membrane lipid barrier. Once in the cell, enzymes known as esterases hydrolyse the esters and release the ligands, which respond to changes in $Ca^{2+}$ concentration in the range $10^{-7}$–$10^{-9}$ M.

[5]The occupation of a binding site is 50 per cent when the concentration (in mol $dm^{-3}$) of species is equal to the dissociation constant (the reciprocal of the association constant).

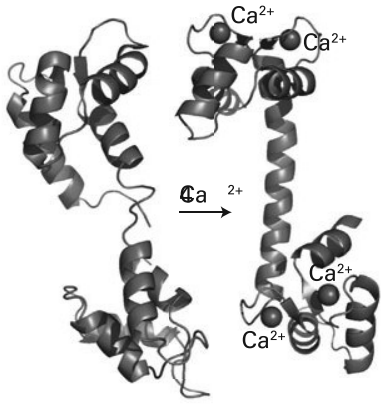
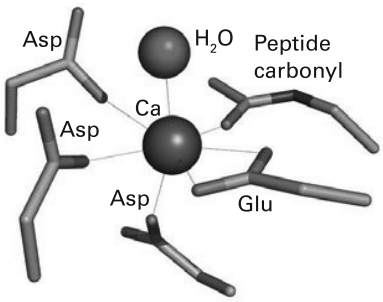
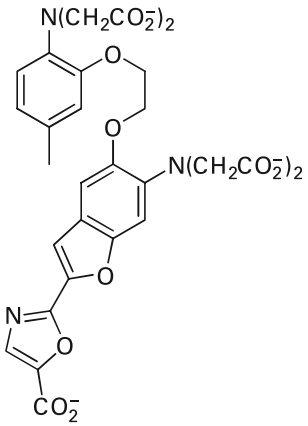



**Figure 27.8** The binding of four $Ca^{2+}$ to apocalmodulin causes a change in the protein conformation, converting it to a form that is recognized by many enzymes. The high proportion of α-helix is typical of proteins that are activated by metal-ion binding.




**12**




**13** FURA-2

**EXAMPLE 27.4 Explaining why calcium is suitable for signalling**

Why is $Ca^{2+}$ more suitable than $Mg^{2+}$ for fast signalling processes in cells?

***Answer*** To answer this question we need to refer to Section 21.1, in which we saw that the ligand-exchange rates of s-block metal ions increase down each group. The exchange of coordinated $H_2O$ molecules is $10^3$—$10^4$ times faster for $Ca^{2+}$ than for $Mg^{2+}$. The speed at which $Ca^{2+}$ can shed its ligands and bind to a target protein is crucial for ensuring fast signalling. As an example, rapid muscle contractions that may protect an organism against sudden attack are initiated by $Ca^{2+}$ binding to troponin C.

***Self-test 27.4*** The $Ca^{2+}$ pump is activated by calmodulin. Explain the significance of this observation. *Hint*: Consider how a feedback mechanism could control $Ca^{2+}$ levels in the cytoplasm.

## 27.5 Zinc in transcription

**Key points:** Zinc fingers are protein structural features produced by coordination of Zn to specific histidine and cysteine residues; a sequence of these fingers enables the protein to recognize and bind to precise sequences of DNA base pairs and plays a crucial role in transferring information from the gene.


**14** **15**

The roles of Zn are either catalytic, which we will deal with later, or structural and regulatory. Unlike Ca and Mg, Zn forms more stable complexes with softer donors, so it is not surprising that it is usually found coordinated in proteins through histidine and cysteine residues. Typical catalytic sites (**14**) commonly have three permanent protein ligands and an exchangeable ligand ($H_2O$), whereas structural Zn sites (**15**) are coordinated by four 'permanent' protein ligands.

**Transcription factors** are proteins that recognize certain regions of DNA and control how the genetic code is interpreted as RNA. It has been known since the 1980s that many DNA-binding proteins contain repeating domains that are folded in place by the binding of Zn and form characteristic folds known as 'zinc fingers' (Fig. 27.9). In a typical case, one side of the finger provides two cysteine-S donors and the other side provides two histidine-N donors and folds as an α helix. Each 'finger' makes recognitory contacts with specific DNA bases. As shown in Fig. 27.10, the zinc fingers wrap around sequences of DNA that they are able to recognize by acting collectively. The high fidelity of transcription factors is the result of a number of such contacts being made along the DNA chain at the beginning of the sequence that is transcribed.

The characteristic residue sequence for a Zn-finger motif is

$$-(\text{Tyr,Phe})-\text{X}-\text{Cys}-\text{X}_{2-4}-\text{Cys}-\text{X}_3-\text{Phe}-\text{X}_5-\text{Leu}-\text{X}_2-\text{His}-\text{X}_{3-5}-\text{His}-$$

where the amino acid X is variable. Aside from the 'classical' $(Cys)_2(His)_2$ zinc finger, others have been discovered that have $(Cys)_3His$ or $(Cys)_4$ coordination, together with more elaborate examples having 'Zn-thiolate clusters', such as the so-called GAL4 transcription factor in which two Zn atoms are linked by bridging cysteine-S ligands (**16**). Various protein folds are produced, with faintly jocular names, such as 'zinc knuckles', joining an

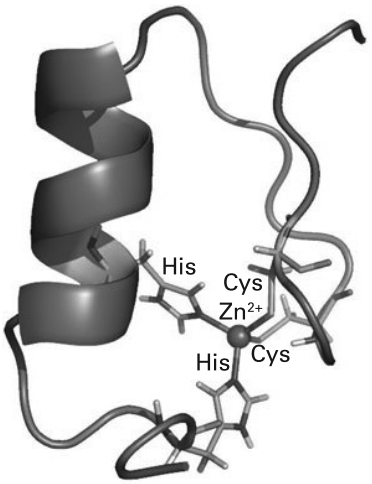



**Figure 27.9** Zinc fingers are protein folds that form a sequence able to bind to DNA. A typical finger is formed by the coordination of Zn(II) to two pairs of amino acid side chains located either side of the 'fingertip'.

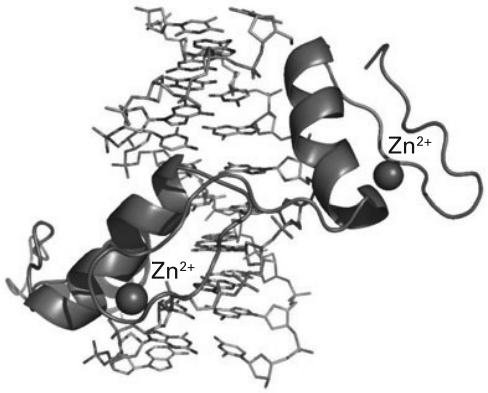
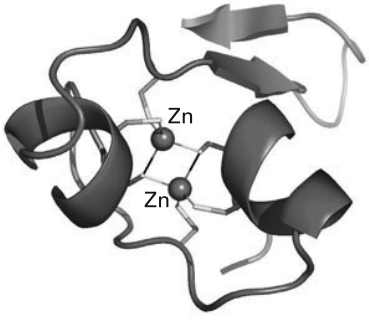



**Figure 27.10** A pair of zinc fingers interacting with a section of DNA.




**16** $Zn_2(Cys)_6$

increasingly large family. Higher order Zn-thiolate clusters are found in proteins known as metallothioneins and some Zn-sensor proteins (see Section 27.16).

Zinc is particularly suited for binding to proteins to hold them in a particular conformation: $Zn^{2+}$ is high in the Irving–Williams series (Section 20.1) and thus forms stable complexes, particularly to S and N donors. It is also redox inactive, which is an important factor because it is crucial to avoid oxidative damage to DNA. Other examples of structural zinc include insulin and alcohol dehydrogenase. The lack of good spectroscopic probes for Zn, however, has meant that even though it is tightly bound in a protein, it is difficult to confirm its binding or deduce its coordination geometry in the absence of direct structural information from X-ray diffraction or NMR. However, some elegant measurements have exploited the ability of $Co^{2+}$, which is coloured and paramagnetic, or $Cd^{2+}$, which has useful NMR properties, to substitute for and report on the Zn site. These substitutions depend on strong similarities between the metal ions: like Zn, $Co^{2+}$ readily forms tetrahedral complexes, whereas Cd lies directly below Zn in the periodic table. For many Zn enzymes, it is found that the surrogate metals are as active as Zn itself (Section 27.9).

## 27.6 Selective transport and storage of iron

**Key points:** The uptake of Fe into organisms involves special ligands known as siderophores; transport in the circulating fluids of higher organisms requires a protein called transferrin; Fe is stored as ferritin.

Iron is essential for almost all life forms; however, Fe is also difficult to obtain, yet any excess presents a serious toxic risk. Nature has at least two problems in dealing with this element. The first is the insolubility of Fe(III), which is the stable oxidation state found in most minerals. As the pH increases, hydrolysis, polymerization, and precipitation of hydrated forms of the oxide occur. Polymeric oxide-bridged Fe(III) is the thermodynamic sink of aerobic Fe chemistry (as seen in a Pourbaix diagram, Section 5.14). The insolubility of rust renders the straightforward uptake by a cell very difficult. The second problem is the toxicity of 'free-Fe' species, particularly through the generation of OH radicals. To prevent Fe from reacting with oxygen species in an uncontrolled manner, a protective coordination environment is required. Nature has evolved sophisticated chemical systems to execute and regulate all aspects, from the primary acquisition of Fe, to its subsequent transport, storage, and utilization in tissue. The 'Fe cycle' as it affects a human is summarized in Fig. 27.11.

### (a) Siderophores

**Siderophores** are small polydentate ligands that have a very high affinity for Fe(III). They are secreted from many bacterial cells into the external medium, where they sequester Fe to give a soluble complex that re-enters the organism at a specific receptor. Once inside the cell, the Fe is released.

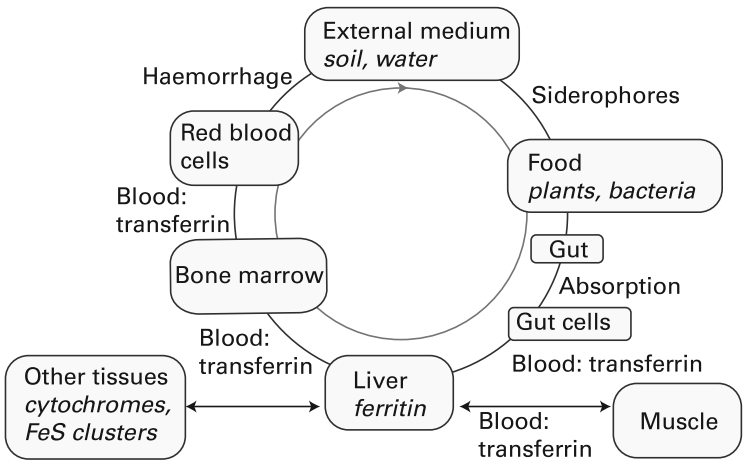
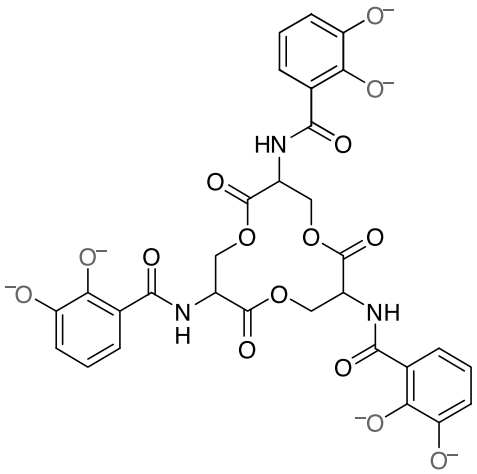
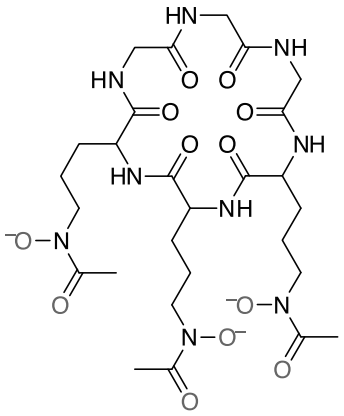



**Figure 27.11** The biological Fe cycle showing how Fe is taken up from the external medium and guarded carefully in its travels through organisms.



**17** Enterobactin



**18** Ferrichrome

Aside from citrate (the Fe(III) citrate complex is the simplest Fe transport species in biology) there are two main types of siderophore. The first type is based on phenolate or catecholate ligands, and is exemplified by enterobactin (**17**), for which the value of the association constant for Fe(III) is $10^{52}$, an affinity so great that enterobactin enables bacteria to erode steel bridges. The second type of siderophore is based on hydroxamate ligands and is exemplified by ferrichrome (**18**), a cyclic hexapeptide consisting of three glycine and three *N*-hydroxyl-l-ornithines.

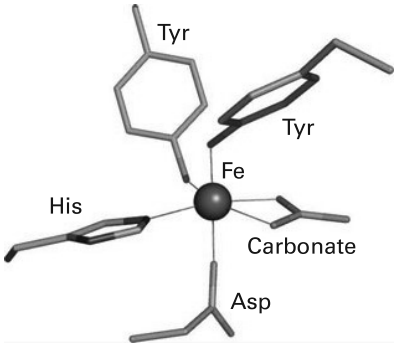
All Fe(III) siderophore complexes are octahedral and high spin. Because the donor atoms are hard O or N atoms and negatively charged, they have a relatively low affinity for Fe(II). Synthetic siderophores are proving to be very useful agents for the control of 'iron overload', a serious condition affecting large populations of the world, particularly South-East Asia (Section 27.17).

### (b) Iron-transport proteins in higher organisms

There are several important, structurally similar Fe-transport proteins known collectively as **transferrins**. The best characterized examples are serum transferrin (in blood plasma), ovotransferrin (in egg-white), and lactoferrin (in milk). The apoproteins are potent antibacterial agents as they deprive microbes of their iron. Transferrins are also present in tears, serving to cleanse eyes after irritation. All these transferrins are glycoproteins (protein molecules modified by covalently bound carbohydrate) with molar masses of about 80 kg $mol^{-1}$ and containing two separated and essentially equivalent binding sites for Fe. Complexation of Fe(III) at each site involves simultaneous binding of $HCO_3^-$ or $CO_3^{2-}$ and release of $H^+$:

$$\text{apo-TF} + \text{Fe(III)} + HCO_3^- \rightarrow \text{TF–Fe(III)–}CO_3^{2-} + H^+$$

where TF denotes transferrin. For each site, the association constant under physiological conditions (pH = 7) is in the range $10^{22}$–$10^{26}$. However, its value depends strongly on the pH and this dependence is the main factor controlling Fe uptake and release.




**19**

Transferrin consists of two very similar parts, termed the **N-lobe** and the **C-lobe** (Fig. 27.12). The protein is a product of gene duplication because the structure of the first half of the molecule can almost be overlaid on the second half. Each half consists of two domains, 1 and 2, which together form a cleft with a binding site for Fe(III). There is a considerable proportion of α helix, resulting in flexibility. Complexation with Fe(III) causes a conformational change consisting of a hinge motion involving domains 1 and 2 at each lobe. Binding of Fe(III) causes the domains to come together.

In each active site (**19**), a single Fe atom is coordinated by widely dispersed amino acid side chains from both domains and the connecting region, hence the change in conformation that occurs. The protein ligands are carboxylate-O (Asp), two phenolate-O (Tyr), and an imidazole-N (His). Only one of the aspartate carboxylate-O atoms is coordinated. The protein ligands form part of a distorted octahedral coordination sphere. The coordination

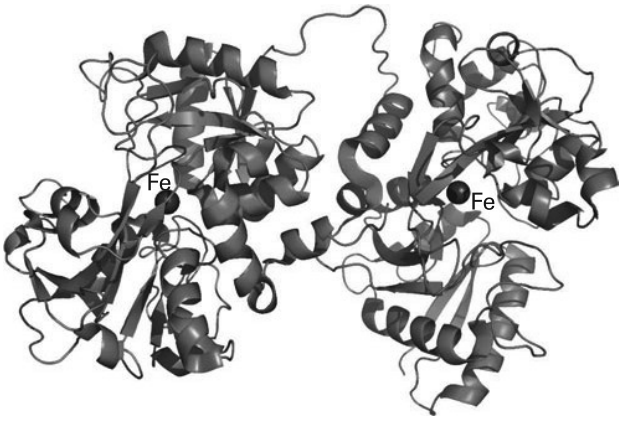



**Figure 27.12** Structure of the Fe-transport protein transferrin: the identical halves of the molecule each coordinate to a single Fe(III) atom (the black spheres) between two lobes. This coordination causes a conformational change that allows transferrin to be recognized by the transferrin receptor.

is completed by bidentate binding to the exogenous carbonate, which is referred to as a **synergistic ligand** because Fe binding depends on its presence. In certain cases phosphate is bound instead of carbonate. As expected from the predominantly anionic ligand set, Fe(III) binds much more tightly than Fe(II). However, ions similar to Fe(III), particularly Ga(III) and Al(III), also bind tightly, so that these metals can use the same transport system to gain access to tissues.

### (c) Release of iron from transferrin

Cells in need of Fe produce large amounts of a protein called the **transferrin receptor** (180 kg $mol^{-1}$), which is incorporated within their plasma membrane. This protein binds Fe-loaded transferrin. The most favoured mechanism for Fe uptake involves the Fe-loaded transferrin receptor complex entering the cell by a process known as **endocytosis**. In endocytosis, a section of the cell membrane is engulfed by the wall, along with its component membrane-bound proteins, to form a vesicle. The pH within this vesicle is then lowered by a membrane-bound $H^+$-pumping enzyme that is also swallowed by the cell. The subsequent release of Fe(III) is probably linked to the coordination of carbonate, which is synergistic in the sense that it is necessary for the binding of Fe but is unstable at low pH. Indeed, from *in vitro* studies it is known that Fe is released by lowering the pH to about 5 for serum transferrin and to 2–3 for lactoferrin. The vesicle then splits and the TF-receptor complex is returned to the plasma membrane by **exocytosis**, and Fe(III), probably now complexed by citrate, is released to the cytoplasm.

### (d) Ferritin, the cellular Fe store

Ferritin is the principal store of non-haem Fe in animals (most Fe is occupied in haemoglobin and myoglobin) and, when fully loaded, contains 20 per cent Fe by mass. It occurs in all types of organism, from mammals to prokaryotes. In mammals, it is found particularly in the spleen and in blood. Ferritins have two components, a 'mineral' core that contains up to 4500 Fe atoms (mammalian ferritin) and a protein shell. Apoferritin (the protein shell devoid of Fe) can be prepared by treatment of ferritin with reducing agents and an Fe(II) chelating ligand (such as 1,10-phenanthroline or 2,2′-bipyridyl). Dialysis then yields the intact shell.

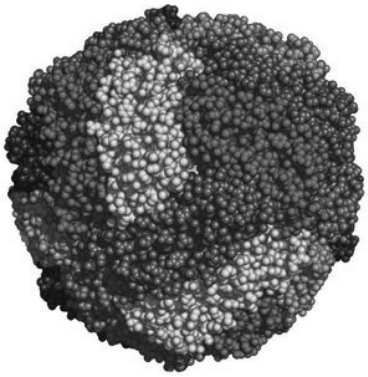
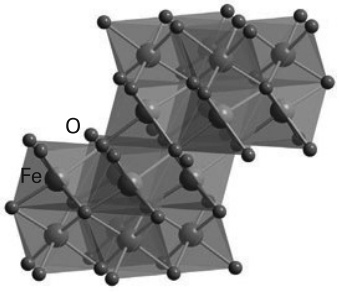


**Figure 27.13** The structure of ferritin, showing the arrangement of subunits that make up the protein shell.

Apoferritins have average molar masses in the range 460 to 550 kg $mol^{-1}$. The protein shell (Fig. 27.13) consists of 24 subunits that link together to form a hollow sphere with twofold, threefold (as shown in the illustration), and fourfold symmetry axes. Each subunit consists of a bundle of four long and one short α helices, with a loop that forms a section of β sheet with a neighbouring subunit. The mineral core is composed of hydrated Fe(III) oxide with varying amounts of phosphate, which helps anchor it to the internal surface. The structure as revealed by X-ray or electron diffraction resembles that of the mineral ferrihydrite, $5Fe_2O_3.9H_2O$, which is based on an hcp array of $O^{2-}$ and $OH^-$ ions, with Fe(III) layered in both the octahedral and tetrahedral sites (**20**).




**20**

The threefold and fourfold symmetry axes of apoferritin are, respectively, hydrophilic and hydrophobic pores. The threefold-axis pores are suited for the passage of ions. However, the

ferrihydrite core is insoluble and Fe must be mobilized. The most feasible mechanism so far proposed for the reversible incorporation of Fe in ferritin involves its transport in and out as Fe(II), perhaps as the $Fe^{2+}$ ion, which is soluble at neutral pH, but more likely some type of 'chaperone' complex. Oxidation to Fe(III) is thought to occur at specific di-iron binding sites known as **ferroxidase centres**, present in each of the subunits. Oxidation to Fe(III) involves the coordination of $O_2$ and inner-sphere electron transfer:

$$2\,Fe(II) + O_2 + 2\,H^+ \rightarrow 2\,Fe(III) + H_2O_2$$

The mechanism by which Fe is released almost certainly involves its reduction back to the more mobile Fe(II).

## 27.7 Oxygen transport and storage

Dioxygen, $O_2$, is a special molecule that has not always been available to biology; in fact, to many life forms it is highly toxic. As the waste product of oxygenic photosynthesis that began with cyanobacteria more than 2 Ga ago, $O_2$ is a biogenic substance, one that owes it existence to solar energy capture by living organisms. As we shall see in Section 27.10, the great thermodynamic advantage of having such a powerful oxidant available undoubtedly led to the evolution of higher organisms that now dominate Earth. Indeed, the requirement for $O_2$ became so important as to necessitate special systems for transporting and storing it. Apart from the difficulty in supplying $O_2$ to buried tissue, there is the problem of achieving a sufficiently high concentration in aqueous environments. This problem is overcome by special metalloproteins known as **$O_2$ carriers**. In mammals and most other animals and plants, these special proteins (myoglobin and haemoglobin) contain an Fe porphyrin cofactor. Animals such as molluscs and arthropods use a Cu protein called haemocyanin, and some lower invertebrates use an alternative type of Fe protein, haemerythrin, which contains a dinuclear Fe site.

### (a) Myoglobin

**Key points:** The deoxy form containing high-spin five-coordinate Fe(II) reacts rapidly and reversibly with $O_2$ to produce low-spin six-coordinate Fe(II); a slow autooxidation reaction releases superoxide and produces Fe(III), which is inactive in binding $O_2$.

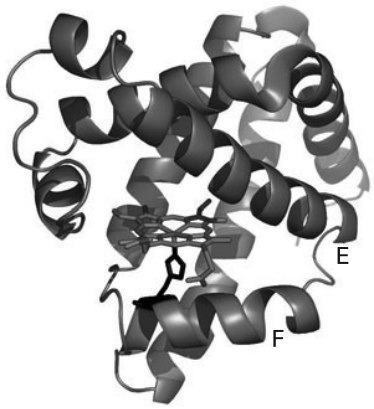



**Figure 27.14** Structure of myoglobin, showing the Fe porphyrin group located between helices E and F.

Myoglobin[6] is an Fe protein (17 kg $mol^{-1}$, Fig. 27.14) that coordinates $O_2$ reversibly and controls its concentration in tissue. The molecule contains several regions of α helix, implying mobility, with the single Fe porphyrin group located in a cleft between helices E and F. Two propionate substituents on the porphyrin interact with solvent $H_2O$ molecules on the surface of the protein. The fifth ligand to the Fe is provided by a histidine-N from helix F, and the sixth position is the site at which $O_2$ is coordinated. In common terminology, the side of the haem plane at which exchangeable ligands are bound is known as the **distal region**, while that below the haem plane is known as the **proximal region**. The histidine on helix F is one of two that are present in all species. Such 'highly conserved' amino acids are a strong indication that evolution has determined that they are essential for function. The other conserved histidine is located on helix E.

Deoxymyoglobin (Mb) is bluish red and contains Fe(II); this is the oxidation state that binds $O_2$ to give the familiar bright red oxymyoglobin (oxyMb). In some instances deoxymyoglobin becomes oxidized to Fe(III), which is called metmyoglobin (metMb) and is unable to bind $O_2$. This oxidation may occur by a ligand substitution-induced redox reaction in which $Cl^-$ ions displace bound $O_2$ as superoxide:

$$Fe^{(II)}O_2 + Cl^- \rightarrow Fe^{(III)}Cl + O_2^-$$

In healthy tissue, an enzyme (methaemoglobin reductase) is available to reduce the met form back to the Fe(II) form.

[6] Myoglobin was the first protein for which the three-dimensional structure was determined by X-ray diffraction. For this achievement, John Kendrew shared the 1962 Nobel Prize for Chemistry with Max Perutz, who solved the structure of haemoglobin.

The Fe in deoxymyoglobin is five-coordinate, high-spin, and lies above the plane of the ring. When $O_2$ binds it is coordinated end-on to the Fe atom, the electronic structure of which is tuned by the F helix histidine ligand (Fig. 27.15). The unbound end of the $O_2$ molecule is fastened by a hydrogen bond to the imidazole-NH of the histidine in helix E. The coordination of $O_2$ (a strong-field π-acceptor ligand) causes the Fe(II) to switch from high-spin (equivalent to $t_{2g}^4 e_g^2$) to low-spin ($t_{2g}^6$) and, with no d electrons in antibonding orbitals, to shrink slightly and move into the plane of the ring. The bonding is often expressed in terms of Fe(II) coordination by singlet $O_2$, in which the doubly occupied antibonding $2\pi_g$ orbital of $O_2$ acts as a σ donor and the empty $2\pi_g$ orbital of $O_2$ accepts an electron pair from the Fe (Fig. 27.16). An alternative description is often considered, in which the bonding is expressed in terms of low-spin Fe(III) coordinated by superoxide, $O_2^-$. With this model, the formation of metmyoglobin by reaction with anions is a simple ligand displacement.

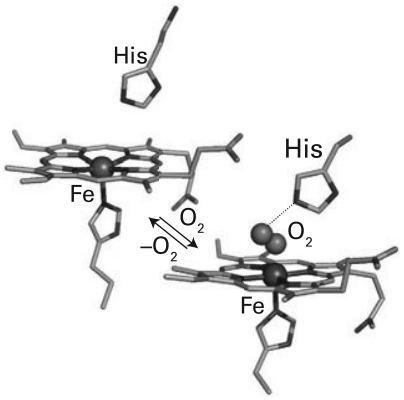



**Figure 27.15** Reversible binding of $O_2$ to myoglobin: coordination by $O_2$ causes the Fe to become low spin and move into the plane of the porphyrin ring.

### (b) Haemoglobin

**Key point: Haemoglobin consists of a tetramer of myoglobin-like subunits, with four Fe sites that bind $O_2$ cooperatively.**

Haemoglobin (Hb, 68 kg mol$^{-1}$, Fig. 27.17) is the $O_2$ transport protein found in special cells known as *erythrocytes* (red blood cells): a litre of human blood contains about 150 g of Hb. Simplistically, Hb can be thought of as a tetramer of myoglobin-like units with a cavity in the middle. There are in fact two types of Mb-like subunits, which differ slightly in their structures, and Hb is referred to as an **$\beta_2\chi_2$ tetramer**.

The $O_2$ binding curves for Mb and Hb are shown in Fig. 27.18: it is highly significant that the curve for Hb is sigmoidal, which indicates that uptake and release of successive $O_2$ molecules is cooperative. At low $O_2$ partial pressure and greater acidity (as in venous blood and muscle tissue following aggressive exercise) Hb has low affinity for $O_2$. This low affinity enables Hb to transfer its $O_2$ to Mb. As the pressure increases, so does the affinity of Hb for $O_2$ and as a result Hb can pick up $O_2$ in the lungs. This change in affinity can be attributed to there being two conformations. The **tensed state** (T) has a low affinity and the **relaxed state** (R) has a high affinity. Deoxy-Hb is T and fully loaded oxy-Hb is R.

To understand the molecular basis of cooperativity we need to refer to Fig. 27.15. Binding of the first $O_2$ molecule to the T-state molecule is weak, but the decrease in the size of the Fe allows it to move into the plane of the porphyrin ring. This motion is particularly important for Hb because it pulls on the proximal histidine ligand and helix F moves. This movement is transmitted to the other $O_2$ binding sites, the effect being to move the other Fe atoms closer to their respective ring planes and thereby convert the protein into the R state. The way is thereby opened for them to bind $O_2$, which they now do with greater ease, although the statistical probability decreases as saturation is approached.

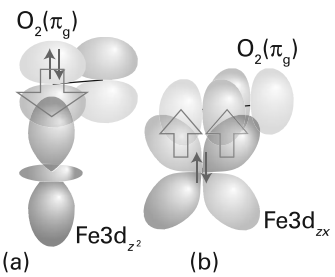



**Figure 27.16** The orbitals used to form the Fe—$O_2$ adduct of myoglobin and haemoglobin. This model considers the $O_2$ ligand to be in a singlet state, in which the full $2\pi_g$ orbital donates an electron pair and the other $2\pi_g$ orbital acts as a p-electron pair acceptor.

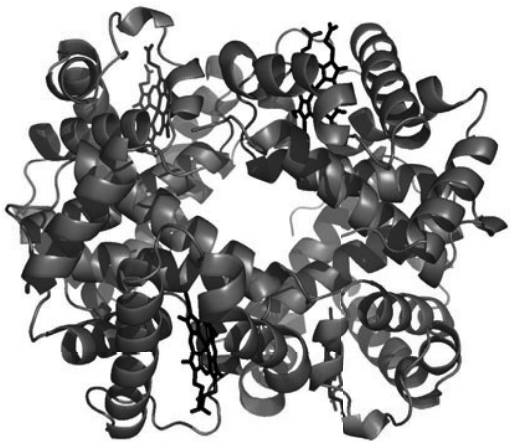


**Figure 27.17** Haemoglobin is an $\alpha_2\beta_2$ tetramer. Its α and β subunits are very similar to myoglobin.

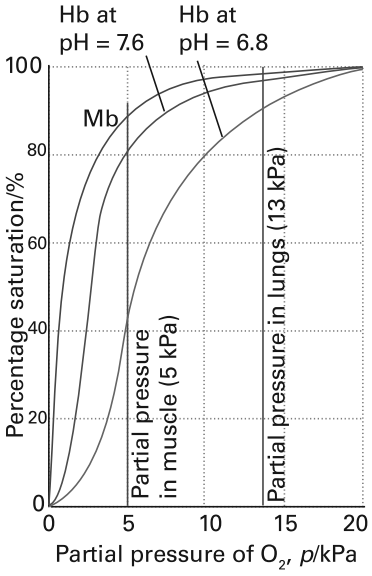



**Figure 27.18** Oxygen binding curves for myoglobin and haemoglobin showing how cooperativity between the four sites in haemoglobin gives rise to a sigmoidal curve. The binding of the first $O_2$ molecule to haemoglobin is unfavourable, but it results in a greatly enhanced affinity for subsequent $O_2$ molecules.

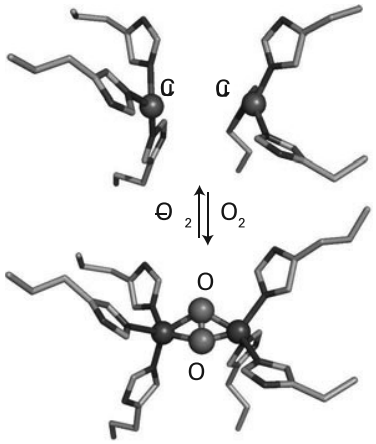



**Figure 27.19** Binding of $O_2$ at the active site of haemocyanin causes the two Cu atoms to be brought closer together. The $O_2$ complex is regarded as a binuclear Cu(II) centre in which the two Cu atoms are bridged by an $\eta^2,\eta^2$-peroxide.

### (c) Other oxygen transport systems

**Key point:** Arthropods and molluscs use haemocyanin and certain marine worms use haemerythrin.

In many organisms, such as arthropods and molluscs, $O_2$ is transported by the Cu protein haemocyanin, which, unlike haemoglobin, is extracellular, as is common for Cu proteins. Haemocyanin is oligomeric, with each monomer containing a pair of Cu atoms in close proximity. Deoxyhaemocyanin (Cu(I)) is colourless but it becomes bright blue when $O_2$ binds.

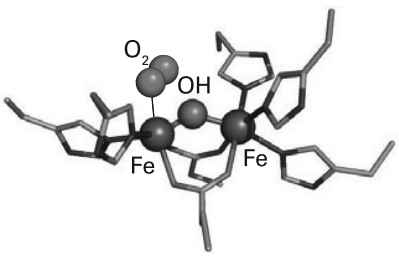
The active site is shown in Fig. 27.19. In the deoxy state, each Cu atom is three-coordinate and bound in a pyramidal array by three histidine residues. The two Cu atoms are so far apart (460 pm) that there is no direct interaction between them. The low coordination number is typical of Cu(I), which is normally two- to four-coordinate. Rapid and reversible coordination of $O_2$ occurs between the two Cu atoms in a bridging dihapto manner ($\mu$-$\eta^2\eta^2$) and the low vibrational wavenumber of the coordinated $O_2$ molecule (750 $cm^{-1}$) shows it has been reduced to peroxide $O_2^{2-}$, with an accompanying lowering of the bond order from 2 to 1. To accommodate the binding of $O_2$, the protein adjusts its conformation to bring the two Cu atoms closer together. The Cu sites become five-coordinate, which is typical of Cu(II).

Haemerythrin is an example of a special class of dinuclear Fe centres that are found in a number of proteins with diverse functions, such as methane monooxygenase, and some ribonucleotide reductases and acid phosphatases. The two Fe atoms in the active site of haemerythrin (**21**) are each coordinated by amino acid side chains but are also linked by two bridging carboxylate groups and a small ligand. In the reduced form, which binds $O_2$ reversibly, this small ligand is an $OH^-$ ion. Coordination of $O_2$ occurs at only one of the Fe atoms and the distal O atom forms a hydrogen bond to the H atom of the bridging hydroxide.

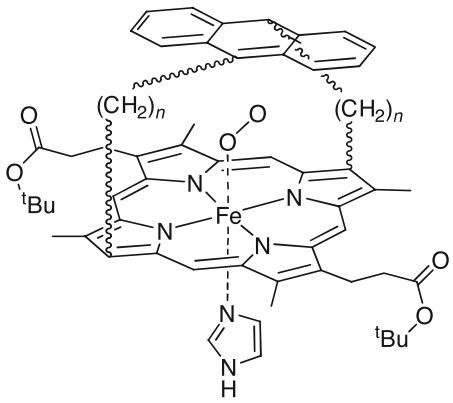
### (d) Reversible $O_2$ binding by small-molecule analogues

**Key point:** Proteins binding $O_2$ reversibly do so by preventing its reduction and eventual O—O bond cleavage. This protection is difficult to achieve with small molecules. Certain elaborate macrocyclic Fe(II) complexes exhibit reversible $O_2$ binding by providing steric hindrance to attack on the coordinated $O_2$.

Much effort has been spent on synthesizing simple complexes that coordinate $O_2$ reversibly and could be used as blood substitutes in special circumstances, such as emergency surgery. The problem is that although $O_2$ reacts with d-block metal ions to form complexes in which the O–O bond is retained (as in superoxo and peroxo species), these products tend to undergo irreversible decomposition involving rapid O–O bond cleavage and formation of water or oxides. Overcoming this problem requires complexes designed to protect the coordinated O–O ligand, preventing it from reacting further. Sterically hindered Fe(II) complexes such as the 'basket' porphyrin (**22**) achieve this protection by preventing a second Fe(II) complex from attacking the distal O atom of the superoxo species to form a bridged peroxo intermediate. As we shall see in Section 27.10, peroxo complexes of Fe(III) tend to undergo rapid O–O bond proteolysis, resulting in formation of $H_2O$ and Fe(IV)=O.




**21**




**22**

Simple Cu complexes that can coordinate $O_2$ reversibly are also rare, but studies have revealed interesting chemistry that is particularly relevant for developing catalysts for oxygenation reactions. Analogues of the dinuclear Cu(I) centre of haemocyanin react with $O_2$ but have a strong tendency to undergo further reactions that involve cleavage of the O—O bond, an example being the rapid equilibrium between μ-$\eta^2$:$\eta^2$ peroxo-dicopper(II) and bis(μ-oxo)copper(III) complexes shown in Fig. 27.20.

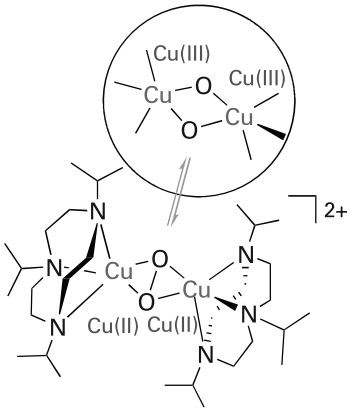



**Figure 27.20** Rapid equilibrium between μ-$\eta^2\eta^2$ peroxo-dicopper(II) and bis (μ-oxo) copper(III) in a model complex for the active site of haemocyanin.

**EXAMPLE 27.5 Identifying how biology compensates for strong competition by CO**

Carbon monoxide is well known to be a strong inhibitor of $O_2$ binding by myoglobin and haemoglobin, yet relative to $O_2$ its binding is much weaker in the protein compared to a simple Fe-porphyrin complex. This suppression of CO binding is important as even trace levels of CO would otherwise have serious consequences for aerobes. Suggest an explanation.

***Answer*** We need to consider how CO and $O_2$, both of which are π-acceptor ligands, differ in terms of the orbitals they use for bonding to a metal atom. The binding of $O_2$ is nonlinear (see Figs 27.15 and 27.16) and the distal O atom is well positioned to form a hydrogen bond to the distal imidazole. By contrast, CO adopts a linear FeCO arrangement and does not participate in the additional bonding.

***Self-test 27.5*** Suggest a reaction sequence accounting for why simple Fe-porphyrin complexes are unable to bind $O_2$ reversibly, but give products that include oxo-bridged dinuclear Fe(III) porphyrin species.

## 27.8 Electron transfer

In all but a few interesting cases, the energy for life stems ultimately from the Sun, either directly in photosynthesis or indirectly by acquiring energy-rich compounds (fuel) from photosynthesizing organisms. Energy can be acquired as a flow of electrons from fuel to oxidant. Important fuels include fats, sugars, and $H_2$, and important biological oxidants include $O_2$, nitrate, and even $H^+$. As estimated from Fig. 27.21, oxidation of sugars by $O_2$ provides a lot of energy (over 4 eV per $O_2$ molecule), and is the reason for the success of aerobic organisms over the anaerobic ones that once dominated the Earth.

### (a) General considerations

**Key points:** Electron flow along electron-transport chains is coupled to chemical processes such as ion (particularly $H^+$) transfer; the simplest electron-transfer centres have evolved to optimize fast electron transfer.

In organisms, electrons are abstracted from food (fuel) and flow to an oxidant, down the potential gradient formed by the sequence of acceptors and donors known as a **respiratory chain** (Fig. 27.22).[7] Apart from flavins and quinones, which are redox-active organic cofactors, these acceptors and donors are metal-containing electron transfer (ET) centres, which fall into three main classes, namely FeS clusters, cytochromes, and Cu sites. These enzymes are generally bound in a membrane, across which the energy from ET is used to sustain a transmembrane proton gradient: this is the basis of the **chemiosmotic theory**. The counterflow of $H^+$, through a rotating enzyme known as ATP synthase, drives the phosphorylation of ADP to ATP. Many membrane-bound redox enzymes are **electrogenic proton pumps**, which means they directly couple long-range ET to proton transfer through specific internal channels.

We shall examine the properties of the three main types of ET centre. The same rules concerning outer-sphere electron-transfer that were discussed in Section 21.12 apply to metal centres in proteins, and we should note that organisms have optimized the structures and properties of these centres to achieve efficient long-range electron transfer.

In the following discussion it will be useful to keep in mind that reduction potentials depend on several factors (Section 5.10). Besides ionization energy and ligand environment (strong donors stabilize high oxidation states and lower the reduction potential; weak donors, π acceptors, and protons stabilize low oxidation states and raise the reduction potential), an active site in a protein is also influenced by the relative permittivity (which

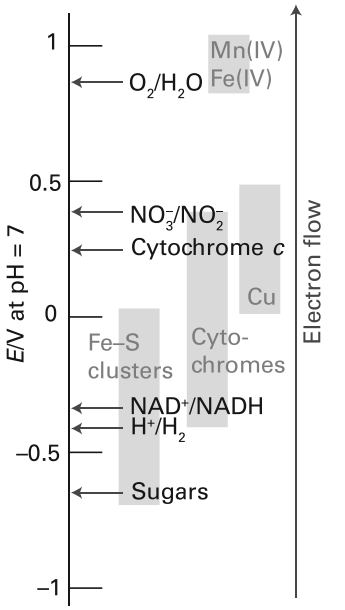



**Figure 27.21** The 'redox spectrum' of life.

[7] An overall current of about 80 A flows through the mitochondrial respiratory chains in an average human.

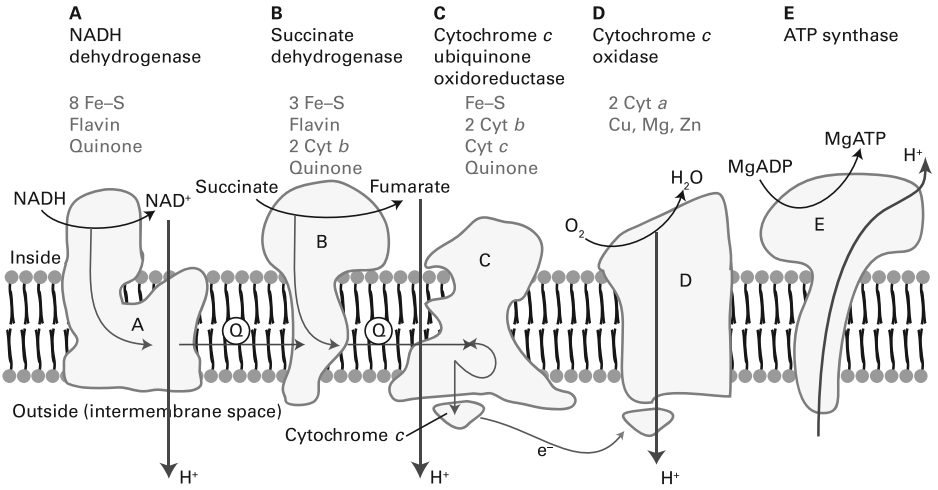
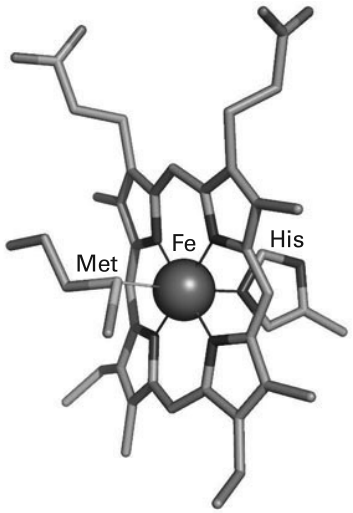



**Figure 27.22** The mitochondrial respiratory electron transfer (ET) chain consists of several metalloenzyme molecules that use the energy of electron transport to transport protons across a membrane. The proton gradient is used to drive ATP synthesis.

stabilizes centres with low overall charge), the presence of neighbouring charges, including those provided by other bound metal ions, and the availability of hydrogen-bonding interactions that will also stabilize reduced states.

When we consider the kinetics of electron transfer, it will similarly be useful to keep in mind that 'efficiency' means that electron transfer is fast even when the reaction Gibbs energy is low and therefore that the reorganization energy $\lambda$ of Marcus theory (Section 21.12) is low. This requirement is met by providing a ligand environment that does not alter significantly when an electron is added and by burying the site so that water molecules are excluded. Intersite distances are generally less than 1.4 nm in order to facilitate electron tunnelling, although it is still debated whether electron transfer in proteins depends mainly on distance alone or whether the protein can provide special pathways.




**23**

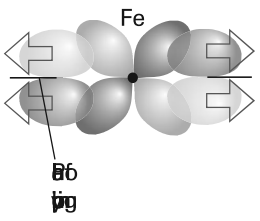



**Figure 27.23** Overlap between the $t_{2g}$ orbitals of the Fe and low-lying empty $\pi^*$ orbitals on the porphyrin effectively extends the Fe orbitals out to the periphery of the ring.

### (b) Cytochromes

**Key points: Cytochromes operate in the potential region −0.3 to +0.4 V; they have a combination of low reorganization energy and extended electron coupling through delocalized orbitals.**

Cytochromes were identified many years ago as cell pigments (hence the name). They contain an Fe porphyrin group and the term 'cytochrome' can refer to both an individual protein and a subunit of a larger enzyme that contains the cofactor. Cytochromes use the $Fe^{3+}/Fe^{2+}$ couple and are generally six-coordinate (**23**), with two stable axial bonds to amino acid donors, and the Fe is usually low spin in both oxidation states. This contrasts with ligand-binding Fe-porphyrin proteins such as haemoglobin, for which the sixth coordination site is either empty or occupied by an $H_2O$ molecule.

A good way to consider the capability of cytochromes for fast electron transfer is to treat the d orbitals of Fe(III) and Fe(II) in terms of an octahedral ligand field and to consider the overlap between the electron-rich but nearly nonbonding $t_{2g}$ orbitals (the configurations are $t_{2g}^5$ and $t_{2g}^6$ in Fe(III) and Fe(II), respectively) and the orbitals of the porphyrin. The electron enters or leaves an orbital having $\pi$ overlap with the $\pi^*$ antibonding molecular orbital on the ring system. This arrangement provides enhanced electron transfer because the d orbitals of the Fe atom are effectively extended out to the edge of the porphyrin ring, so decreasing the distance over which an electron must transfer between redox partners (Fig. 27.23).

The paradigm of cytochromes is **mitochondrial cytochrome *c*** (12 kg mol$^{-1}$, Fig. 27.24). This cytochrome is found in the mitochondrial intramembrane space, where it supplies electrons to cytochrome *c* oxidase, the enzyme responsible for reducing $O_2$ to $H_2O$ at the end of the energy-transducing respiratory chain (Section 27.10). The fifth and sixth ligands to Fe in cytochrome *c* are histidine (imidazole-N) and methionine (thioether-S, **23**). Methionine is not a common ligand in metalloproteins but because it is a neutral, soft

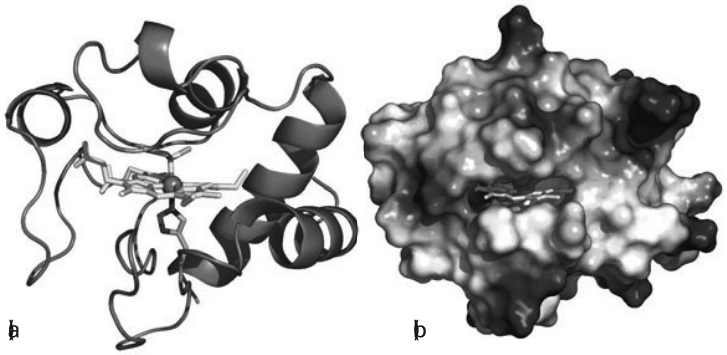
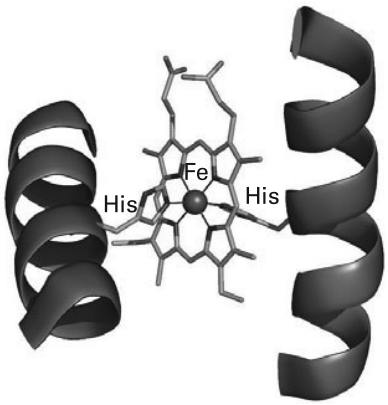



**Figure 27.24** Different views (but from the same viewpoint) of mitochondrial cytochrome *c*. (a) The secondary structure and the position of the haem cofactor. (b) The surface charge distribution that guides the docking with its natural redox partners (red and blue areas represent patches of negative and positive charge, respectively).

donor it is expected to stabilize Fe(II) rather than Fe(III). The reduction potential of cytochrome *c* is +0.26 V, at the higher end of values for cytochromes in general. Cytochromes vary in the identity of the axial ligands as well as the structure of the porphyrin ligand (the notation *a, b, c, d,…* defines positions of absorption maxima in the visible region, but also refers to variations in the substituents on the porphyrin ring). Many cytochromes, in particular those sandwiched between membrane-spanning helices, have bis(histidine) axial ligation (**24**).




**24**

In cytochrome *c*, the edge of the porphyrin ring is exposed to solvent and is the most likely site for electrons to enter or leave. Specific protein–protein interactions are important for obtaining efficient electron transfer and the region around the exposed edge of the porphyrin ring in cytochrome *c* provides a pattern of charges that are recognized by cytochrome *c* oxidase and other redox partners. One example in particular has been well studied, that of cytochrome *c* with yeast cytochrome *c* peroxidase. The driving force for electron transfer from either of the two catalytic intermediates of peroxidase (Section 27.10) to each reduced cytochrome *c* is approximately 0.5 V. Figure 27.25 shows the structure of a bimolecular complex formed between cytochrome and cytochrome *c* peroxidase. Electrostatic interactions guide the two proteins together within a distance that is favourable for fast electron tunnelling between cytochrome *c* and two redox centres on the peroxidase, the haem cofactor and tryptophan-191 (Section 27.10).

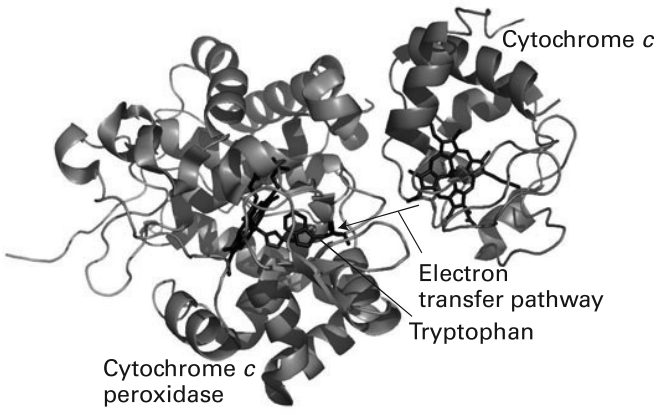



**Figure 27.25** The bimolecular ET complex between cytochrome *c* and cytochrome *c* peroxidase produced by co-crystallization of cytochrome *c* with the Zn derivative of cytochrome *c* peroxidase. The orientation suggests an electron transfer pathway between the haem groups of cytochrome *c* and cytochrome *c* peroxidase that includes tryptophan.

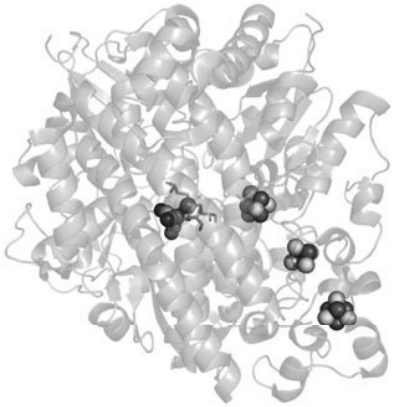
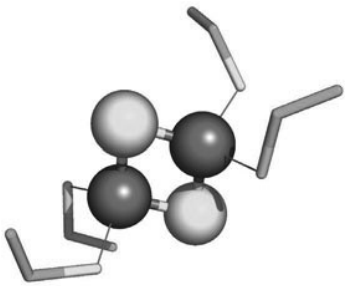
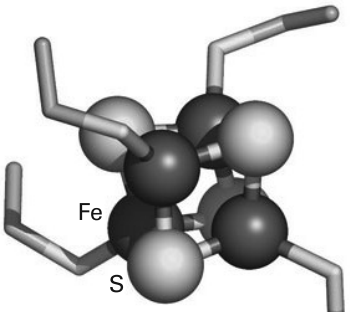


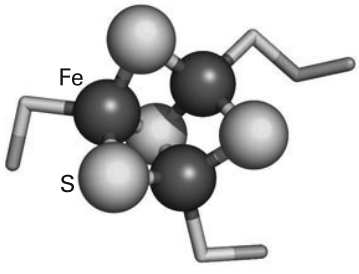
**Figure 27.26** A series of three Fe–S clusters provides a long-range electron transfer pathway to the buried active site in hydrogenases.



**25** [2Fe–2S]




**26** [4Fe–4S]




**27** [3Fe–4S]

### (c) Iron–sulfur clusters

**Key points:** Iron–sulfur clusters generally operate at more negative potentials than cytochromes; they are composed of high-spin Fe(III) or Fe(II) with sulfur ligands in a mainly tetrahedral environment.

Iron–sulfur clusters are widespread in biology, although their importance was not established as early as cytochromes on account of their lack of distinctive optical characteristics. By convention, FeS clusters are represented by square brackets showing how many Fe and non-protein S atoms are present, as in [2Fe–2S] (**25**), [4Fe–4S] (**26**), and [3Fe–4S] (**27**). The efficacy of FeS clusters as fast ET centres is largely due to their being able to delocalize the added electron to varying degrees, which minimizes bond length changes and decreases the reorganization energy. The presence of sulfur ligands to provide good lead-in groups is also important. Small ET proteins containing FeS clusters are known as **ferredoxins**, whereas in many large enzymes, FeS clusters are arranged in a relay, less than 1.5 nm apart, to link remote redox sites in the same molecule. The relay concept is illustrated in Fig. 27.26 with a class of enzymes known as **hydrogenases**, which we discuss further in Section 27.14.

In nearly all cases, the Fe atoms are tetrahedrally coordinated by cysteine thiolate ($RS^-$) groups as the protein ligands. The overall assembly, including the protein ligands, is known as an 'FeS centre'. Examples are known in which one or more of the Fe atoms is coordinated by non-thiolate amino acid ligands, such as carboxylate, imidazole, and alkoxyl (serine), or by an exogenous ligand such as $H_2O$ or $OH^-$, and the coordination number about the Fe subsite may be increased to six. The cubane [4Fe–4S] (**26**) and cuboidal [3Fe–4S] (**27**) clusters are obviously closely related, and may even interconvert within a protein by the addition or removal of Fe from one subsite. Larger clusters also occur, such as the 'super clusters' [8Fe–7S] and [Mo–7Fe–8S–X] found in nitrogenase (Section 27.13).

Despite the presence of more than one Fe atom, FeS clusters generally carry out single electron transfers and are good examples of mixed-valence systems comprising Fe(III) and Fe(II). The redox state of a cluster is commonly represented by summing the charges due to Fe (3+ or 2+, respectively) and S atoms (2−) and the resultant overall charge, which is referred to as the **oxidation level**, is written as a superscript.

| | | |
|---|---|---|
| $[2Fe{-}2S]^{2+} + e^- \rightarrow [2Fe{-}2S]^+$ | | $E^\ominus = 0$ to $-0.4$ V |
| 2 Fe(III) | {Fe(III):Fe(II)} | |
| $S = 0$ | $S = \frac{1}{2}$ | |
| $[3Fe{-}4S]^+ + e^- \rightarrow [3Fe{-}4S]$ | | $E^\ominus = +0.1$ to $-0.4$ V |
| 3Fe(III) | {2Fe(III):Fe(II)} | |
| $S = \frac{1}{2}$ | $S = 2$ | |
| $[4Fe{-}4S]^{2+} + e^- \rightarrow [4Fe{-}4S]^+$ | | $E^\ominus = -0.2$ to $-0.7$ V |
| {2Fe(III):2Fe(II)} | {Fe(III):3Fe(II)} | |
| $S = 0$ | $S = \frac{1}{2}$ | |

Most FeS centres have negative reduction potentials (usually more negative than −0.2 V) so the reduced forms are good reducing agents: exceptions are [4Fe–4S] clusters that operate instead between the +3 and +2 oxidation states (these are called 'HiPIP' centres because they were originally discovered in a protein called *high-potential iron protein* for which the reduction potential is 0.35 V) and so-called **Rieske centres**, which are [2Fe–2S] clusters having one Fe subsite coordinated by two neutral imidazole ligands rather than cysteine (**28**). Coordination by histidine stabilizes the iron as Fe(II) and usually raises the reduction potential to a much more positive value (above +0.2 V). The half-reactions have been written to include the spin states of FeS clusters: individual Fe atoms are high spin, as expected for tetrahedral coordination by $S^{2-}$, and different magnetic states arise from ferromagnetic and antiferromagnetic coupling (Section 20.8). These magnetic properties are very important, as they allow the centres to be investigated by EPR (Section 8.6).

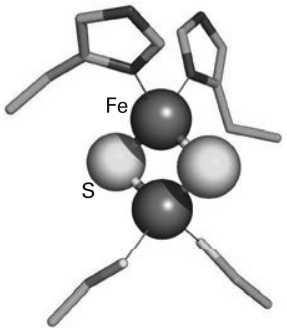
A major question is how FeS centres are synthesized and inserted into the protein. This process has been studied mostly in prokaryotes, from which it is known that specific proteins are involved in the supply and transport of Fe and S atoms, their assembly into clusters, and their transfer to target proteins. Free sulfide ($H_2S$, $HS^-$, or $S^{2-}$) in a cell is highly poisonous, so it is produced only when required by an enzyme called cysteine desulfurase, which breaks down cysteine to yield $S^{2-}$ ions and alanine.

### (d) Copper electron-transfer centres

**Key point:** The protein overcomes the large inherent difference in preferred geometries for Cu(II) and Cu(I) by constraining Cu in a coordination environment that does not change upon electron transfer.

The so-called 'blue' Cu centre is the active site of a number of small electron-transfer proteins as well as larger enzymes (the blue Cu oxidases) that contain, in addition, other Cu sites. Blue Cu centres have reduction potentials for the Cu(II)/Cu(I) redox couple that lie in the range 0.15−0.8 V and so they are generally more oxidizing than cytochromes. The name stems from the intense blue colour of pure samples in the oxidized state, which arises from ligand(thiolate)-to-metal charge transfer. In all cases, the Cu is shielded from solvent water and coordinated by a minimum of two imidazole-N and one cysteine-S in a nearly trigonal planar manner, with one or two longer bonds to axial ligands. The most studied examples are plastocyanin (Fig. 27.27), a small electron carrier protein in chloroplasts, and azurin, a bacterial electron carrier. These small proteins have a β-barrel structure, which holds the Cu coordination sphere in a rigid geometry. Indeed, the crystal structures of oxidized, reduced (**29**, numbers refer to bond distances in pm), and apo forms reveal that the ligands remain in essentially the same position in all cases. As a result, the blue Cu centre is well suited to undergo fast and efficient electron transfer because the reorganization energy is small.

The dinuclear Cu centre known as $Cu_A$ is present in cytochrome *c* oxidase and $N_2O$ reductase. The two Cu atoms (**30**) are each coordinated by two imidazole groups and a pair of cysteine thiolate ligands act as bridging ligands. In the reduced form, both Cu atoms are Cu(I). This form undergoes one-electron oxidation to give a purple, paramagnetic species in which the unpaired electron is shared between the two Cu atoms. Once again, we see how delocalization assists electron transfer because the reorganization energy is lowered.




**28** Rieske [2Fe–2S] centre


**Figure 27.27** The plastocyanin molecule.

**EXAMPLE 27.6** Explaining the function of ET centres

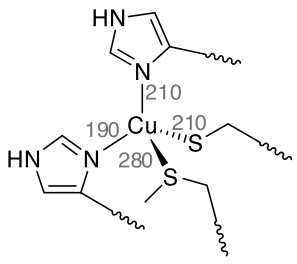
The reduction potential of Rieske FeS centres is very pH dependent, unlike the standard FeS centres that have only thiolate ligation. Suggest an explanation.

***Answer*** We need to refer back to Sections 5.6 and 5.14 to see how protonation equilibria influence reduction potentials. Each of the two imidazole ligands that coordinate one of the Fe atoms in the Rieske [2Fe−2S] cluster is electrically neutral at pH = 7 and the proton located on the noncoordinating N atom is easily removed. The $pK_a$ depends on the oxidation level of the cluster, and there is a large region of pH in which the imidazole ligands are protonated in the reduced form but not in the oxidized form. As a result, the reduction potential depends on pH.

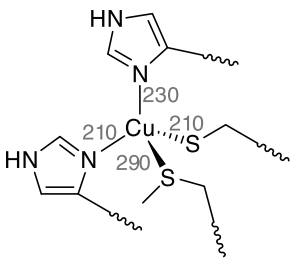
***Self-test 27.6*** Simple Cu(II) compounds show a large EPR hyperfine coupling to the Cu nucleus ($I = \frac{3}{2}$ for $^{65}Cu$ and $^{63}Cu$), whereas the EPR spectra of blue Cu proteins show a much smaller hyperfine coupling. What does this suggest about the nature of the ligand coordination at blue Cu centres?

## Catalytic processes

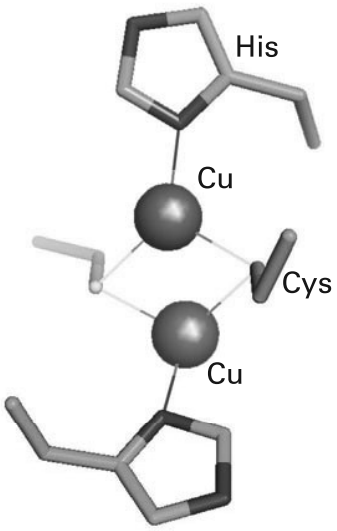
The classic role of enzymes is as highly selective catalysts for the myriad chemical reactions that take place in organisms and sustain the activities of life. In this section we view some of the most important examples in terms of the suitability of certain elements for their roles.




**29a** Oxidized plastocyanin




**29b** Reduced plastocyanin




**30**

## 27.9 Acid–base catalysis

Biological systems rarely have the extreme pH conditions under which catalysis by free $H^+$ or $OH^-$ can occur; indeed, the result would be indiscriminate because all hydrolysable bonds would be targets. One way that organisms have solved this problem has been to harness properties of certain metal ions and build them into protein structures designed to accomplish specific (Brønsted) acid–base reactions (Section 4.1).

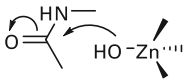
Organisms make extensive use of Zn for achieving acid–base catalysis, but not to the exclusion of other metals. For example, in addition to the numerous enzymes that feature Fe(II) and Fe(III), Mg(II) serves as the catalyst in pyruvate kinase (phosphate ester hydrolysis) and ribulose bisphosphate carboxylase ($CO_2$ incorporation into organic molecules), Mn is the catalyst in arginase (for the hydrolysis of arginine, yielding urea and L-ornithine), and Ni(II) is the active metal in urease (for the hydrolysis of urea, yielding ammonia and, ultimately, carbon dioxide). Because of their importance to industry and medicine, many of these enzymes have been studied in great detail and model systems have been synthesized in efforts to reproduce catalytic properties and understand the mode of action of inhibitors. Many of these sites (including arginase-[Mn,Mn] and urease-[Ni,Ni]) contain two or more metal ions in an arrangement unique to the protein and difficult to model with simple ligands.

### (a) Zinc enzymes

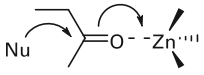
**Key point:** Zinc is well suited for catalysing acid–base reactions as it is abundant, redox inactive, forms strong bonds to donor groups of amino acid residues, and exogenous ligands such as $H_2O$ are exchanged rapidly.

A $Zn^{2+}$ ion has high rates of ligand exchange and its polarizing power means that the $pK_a$ of a coordinated $H_2O$ molecule is quite low. Combined with strong binding to protein ligands, rapid ligand exchange (coordinated $H_2O$ or substrate molecules), a reasonably high electron affinity, flexibility of coordination geometry, and no complicating redox chemistry, Zn is well suited to its role in catalysing specific acid–base reactions. The large family of Zn enzymes include carbonic anhydrase, carboxypeptidases, alkaline phosphatase, β-lactamase (responsible for penicillin resistance in bacteria), and alcohol dehydrogenase. Typically, the Zn is coordinated by three amino acid ligands (in contrast to zinc fingers, which have four) and one exchangeable $H_2O$ molecule (**14**).

The mechanisms of Zn enzymes are normally discussed in terms of two limiting cases. In the **Zn-hydroxide mechanism**, the Zn functions by promoting deprotonation of a bound water molecule, so creating an optimally positioned $OH^-$ nucleophile that can go on to attack the carbonyl C atom:




In the **Zn-carbonyl mechanism**, the Zn ion acts directly as a Lewis acid to accept an electron pair from the carbonyl O atom, and its role is therefore analogous to $H^+$ in acid catalysis:




Similar reactions occur with other X=O groups, particularly the P=O of phosphate esters. There is an obvious advantage of achieving such catalysis at Zn or some other acid species that is anchored in a stereoselective environment.

The formation and transport of $CO_2$ is a fundamental process in biology. The solubility of $CO_2$ in water depends on its hydration and deprotonation to form $HCO_3^-$. However, the uncatalysed reaction at pH = 7 is very slow, the forward process occurring with a rate constant of less than $10^{-3}$ $s^{-1}$. Because turnover of $CO_2$ by biological systems is very high, such a rate is far too slow to sustain vigorous aerobic life in a complex organism. In photosynthesis, only $CO_2$ can be used by the enzyme known as 'rubisco' (Section 27.9(b)) so rapid dehydration of $HCO_3^-$ is essential and is one of the first steps in the production of biomass. The $CO_2$/ $HCO_3^-$ equilibrium is also important (in addition to its role in $CO_2$ transport) because it provides a way of regulating tissue pH.

In 1932 an enzyme, carbonic anhydrase (CA, or carbon dioxide dehydratase) was identified that catalyses this reaction with a remarkable rate enhancement, and it was found to contain one Zn atom per molecule. It is now known that there are several forms of CA, all of which are monomers with molar mass close to 30 kg mol$^{-1}$. The best-studied enzyme is CA II from red blood cells, which has a turnover frequency for $CO_2$ hydration of about $10^6$ s$^{-1}$, making it one of the most active of all enzymes. The crystal structure of human CA II shows that the Zn atom is located in a conical cavity about 1.6 nm deep, which is lined with several histidine residues. The Zn is coordinated by three His-N ligands and one $H_2O$ molecule in a tetrahedral arrangement (Fig. 27.28). The neutral N-ligands lower the p$K_a$ of the bound $H_2O$ by about 3 units (compared to the aqua ion), so creating a high local concentration of $OH^-$ as attacking nucleophile. Other groups in the active site pocket, including noncoordinating histidines and ordered water molecules, are important for mediating proton transfer (which is the rate-limiting factor) and for binding the $CO_2$ substrate (which does not coordinate to Zn). Carbonic anhydrases show some variation in their Zn coordination environment, with some CAs from higher plants having two cysteines and one histidine.

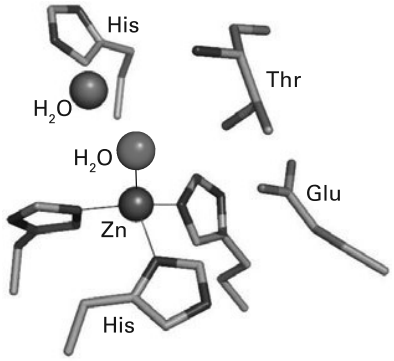
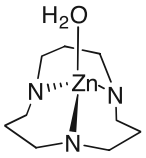



**Figure 27.28** The active site of carbonic anhydrase.

The mechanism of action of CA (Fig. 27.29) is best described in terms of a Zn-hydroxide mechanism. Proton transfers are very fast, aided by a hydrogen-bonding network that extends from the protein surface to the active site. The key feature is the acidity of the $H_2O$ molecule coordinated to Zn, as the coordinated $HO^-$ ion that is produced after deprotonation is sufficiently nucleophilic to attack a nearby $CO_2$ molecule bound noncovalently. This attack results in a coordinated $HCO_3^-$ ion, which is then released. Small analogues of CA that have been studied, such as (**31**), reproduce the substrate binding and acid–base properties of the enzyme, but their turnover frequencies are orders of magnitude lower.




**31**

Carboxypeptidase (CPD, 34.6 kg mol$^{-1}$) is an exopeptidase, an enzyme that catalyses the hydrolysis of C-terminal amino acids containing an aromatic or bulky aliphatic side chain. There are two types of Zn-containing enzyme, and both are synthesized as inactive precursors in the pancreas for secretion into the digestive tract. The better studied is CPD A, which acts on terminal aromatic residues; whereas CPD B acts on basic residues. The X-ray structure of CPD shows that the Zn is located to one side of a groove in which the substrate is bound. It is coordinated by two histidine-N ligands, one glutamate-$CO_2^-$ (bidentate), and one weakly bound $H_2O$ molecule (Fig. 27.30). Structures of the enzyme

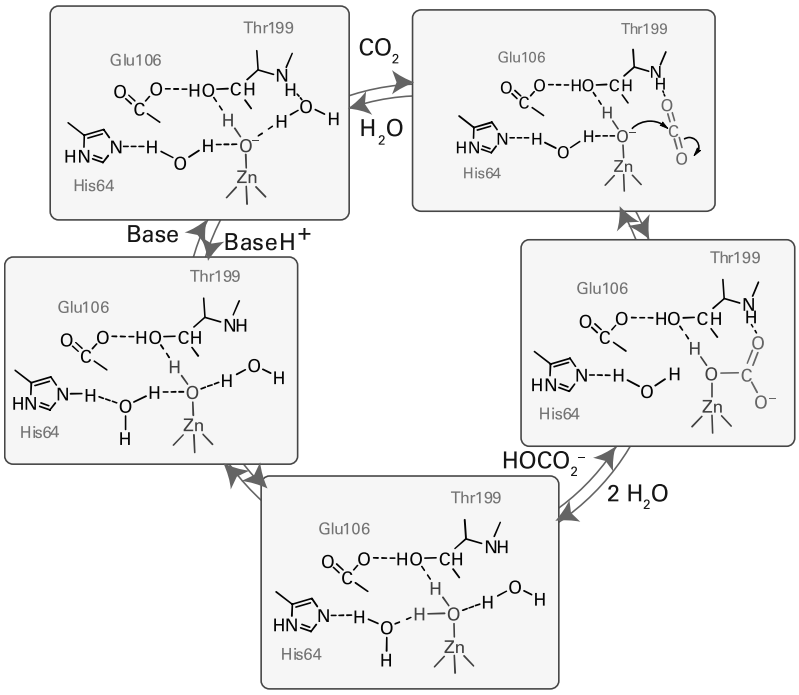



**Figure 27.29** The mechanism of action of carbonic anhydrase indicating the importance of proton transfer in this very fast reaction.

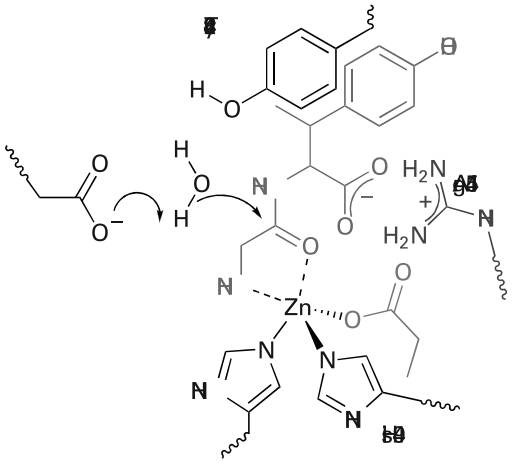
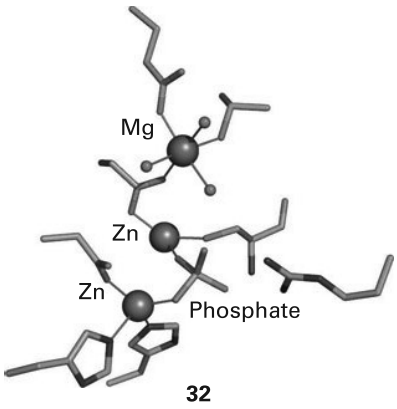


**Figure 27.30** Structure of the active site of carboxypeptidase with a peptide inhibitor (red) bound to it.

obtained in the presence of glycyl inhibitors show that the $H_2O$ molecule has moved away from the Zn atom, which has become coordinated instead to the carbonyl-O of the glycine, suggesting a Zn-carbonyl mechanism. The guanidinium group of a nearby arginine binds the terminal carboxyl group, while the tyrosine provides aromatic/hydrophobic recognition.

Alkaline phosphatase (AP) introduces us to catalytic Zn centres that contain more than one metal atom. The enzyme catalyses the hydrolysis of phosphate monoesters:

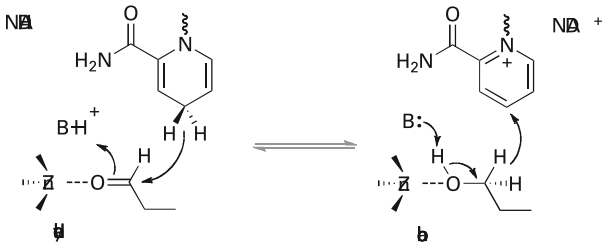
$$R-OPO_3^{2-} + H_2O \rightarrow ROH + HOPO_3^{2-}$$




**32**

It occurs in tissues as diverse as the intestine and bone, where it is found in the membranes of osteoblasts, cells that form the sites of nucleation of hydroxyapatite crystals. AP catalyses the general breakdown of organic phosphates, including ATP, to provide the phosphate required for bone growth. As its name implies, its optimum pH is in the mild alkali region. The active site of AP contains two Zn atoms located only about 0.4 nm apart, with a Mg ion nearby. The crystal structure of the enzyme–phosphate complex (**32**) reveals that the phosphate ion (the product of the normal reaction) bridges the two Zn atoms.

Alcohol dehydrogenase (ADH) is discussed here even though it is classed as a redox enzyme because the role of Zn once again is as a Lewis acid. The reaction catalysed is the reduction of $NAD^+$ by alcohol:



The Zn activates the C–OH group towards transfer of the H as a hydride entity to a molecule of $NAD^+$. It is easy to visualize this reaction in the opposite direction, in which the Zn atom polarizes the carbonyl group and induces attack by the nucleophilic hydridic H atom from NADH. Alcohol dehydrogenase is an $\alpha_2$ dimer that contains both catalytic and structural Zn sites.

Cadmium, the element below Zn in Group 12 and normally regarded as highly toxic, is now recognized as being an essential nutrient for certain organisms. In 2005, a carbonic anhydrase isolated from the marine phytoplankton *Thalassiosira weissflogii* was discovered to contain Cd at its active site. In contrast to cases in which Cd is simply able to substitute for Zn, this enzyme is specific for $Cd^{2+}$. The surface waters in which *Thalassiosira weissflogii* grows are extremely low in $Zn^{2+}$ and its growth in the laboratory is stimulated by adding $Cd^{2+}$.

### (b) Magnesium enzymes

**Key points:** The major direct catalytic function of magnesium is as the catalytic centre of ribulose bisphosphate carboxylase.

The $Mg^{2+}$ cation confers less polarization of coordinated ligands than $Zn^{2+}$ (we often refer to $Mg^{2+}$ as being a 'weaker acid' than $Zn^{2+}$); however, compared to Zn it is much more mobile and cells contain high concentrations of uncomplexed $Mg^{2+}$ ions. Its major role in enzyme catalysis is as the Mg–ATP complex (**1**), which is the substrate in **kinases**, the enzymes that transfer phosphate groups thereby activating the target compound or causing it to change its conformation. Kinases are controlled by calmodulin (Section 27.4) and other proteins, so they are part of the signalling mechanism in higher organisms.

An important example of an Mg enzyme in which Mg acts separately from ATP is ribulose 1,5-bisphosphate carboxylase, commonly known as 'rubisco'. This enzyme, the most abundant in the biosphere, is responsible for the production of biomass by oxygenic photosynthetic organisms and removal of $CO_2$ from the atmosphere (to the extent, globally, of over $10^{11}$ t of $CO_2$ per year). Rubisco is an enzyme of the **Calvin cycle**, the stages of photosynthesis that can occur in the dark, in which it catalyses the incorporation of $CO_2$ into a molecule of ribulose 1,5-bisphosphate (Fig. 27.31). The $Mg^{2+}$ ion is octahedrally coordinated by carboxylate groups from glutamate and aspartate residues, three coordinated $H_2O$ molecules, and a carbamate derived from a lysine residue. The carbamate is formed by a reaction between $CO_2$ and the side-chain $-NH_2$, in an activation process that is necessary for $Mg^{2+}$ to bind. In the catalytic cycle, the binding of ribulose 1,5-bisphosphate displaces two $H_2O$ molecules, and proton abstraction assisted by the carbamate results in a coordinated enolate. This intermediate reacts with $CO_2$, forming a new C–C bond, then the product is cleaved to yield two new three-carbon species and the cycle continues. The reactive enolate will also react with $O_2$, in which case the result is an oxidative degradation of substrate: for this reason the enzyme is often called ribulose 1,5-bisphosphate carboxylase-oxygenase. We note the difference from Zn, which would favour ligation by softer ligands and a lower coordination number. Rubisco requires a metal ion that combines good Lewis acidity with weak binding and high abundance.

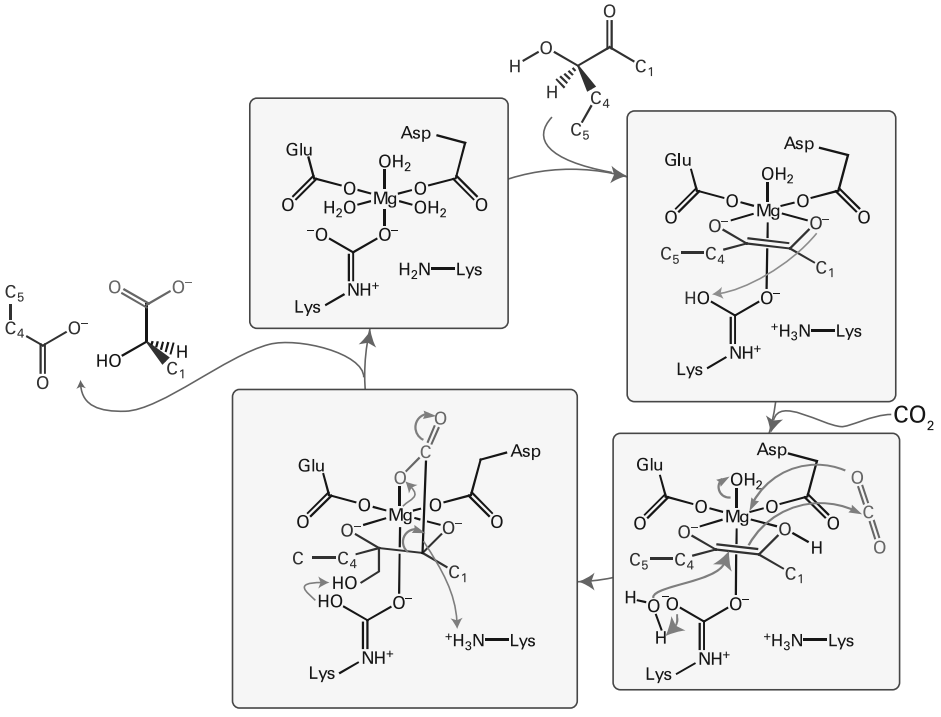
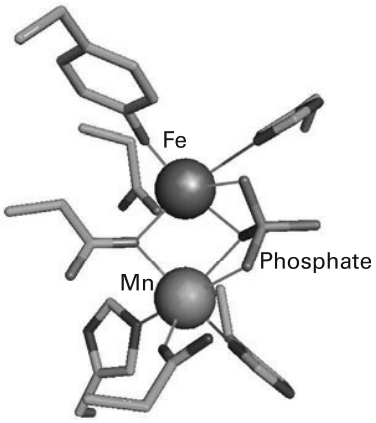



**Figure 27.31** Mechanism of action of ribulose 1,5-bisphosphate carboxylase, the enzyme responsible for removing $CO_2$ from the atmosphere and 'fixing' it in organic molecules in plants.

### (c) Iron enzymes

**Key points:** Acid phosphatases contain a dinuclear metal site containing Fe(III) in conjunction with Fe, Zn, or Mn; aconitase contains a [4Fe–4S] cluster, one subsite of which is modified to manipulate the substrates.




**33**

Acid phosphatases, sometimes known as 'purple' acid phosphatases (PAPs) on account of their intense colour, occur in various mammalian organs, particularly the bovine spleen and porcine uterus. Acid phosphatases catalyse hydrolysis of phosphate esters, with optimal activity under mild acid conditions. They are involved in bone maintenance and hydrolysis of phosphorylated proteins (therefore they are important in signalling). They may also have other functions, such as Fe transport. The pink or purple colours of acid phosphatases are due to a tyrosinate → Fe(III) charge-transfer transition at 510–550 nm ($\varepsilon = 4000\ \mathrm{dm^3\ mol^{-1}\ cm^{-1}}$). The active site contains two Fe atoms linked by ligands, similar to haemerythrin (**21**). Acid phosphatases are inactive in the oxidized {Fe(III)Fe(III)} state in which they are often isolated. In the active state, one Fe is reduced to Fe(II). Both Fe atoms are high spin and remain so throughout the various stages of reactions.

Acid phosphatases also occur in plants, and in these enzymes the reducible Fe is replaced by Zn or Mn. The active site of an acid phosphatase from sweet potato (**33**) shows how phosphate becomes coordinated to both Fe(III) and Mn(II) ions. In the mechanism shown in Fig. 27.32, rapid binding of the phosphate group of the ester occurs to the M(II) subsite, then the P atom is attacked by an $OH^-$ ion that is formed at the more acidic Fe(III) subsite. The FeZn centre is also found in an important enzyme called *calcineurin*, which catalyses the phosphorylation of serine or threonine residues on certain protein surfaces, in particular a transcription factor involved in controlling the immune response. Calcineurin is activated by $Ca^{2+}$ binding, both directly and through calmodulin.

Aconitase is an essential enzyme of the **tricarboxylic acid cycle** (also known as the Krebs cycle, or citric acid cycle), the main source of energy production in higher organisms, where it catalyses the interconversion of citrate and isocitrate in a reaction that formally involves dehydration and rehydration, and proceeds through an intermediate, aconitate, which is released in small amounts:

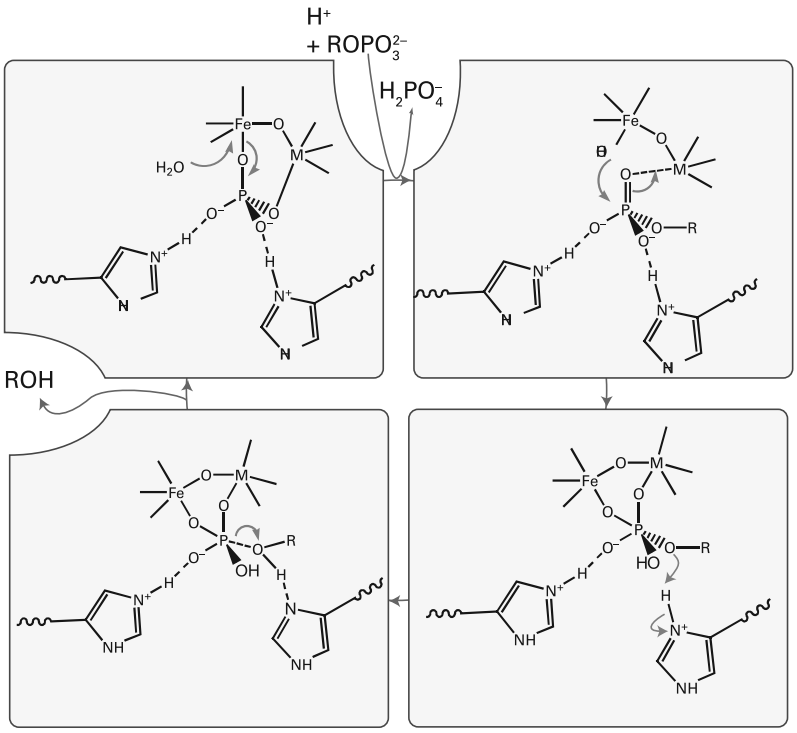
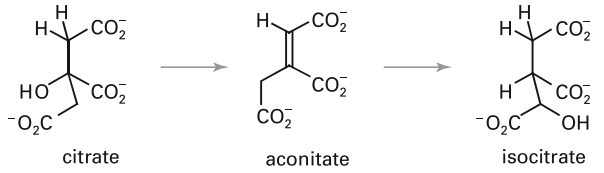
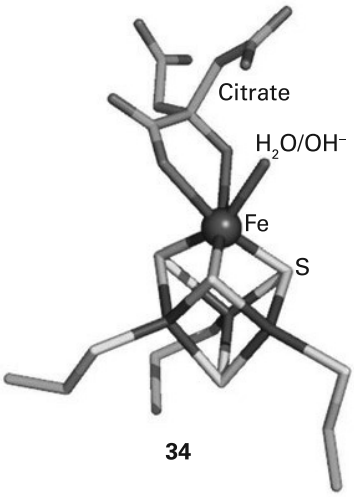



**Figure 27.32** Proposed mechanism of action of acid phosphatase. The metal site M(II) is occupied by Fe (most common in animals) or by Mn or Zn (in plants).




The active form of the enzyme contains a [4Fe–4S] cluster, which degrades to [3Fe–4S] when the enzyme is exposed to air. The site of catalysis is the Fe atom that is lost on oxidation. The structure shows that this unique subsite is not coordinated by a protein ligand but by an $H_2O$ molecule, which explains why this Fe is more readily removed.

A plausible mechanism for the action of aconitase, based on structural, kinetic, and spectroscopic evidence, involves the binding of citrate to the active Fe subsite, which increases its coordination number to 6. An intermediate in the catalytic cycle is 'captured' for X-ray diffraction investigation, using a site-directed mutant that can bind the citrate but cannot complete the reaction (**34**). The Fe atom polarizes a C–O bond and OH is abstracted, while a nearby base accepts a proton. The substrate now swings round, and the OH and H are reinserted onto different positions. A form of aconitase that is found in cytoplasm has another intriguing role, that of an Fe sensor (Section 27.15).




**34**

## 27.10 Enzymes dealing with $H_2O_2$ and $O_2$

In Section 27.7 we saw how organisms have evolved systems that transport $O_2$ reversibly and deliver it unchanged to where it is required. In this section we describe how $O_2$ is reduced catalytically, either for production of energy or synthesis of oxygenated organic molecules. We start by considering a simpler case, that of the reduction of hydrogen peroxide, as this discussion introduces Fe(IV) as a key intermediate in so many biological processes. We end by completing a remarkable cycle, the production of $O_2$ from $H_2O$, catalysed by a unique Mn/Ca cluster.

### (a) Peroxidases

**Key points:** Peroxidases catalyse reduction of hydrogen peroxide; they provide important examples of Fe(IV) intermediates that can be isolated and characterized.

Haem-containing peroxidases, as exemplified by horseradish peroxidase (HRP) and cytochrome *c* peroxidase (CcP), catalyse the reduction of hydrogen peroxide:

$$H_2O_2(aq) + 2e^- + 2H^+(aq) \rightarrow 2H_2O(l)$$

The intense chemical interest in these enzymes lies in the fact that they are the best examples of Fe(IV) in chemistry. Iron(IV) is an important catalytic intermediate in numerous biological processes involving oxygen. Catalase, which catalyses the thermodynamically favourable disproportionation of $H_2O_2$ and is one of the most active enzymes known, is also a peroxidase. The active site of yeast cytochrome *c* peroxidase shown in Fig. 27.33 indicates how the substrate is manipulated during the catalytic cycle. The proximal ligand is the imidazole side chain of a histidine and the distal pocket, like myoglobin, also contains an imidazole side chain, but there is also a guanidinium group from arginine.

The catalytic cycle shown in Fig. 27.34 starts from the Fe(III) form. A molecule of $H_2O_2$ coordinates to Fe(III) and the distal histidine mediates proton transfer so that both H atoms are placed on the remote O atom. The simultaneous bond polarization by the guanidinium side chain results in heterolytic cleavage of the O–O bond: one half leaves as $H_2O$ and the other remains bound to the Fe atom to produce a highly oxidizing intermediate. Although it is instructive to regard this system as a trapped O atom (or an $O^{2-}$ ion bound to Fe(V)), detailed measurements by EPR and Mössbauer spectroscopy show that this highly oxidizing intermediate (which is known historically as 'Compound I') is in fact Fe(IV) and an organic cation radical. In HRP the radical is located on the porphyrin ring whereas in cytochrome peroxidase it is located on nearby peptide residue tryptophan-191. Descriptions of the Fe−O bonding range from Fe(IV)=O ('ferryl') to Fe(IV)−O···H, in which the O atom is either protonated or linked by a hydrogen bond to a donor group.

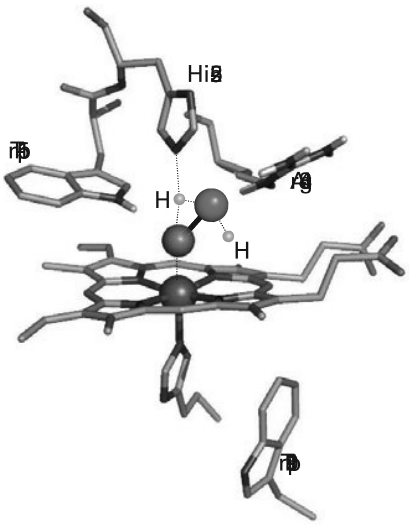



**Figure 27.33** The active site of yeast cytochrome *c* peroxidase showing amino acids essential for activity and indicating how peroxide is bound in the distal pocket.

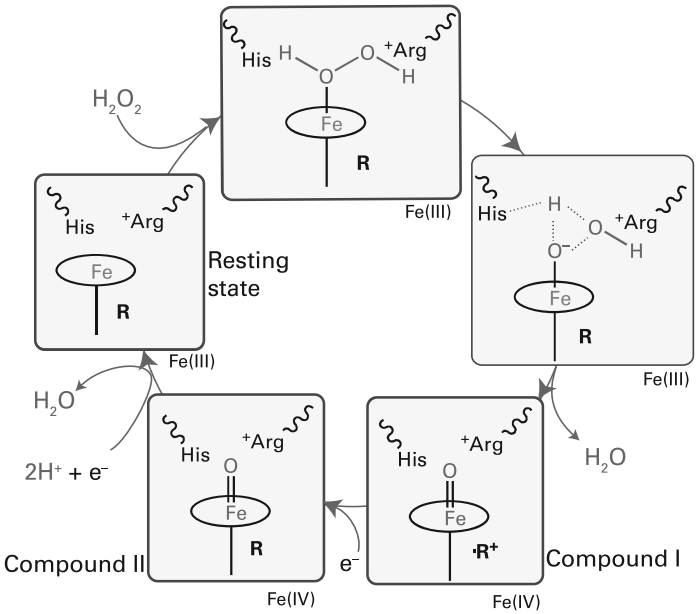



**Figure 27.34** The catalytic cycle of haem-containing peroxidases.

Compound I is reduced back to the resting Fe(III) state by two one-electron transfers from either organic substrates or cytochrome *c* (Fig. 27.25).

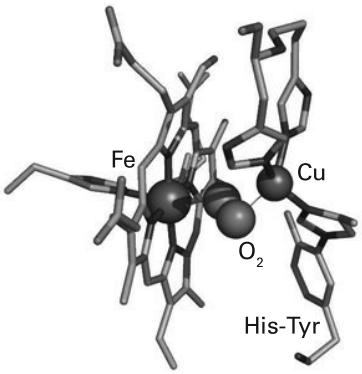
### (b) Oxidases

**Key points:** Oxidases are enzymes that catalyse the reduction of $O_2$ to water or hydrogen peroxide without incorporation of O atoms into the oxidizable substrate; they include cytochrome c oxidase, the enzyme that is a basis for all higher life forms.

Cytochrome *c* oxidase is a membrane-bound enzyme that catalyses the four-electron reduction of $O_2$ to water, using cytochrome *c* as the electron donor. The potential difference between the two half-cell reactions is over 0.5 V but this value does not reflect the true thermodynamics because the actual reaction catalysed by cytochrome *c* oxidase is:

$$O_2(g) + 4e^- + 8H^+(\text{inside}) \rightarrow 2H_2O(l) + 4H^+(\text{outside})$$

This reaction includes four $H^+$ that are not consumed chemically but are 'pumped' across the membrane against a concentration gradient. Such an enzyme is called an **electrogenic ion pump** (or *proton pump*). In eukaryotes, cytochrome oxidase is located in the inner membrane of mitochondria and has many subunits (Fig. 27.35), although a simpler enzyme is produced by some bacteria. It contains three Cu atoms and two haem-Fe atoms, as well as a Mg atom and a Zn atom. The Cu and Fe atoms are arranged in three main sites. The active site for $O_2$ reduction consists of a myoglobin-like Fe-porphyrin (haem-$a_3$) that is situated close to a 'semi-haemocyanin-like' Cu (known as $Cu_B$) coordinated by three histidine ligands (**35**). One of the histidine imidazole ligands to the Cu is modified by formation of a covalent bond to an adjacent tyrosine. Electrons are supplied to the dinuclear site by a second Fe porphyrin (haem-*a*) that is six-coordinate, as expected for an electron-transfer centre. These centres are located in subunit 1. Subunit 2 contains the dinuclear $Cu_A$ centre that was described in Section 27.8, which is believed to be the immediate acceptor of the electron arriving from cytochrome *c*. The electron transfer sequence is therefore




**35**

$$\text{Cytochrome } c \rightarrow Cu_A \rightarrow \text{haem } a \rightarrow \text{binuclear site}$$

The enzyme contains two proton-transfer channels, one of which is used to supply the protons needed for $H_2O$ production while the other is used for protons that are being pumped across the membrane. Figure 27.36 shows the proposed catalytic cycle.

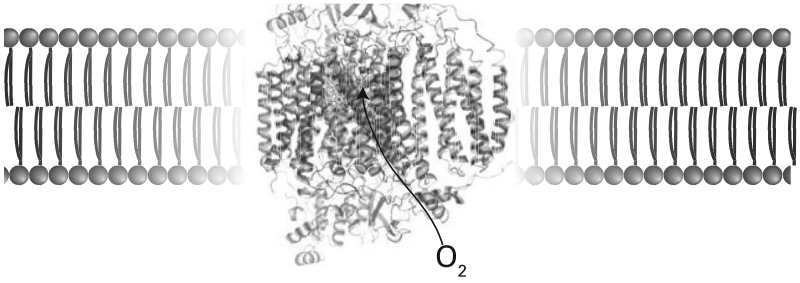



**Figure 27.35** The structure of cytochrome *c* oxidase as it occurs in the membrane, showing the locations of the redox centres and the sites for reaction with $O_2$ and cytochrome *c*. See (**33**) for a more detailed view of the active site.

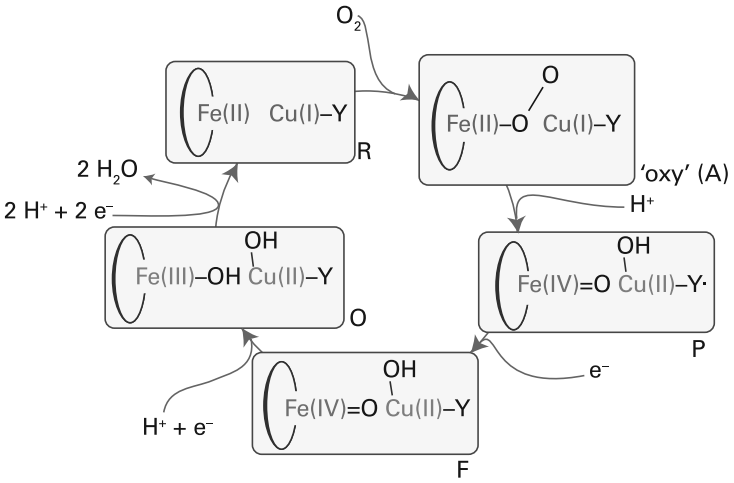
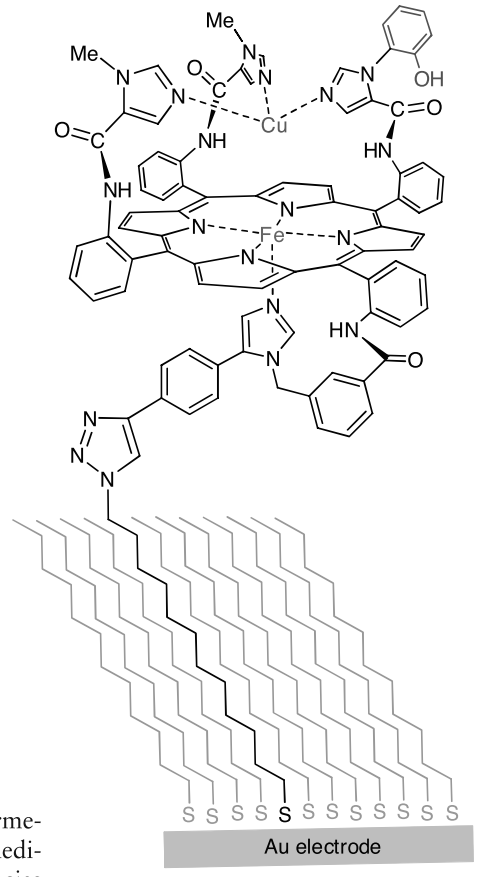



**Figure 27.36** The catalytic cycle of cytochrome *c* oxidase. The intermediates are labelled according to current convention. Electrons are provided from the other haem and $Cu_A$. During the cycle, an additional four $H^+$ are pumped across the membrane.

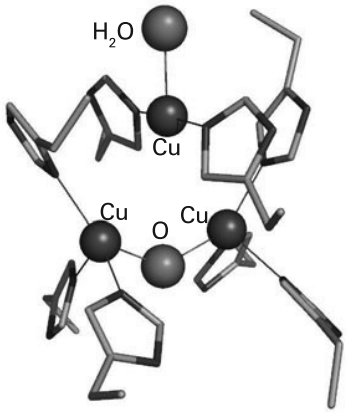



**36**

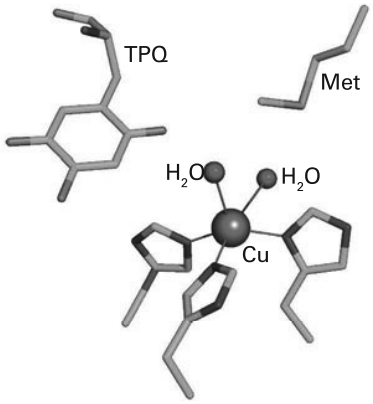
Starting from the state in which the active site is Fe(II)−Cu(I), $O_2$ binds to give an intermediate (oxy) that resembles oxymyoglobin. However, unlike oxymyoglobin, this intermediate takes up the other electron that is immediately available, producing a peroxy species that quickly breaks down to give an intermediate known as P. Species P has been trapped and studied by optical and EPR spectroscopy, which show that it contains Fe(IV) and an organic radical that may be located on the unusual His−Tyr pair (Y·). The oxido-Fe(IV) (ferryl) group is formed by heterolytic cleavage of $O_2$, producing a water molecule. The role of a cation radical is again noted: without this radical, the Fe would have to be assigned as Fe(V).

It is vital that intermediates such as peroxide are not released during conversion of $O_2$ to water. Studies with an elaborate model complex (**36**) which can be attached to an electrode show that the presence of the phenol is crucial because it allows all four electrons necessary for the reduction of the $O_2$ to be provided rapidly without relying on long-range electron transfer, which is slow through the long-chain aliphatic linker. If the phenolic −OH group is replaced by $-OCH_3$, hydrogen peroxide is released during $O_2$ reduction because the methoxy derivative is unable to form an oxidized radical.

The blue Cu oxidases contain a blue Cu centre that removes an electron from a substrate and passes it to a trinuclear Cu site that catalyses the reduction of $O_2$ to $H_2O$. Two examples, ascorbate oxidase and a larger class known as laccases, are well characterized, whereas another protein, ceruloplasmin, occurs in mammalian tissue and is the least well understood. Ascorbate oxidase occurs in the skins of fruit such as squash. Its role may be twofold: to protect the flesh of the fruit from $O_2$ and to oxidize phenolic substrates to intermediates that will form the skin of the fruit. Laccases are widely distributed, particularly




**37**




**38**

in plants and fungi, from which they are secreted to catalyse the oxidation of phenolic substrates. The active site at which $O_2$ is reduced (**37**) is well buried. It contains a pair of Cu atoms linked in the oxidized form by a bridging O atom, with a third Cu atom situated very close by, completing an almost triangular arrangement.

Amine oxidases catalyse the oxidation of amines to aldehydes by using just a single Cu atom that shuttles between Cu(II) and Cu(I), yet the enzyme carries out a two-electron reduction of $O_2$, producing a molecule of $H_2O_2$. The problem is overcome because, like cytochrome *c* peroxidase and cytochrome *c* oxidase, amine oxidases have an additional oxidizing source located near to the metal, in this case a special cofactor called topaquinone (TPQ), which is formed by post-translational oxidation of tyrosine (**38**).

**EXAMPLE 27.7 Interpreting reduction potentials**

The four-electron reduction potential for $O_2$ is +0.82 V at pH = 7. Cytochrome *c*, the electron donor to cytochrome *c* oxidase, has a reduction potential of +0.26 V, whereas the organic substrates of fungal laccases often have values as high as +0.7 V. What is the significance of these data in terms of energy conservation?

***Answer*** Although cytochrome *c* oxidase and laccase both catalyse the efficient four-electron reduction of $O_2$, we need to consider their different biological functions. Laccases are efficient catalysts of phenol oxidation, the driving force being small. Cytochrome oxidase is a proton pump and approximately 2 eV (4 × 0.56 eV) of Gibbs energy is available from oxidation of cytochrome *c* to drive proton transfer across the mitochondrial inner membrane.

***Self-test 27.7*** Before the discovery of the unusual active site structures in amine oxidase and another Cu enzyme called galactose oxidase, Cu(III) was proposed as a catalytic intermediate. What properties would be expected of this state ?

### (c) Oxygenases

**Key points: Oxygenases catalyse the insertion of one or both O atoms derived from $O_2$ into an organic substrate; monooxygenases catalyse insertion of one O atom while the other O atom is reduced to $H_2O$; dioxygenases catalyse the incorporation of both O atoms.**

Oxygenases catalyse the insertion of one or both O atoms of $O_2$ into substrates, whereas with the $Fe^-$ and $Cu^-$ containing oxidases both O atoms end up as $H_2O$. Oxygenases are often referred to as *hydroxylases* when the O atom is inserted into a C–H bond. Most oxygenases contain Fe, the rest contain Cu or flavin, an organic cofactor. There are many variations. Monooxygenases catalyse reactions of the type

$$R-H+O_2+2H^++2e^-\rightarrow R-O-H+H_2O$$

in which electrons are supplied by an electron donor such as an FeS protein. Monoxygenases can also catalyse the epoxidation of alkenes. Dioxygenases catalyse the insertion of both atoms of $O_2$ into substrates, and no additional electron donor is required. Two C–H bonds on the same molecule may be oxygenated:

$$H-R-R'-H + O_2 \rightarrow H-O-R-R'-O-H$$

The Fe enzymes are divided into two main classes, haem and non-haem. We discuss the haem enzymes first, the most important type being cytochrome P450.

Cytochrome P450 (or just P450) refers to an important and widely distributed group of haem-containing monooxygenases. In eukaryotes, they are localized particularly in mitochondria, and in higher animals they are concentrated in liver tissue. They play an essential role in biosynthesis (for example steroid transformations), such as the production of progesterone. The designation 'P450' arises from the intense absorption band that appears at 450 nm when solutions containing the enzyme or even crude tissue extracts are treated with a reducing agent and carbon monoxide, which produces the Fe(II)–CO complex. Most P450s are complex membrane-bound enzymes that are difficult to isolate. Much of what we know about them stems from studies carried out with an enzyme $P450_{cam}$, which is isolated from the bacterium *Pseudomonas putida*.

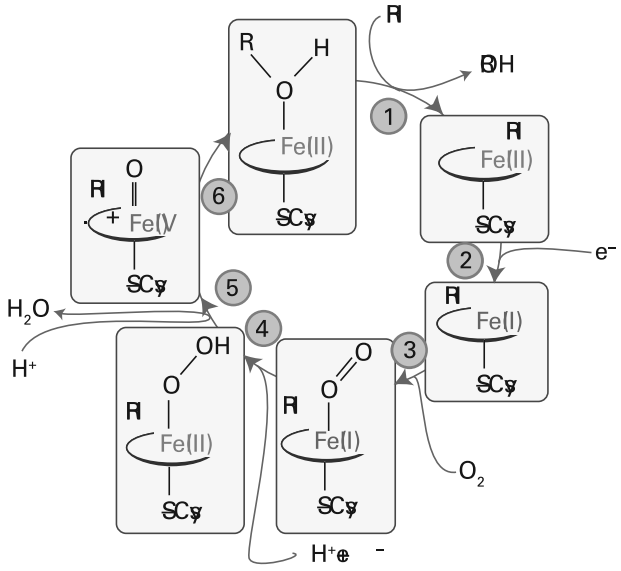
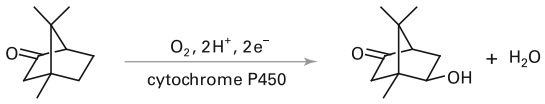



**Figure 27.37** The catalytic cycle of cytochrome P450.

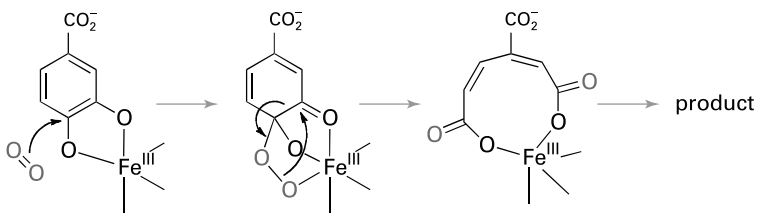
This organism uses camphor as its sole source of carbon, and the first stage is oxygenation of the 5-position:



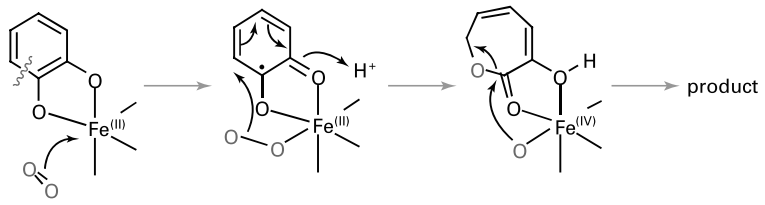

The catalytic cycle has been studied using a combination of kinetic and spectroscopic methods (Fig. 27.37). Starting from the resting enzyme, which is Fe(III), the binding of the substrate in the active site pocket (1) induces release of the coordinated $H_2O$ molecule. This step is detected as a change in spin state from low spin ($S = \frac{1}{2}$) to high spin ($S = \frac{5}{2}$) and the reduction potential increases, causing an electron to be transferred (2) from a small [2Fe–2S]-containing protein known as putidaredoxin. The five-coordinate Fe(II) that is formed resembles deoxymyoglobin and binds $O_2$ (3). Unlike in myoglobin, addition of a second electron is both thermodynamically and kinetically favourable. The subsequent reactions (4–6) are very fast, but it is thought that an Fe(III) peroxide intermediate is formed that undergoes rapid heterolytic O–O cleavage to produce a species similar to Compound I of peroxidases. In what is known as the **oxygen rebound mechanism**, the Fe(IV)=O group abstracts an H atom from the substrate and then inserts it back as an OH radical. This process is remarkable, as it amounts to the 'taming' of an O atom or OH radical by its attachment to Fe.

Other P450s are thought to operate by similar mechanisms but differ in the architecture of the active site pocket. That site, unlike the site in peroxidases, is predominantly hydrophobic, with specific polar groups present to orient the organic substrate so the correct R–H bond is brought close to the Fe=O entity.

Non-haem oxygenases are widely distributed and are usually dioxygenases. Most contain a single Fe atom at the active site and are classified according to whether the active species in the protein is Fe(III) or Fe(II). In the Fe(III) enzymes, which are also (historically) known as **intradiol oxygenases**, the Fe atom functions as a Lewis acid catalyst and activates the organic substrate towards attack by noncoordinating $O_2$:



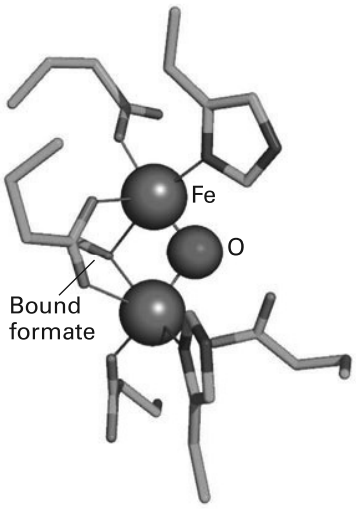
By contrast, in the Fe(II) enzymes, which are known historically as **extradiol oxygenases**, the Fe binds $O_2$ directly and activates it to attack the organic substrate:




The Fe(III) enzymes are exemplified by protocatechuate 3,4-dioxygenase: the Fe is high spin and tightly coordinated by a set of protein ligands that includes two His-N and two Tyr-O, the latter hard donors being particularly suitable for stabilizing Fe(III) relative to Fe(II). The Fe(III) enzymes are deep red due to an intense tyrosinate-to-Fe(III) charge transfer transition. The Fe(II) enzymes are exemplified by catechol 2,3-dioxygenase: the Fe is high spin and coordinated within the protein by a set of ligands that includes two His-N and one carboxylate group. The binding is weak, reflecting the low position of Fe(II) in the Irving–Williams series (Section 20.1). This weak binding, together with the difficulty of observing useful spectroscopic features (such as EPR spectra), has made these enzymes much more difficult to study than the Fe(III) enzymes.

A particularly important class of Fe(II) oxygenases use a molecule of 2-oxoglutarate as a second substrate:

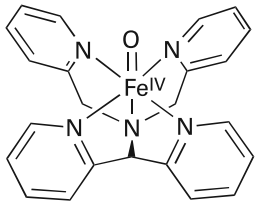

The principle of **oxo-glutarate-dependent oxygenases** is that the transfer of one O atom of $O_2$ to 2-oxoglutarate (also known as α-ketoglutarate) results in its irreversible decarboxylation, thus driving insertion of the other O atom into the primary substrate. Examples include enzymes that serve in cell signalling by modifying an amino acid in certain transcription factors (Section 27.15).




**39**

Oxygenases play a crucial role in the metabolism of methane, a greenhouse gas. Of all hydrocarbons, methane contains the strongest C–H bonds and is the most difficult to activate. Methane-metabolizing bacteria produce two types of enzyme that catalyse the conversion of methane to methanol (a more useful chemical and fuel) and thus attract much industrial interest. One is a membrane-bound enzyme that contains Cu atoms. This enzyme, known as 'particulate' methane monooxygenase (p-mmo), is expressed when high levels of Cu are available. The other enzyme, soluble methane monooxygenase (s-mmo), contains a dinuclear Fe active site (**39**) that is related to haemerythrin (**21**) and acid phosphatase (**33**). The mechanisms are not established but Fig. 27.38 shows a plausible catalytic cycle for s-mmo. The intermediate Fe(IV) species that is proposed differs from those we have encountered up to now, as the $O_2$-derived oxido ligands are bridging rather than terminal.

Despite the importance of Fe(IV) as an enzyme intermediate, small Fe(IV) complexes that could provide important models for understanding the enzymes have been elusive. The easiest to prepare are haem analogues in which Fe is equatorially ligated by porphyrin and which can be formed by reacting the Fe(II) or Fe(III) forms with a peroxo acid. Small models of non-haem Fe(IV) species have now been prepared. The mononuclear complex (**40**) containing the pentadentate pentaaza ligand *N,N*-bis(2-pyridylmethyl)-*N*-bis(2-pyridyl)methylamine is formed by treating the Fe(II) complex with the oxo-transfer agent iodosylbenzene. It is fairly stable at room temperature and has been structurally characterized by X-ray diffraction. A powerful oxidizing agent, it can also be generated in acetonitrile solution by bulk electrolysis of the Fe(II) complex in the presence of water, and the standard potential for Fe(IV)/Fe(III) is estimated to be 0.9 V relative to the ferrocinium/ferrocene couple. Complex (**40**) and similar species are paramagnetic ($S = 1$) and show characteristic absorption bands in the near-infrared. The Fe–O bond length in (**40**) is 164 pm, which is fully consistent with a multiple bond in which the O atom is acting as a π donor. Complex (**40**) is able to oxygenate C–H bonds in a variety of hydrocarbons,




**40**

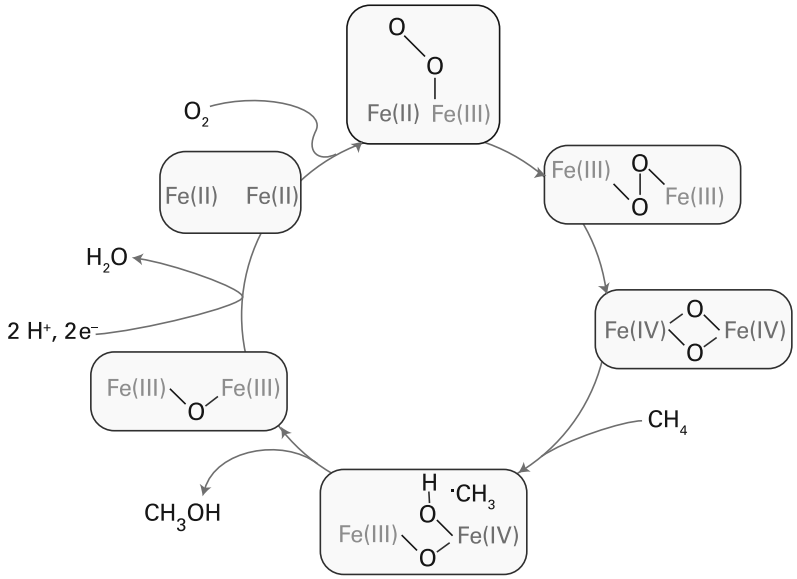



**Figure 27.38** A plausible catalytic cycle for methane monooxygenase.

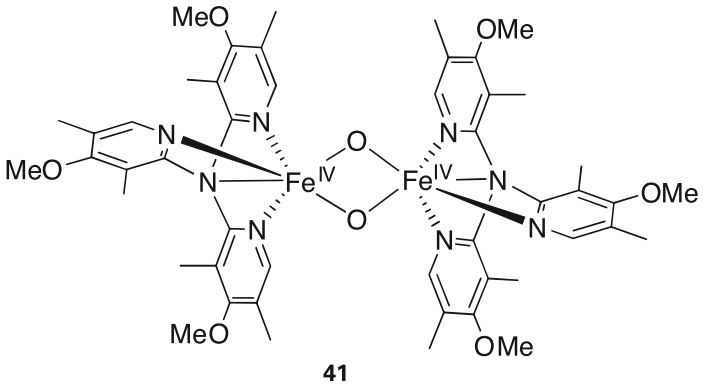
including cyclohexane. The bis(μ-oxo)Fe(IV) complex (**41**) has been proposed as a structural analogue of the reactive intermediate formed in s-mmo.

Tyrosinase and catechol oxidase, two enzymes responsible for producing melanin-type pigments, each contain a strongly coupled dinuclear Cu centre that coordinates $O_2$ in a manner similar to haemocyanin. However, unlike in haemocyanin, the ligands σ*-to-Cu charge transfer is enhanced sufficiently to activate the coordinated $O_2$ for electrophilic attack at a phenolic ring of the substrate. The structure of the active site of catechol oxidase complexed with the inhibitor phenolthiourea (**42**) shows how the phenol ring of the substrate can be oriented in close proximity to a bridging $O_2$. Copper enzymes are also responsible for the production of important neurotransmitters and hormones, such as dopamine and noradrenaline. These enzymes contain two Cu atoms that are well separated in space and uncoupled magnetically.

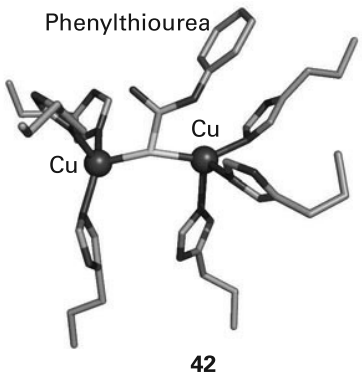
### (d) Photosynthetic $O_2$ production

**Key points:** Biological solar energy capture by photoactive centres results in the generation of species with sufficiently negative reduction potentials to reduce $CO_2$ to produce organic molecules; in higher plants and cyanobacteria, the electrons are derived from water, which is converted to $O_2$ by a complex catalytic centre containing four Mn atoms and one Ca atom.

**Photosynthesis** is the production of organic molecules using solar energy. It is conveniently divided into the **light reactions** (the processes by which electromagnetic energy is trapped) and the **dark reactions** (in which the energy acquired in the light reactions is used to convert $CO_2$




41




42

and $H_2O$ into carbohydrates). We have already mentioned the most important of the dark reactions, the incorporation of $CO_2$ into organic molecules, which is catalysed by rubisco. In this section, we describe some of the roles that metals play in the light reactions.

The basic principle of photochemical energy capture, applied in a technology to produce $H_2$ from water, was described in Chapter 10 (Box 10.3). We can view photosynthesis in an analogous way in that $H_2$ is 'stored' by reaction with $CO_2$. In biology, photons from the Sun excite pigments present in giant membrane-bound proteins known as **photosystems**. The most important pigment, chlorophyll, is a Mg complex that is very similar to a porphyrin (8). Most chlorophyll is located in giant proteins known as **light-harvesting antennae**, the name perfectly describing their function, which is to collect photons and funnel their energy to enzymes that convert it into electrochemical energy. This energy conversion uses further chlorophyll complexes that become powerful reducing agents when excited by light. Each electron released by excited chlorophyll travels rapidly down a sequence of protein-bound acceptors, including FeS clusters, and (through the agency of ferredoxin and other redox enzymes) is eventually used to reduce $CO_2$ to carbohydrate. Immediately after releasing an electron, the chlorophyll cation, a powerful oxidant, must be rapidly reduced by using an electron from another site to avoid wasting the energy by recombination (simple reversal of electron flow). In 'oxygenic' photosynthesis, which occurs in green algae, cyanobacteria, and most importantly in green plants, each such 'restoring' electron is provided from a water molecule, resulting in production of $O_2$.

In green plants, photosynthesis occurs in special organelles known as *chloroplasts*. Plant chloroplasts have two photosystems, I and II, operating in series, that allow low-energy light (approximately 680–700 nm, >1 eV) to span the large potential range (>1 V) within which water is stable. The arrangement of proteins is depicted in Fig. 27.39. Some of the energy of the photosynthetic electron transfer chain is used to generate a transmembrane proton gradient which in turn drives the synthesis of ATP, as in mitochondria. Photosystem I lies at the low-potential end, its electron donor is the blue Cu protein plastocyanin that has been reduced using the electrons generated by photosystem II; in turn, the electron donor to photosystem II is $H_2O$. Thus green plants dispose of the oxidizing power by converting $H_2O$ into $O_2$. This four-electron reaction is remarkable because no intermediates are released. The catalyst, called the 'oxygen evolving centre' (OEC) also has a special significance because its action, commencing over 2 Ga ago, has provided essentially all the $O_2$ we have in the atmosphere. The OEC is the only enzyme active site known to produce an

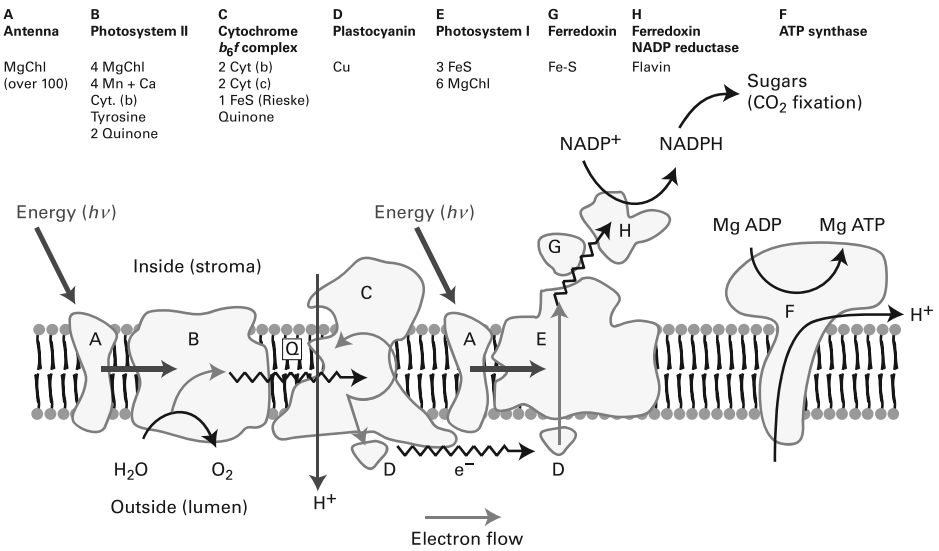
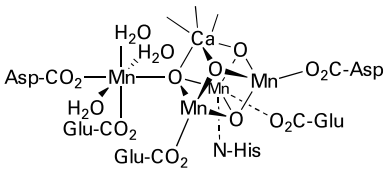



**Figure 27.39** The arrangement of proteins in the photosynthetic electron-transport chain (The Mg–chlorophyll complex is represented 'MgChl'.) **A**. Antenna ('light harvesting') complex. **B**. Photosystem II. **C**. The 'cytochrome $b_6f$ complex' (this is similar to **complex III** in the mitochondrial ET chain). **D**. Plastocyanin (soluble). **E**. Photosystem I. **F**. ATPase. **G**. Ferredoxin (Fe—S). **H**. Ferredoxin–$NADP^+$ reductase (flavin). Blue arrows show transfer of energy. Note how the overall transfer of electrons is from Mn (high potential) to FeS (low potential): this apparently 'uphill' flow reflects the crucial input of energy at each photosystem.

O–O bond from two $H_2O$ molecules, and there is much interest in producing functional models of this catalyst for photochemical water splitting (Box 10.3).

The OEC is a metal oxide cluster, containing four Mn atoms and one Ca atom, that is located in subunit D1 of photosystem II. Subunit D1 has long attracted interest because the cell replaces it at frequent intervals as it quickly becomes worn out by oxidative damage. X-ray diffraction data indicate that the metal atoms are arranged as a [3MnCa−4O] cubane connected to a fourth 'dangling' Mn (**43**). The OEC exploits the oxidizing abilities of Mn(IV) and Mn(V), coupled with that of a nearby tyrosine residue, to oxidize $H_2O$ to $O_2$. Successive photons received by photosystem II result in the OEC being progressively oxidized (the acceptor, an oxidized chlorophyll known as $P680^+$, has a reduction potential of approximately 1.3 V) through a series of states designated S0 to S4, as shown in Fig. 27.40. Apart from S4, which rapidly releases $O_2$ and has not been isolated, these states are identified in kinetic studies by their characteristic spectroscopic properties, for example S2 shows a complex multi-line EPR spectrum. Note that the Mn ligands are hard O-atom donors and Mn(III) ($d^4$), Mn(IV) ($d^3$), and Mn(V) ($d^2$) are hard metal ions.



**43**

Based on the available structural evidence, different models have been proposed for the mechanism of $O_2$ evolution from two $H_2O$ molecules. The overriding barrier to $O_2$ formation lies in forming the weak peroxidic O–O bond, following which the formation of O=O is energetically easy (Section 16.1 and *Resource section 3*). First, as the Mn sites are progressively oxidized, coordinated $H_2O$ molecules become increasingly acidic and lose protons, progressing from $H_2O$ through $OH^-$ to $O^{2-}$. Second, computational studies suggest that Mn(V)=O is best regarded as Mn(IV)–O•, in which the oxido ligand is electron deficient and has appreciable radical character. An $H_2O$ or $OH^-$ ligand coordinated at one Mn subsite (or the Ca) could attack such an electron-deficient O-ligand, perhaps formed at the 'dangling' Mn that is not part of the cubane. Such an attack by coordinated $H_2O$ or $OH^-$ on Mn(IV)−O• would result in a Mn(III)-peroxide species that is easily converted to $O_2$ by using the reservoir of oxidizing power that has accumulated on the cluster. The presence of the $Ca^{2+}$ is essential, and the only metal ion that can be substituted is $Sr^{2+}$. A possible role for the Ca is that it provides a site that will remain permanently in the +2 oxidation state and provide a rapid and stable binding site for incoming $H_2O$, whereas if this subsite were occupied by a fifth Mn atom, the latter would certainly become oxidized and the advantage would be lost.

## 27.11 The reactions of cobalt-containing enzymes

**Key points:** Nature uses cobalt in the form of complexes with a macrocyclic ligand, known as corrin. Complexes in which the fifth ligand is a benzimidazole that is covalently linked to the corrin ring are known as cobalamins. Cobalamin enzymes catalyse methyl transfer and dehalogenation.

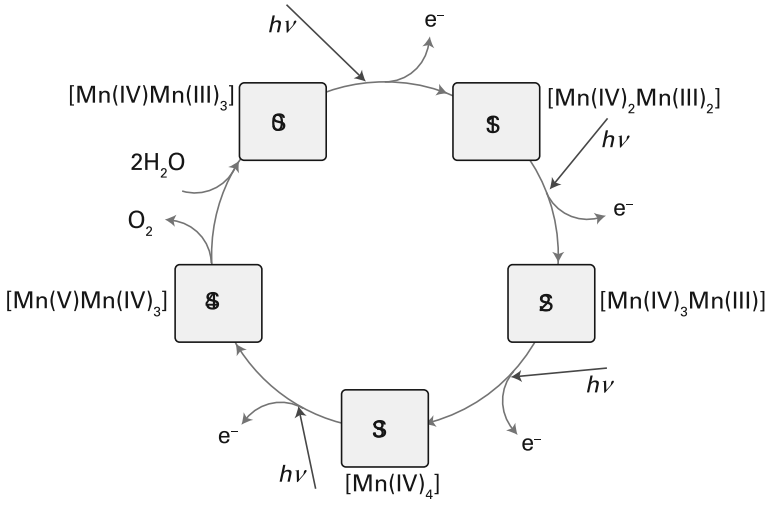
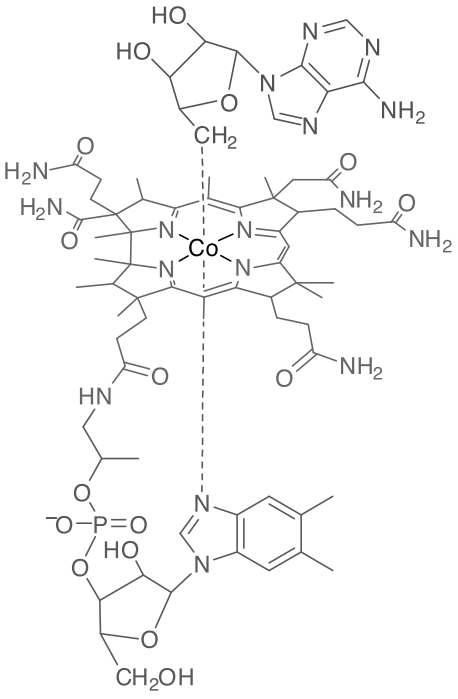



**Figure 27.40** The S-cycle for evolution of $O_2$ by successive one-electron oxidations of the [4MnCa−4O] cluster of photosystem II. The formal oxidation numbers of the Mn components are indicated, but $H^+$ transfers are omitted. In chloroplasts that have become adapted to dark conditions, the cycle 'rests' in the S1 state.

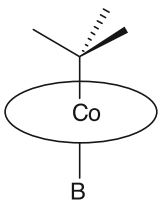
**In Coenzyme $B_{12}$ the sixth ligand is deoxyadenosine, which is coordinated through a Co—C bond; enzymes containing coenzyme $B_{12}$ catalyse radical-based rearrangements.**

Cobalt macrocycle complexes are cofactors in enzymes that catalyse methyl transfer reactions and they are also important for dehalogenation and radical-based rearrangements (for example, isomerizations). The macrocycle is a corrin ring (**9**), which is similar to porphyrin (**8**), except there is less conjugation and it has a smaller ring (15-membered instead of 16-membered). The five-coordinate complex known as **cobalamin** includes a fifth nitrogen donor in one of the axial positions: usually this ligand is a dimethylbenzimidazole that is covalently linked to the corrin ring through a nucleotide, but a histidine residue is also commonly encountered. The more elaborate structure known as **coenzyme $B_{12}$** (**44**) is an important enzyme cofactor for radical rearrangements: the sixth ligand, R, is 5′-deoxyadenosine, which is bonded to the Co atom through the $-CH_2-$ group, making coenzyme $B_{12}$ a rare example of a naturally occurring organometallic compound.[8] The sixth ligand is exchangeable and the complex is ingested in the form of species such as aquacobalamin, hydroxocobalamin, or cyanocobalamin, known generally as vitamin $B_{12}$. Cobalamin is essential for higher organisms (the human requirement is only a few milligrams per day) but it is synthesized only by microorganisms. Like Fe porphyrins, the Co corrins are enzyme cofactors and exert their activities when bound within a protein.

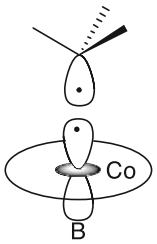
The Co atom can exist in three oxidation states under physiological conditions, Co(III), Co(II), and Co(I), all of which are low spin. The electronic structure of Co is crucial to its biological activity. As expected, the Co(III) form ($d^6$) is an 18-electron, six-coordinate species (**45**). The Co(II) form (**46**) is 17-electron, five-coordinate and has its unpaired electron in the $d_{z^2}$ orbital. These species are termed 'base-on' forms because the fifth nitrogen ligand is coordinated. The Co(I) form (**47**) is a classic 16-electron, four-coordinate square-planar species, due to dissociation of both axial ligands. The square-planar structure is a 'base-off' form.



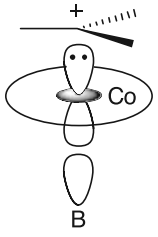

**44** Coenzyme $B_{12}$




**45**




**46**

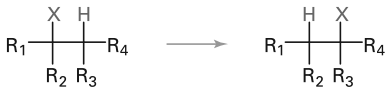



**47**

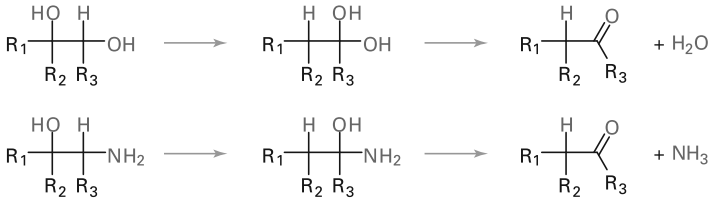
[8] Coenzyme $B_{12}$ was one of the earliest molecules to be structurally characterised by X-ray diffraction methods (Dorothy Crowfoot Hodgkin, Nobel Prize for Chemistry, 1964). In 1973, Robert Woodward and Albert Eschenmoser published the total synthesis of $B_{12}$, the most complex natural product to be synthesized at that time, involving almost 100 steps.

Methyl transfer reactions of cobalamins exploit the high nucleophilicity of square-planar Co(I). A particularly important example is methionine synthase, which is responsible for the biosynthesis of methionine. Methionine is produced by transferring a $CH_3$ group, derived from the methyl carrier methyl hydrofolate, to homocysteine. Not only is methionine an essential amino acid, but also accumulation of homocysteine (which occurs if activity is impaired) is associated with serious medical problems. The mechanism involves a 'base-on/base-off' cycle in which Co(I) abstracts an electrophilic $-CH_3$ group (effectively $CH_3^+$) from a quaternary N atom on $N^5$-tetrahydrofolate to produce methylcobalamin, which then transfers $-CH_3$ to homocysteine (Fig. 27.41). Methylcobalamin is the methyl-transferring cofactor for a wide variety of biosynthetic pathways, including the production of antibiotics. In anaerobic microbes, methylcobalamin is involved in the synthesis of acetyl coenzyme A, an essential metabolite, and in production of methane by methanogens.

Radical-based rearrangements catalysed by coenzyme $B_{12}$ (but see Box 27.1) include isomerizations (mutases) and dehydration or deamination (lyases). The generic reaction is




Dehydration and deamination occur after two $-OH$ or $-OH$ and $-NH_2$ become placed on the same carbon atom and hence are triggered by isomerization:




Radical-based rearrangements occur by a mechanism involving initiation of radical formation that begins with enzyme-induced weakening of the Co–C (adenosine) bond. In the free state, the Co–C bond dissociation energy is about 130 kJ $mol^{-1}$, but when bound in the

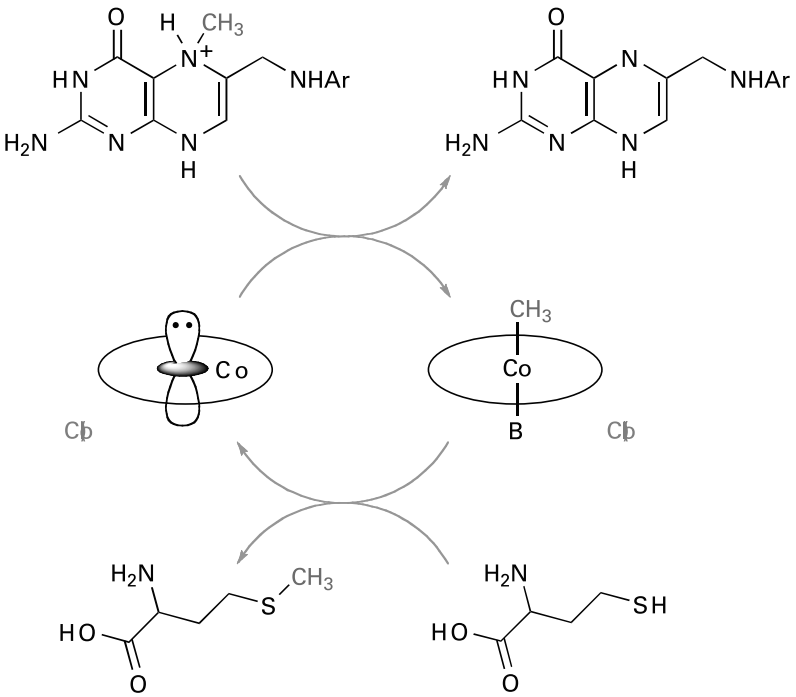
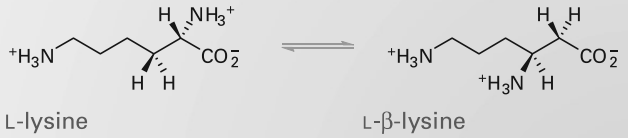



**Figure 27.41** The mechanism of methionine synthase. Co(I) is a strong nucleophile and attacks the electrophilic quaternary-$CH_3$ group on the methyl carrier tetrahydrofolate. The resulting Co(III) methyl complex transfers $CH_3^+$ to homocysteine.

## BOX 27.1 Iron–sulfur enzymes in radical reactions

The discovery in 1970 of an enzyme, lysine 2,3-aminomutase, which catalyses the radical-based rearrangement of an amino acid without any involvement of coenzyme $B_{12}$, initiated development of a whole new area of biochemistry that has led to $B_{12}$ being relegated to second place in regard to catalyzing these rearrangements. Lysine 2,3-aminomutase belongs to a large class of FeS enzymes now known as the radical S-adenosylmethionine (SAM) superfamily. Radical SAM enzymes include those responsible for synthesis of essential vitamins, such as vitamin H (biotin), vitamin $B_1$ (thiamine), haem, and molybdopterin (Section 27.12), as well as those that undertake routine repair of DNA.

Interconversion of L-lysine and L-β-lysine involves migration of the α-amino group to the β-carbon atom. L-β-lysine is required by certain bacteria for antibiotic synthesis.




As with $B_{12}$ enzymes, this reaction involves a 5′-deoxyadenosyl radical, but in radical SAM enzymes the radical is generated by reductive cleavage of the S-adenosylmethionine cation using a special [4Fe–4S] cluster. The reaction sequence begins with reduction of the [4Fe–4S] cluster: $[4Fe\text{–}4S]^+$ is a powerful reductant and SAM is reductively cleaved at the tertiary $S^+$ to produce methionine, which remains coordinated to the special Fe of the cluster, and the deoxyadenosyl radical, which now abstracts a hydrogen atom from lysine and induces rearrangement. The X-ray structure of the precursor (Fig. B27.1) reveals how the different groups are arranged in space. Based on various lines of spectroscopic evidence, likely mechanisms for forming a 5′-deoxyadenosyl radical (Fig. B27.2) involve the tertiary S attacking the unique Fe subsite (a) or a cluster S atom (b). In either case this attack is followed by rapid electron transfer and bond cleavage.

The amino acid part sequence —C*xxx*C*xx*C— is the characteristic coordination motif for the [4Fe–4S] cluster in a radical SAM protein. More than 2000 proteins have now been identified by searching for the equivalent base sequence occurring in genes.

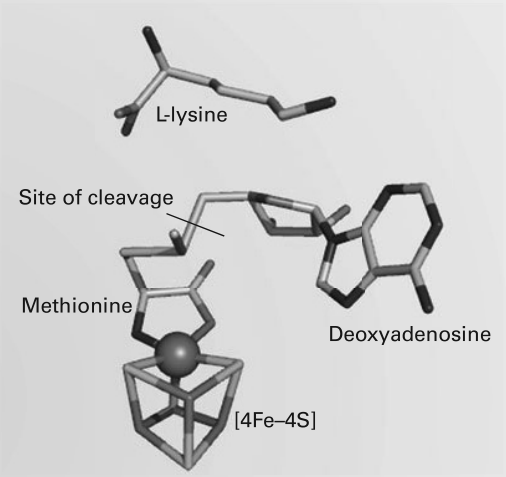



**Figure B27.1** Structure of the active site of lysine 2,3-aminomutase, showing the arrangement of the precursor state in which S-adenosylmethionine is coordinated to the [4Fe–4S] cluster. Lysine, the substrate, is held close by.

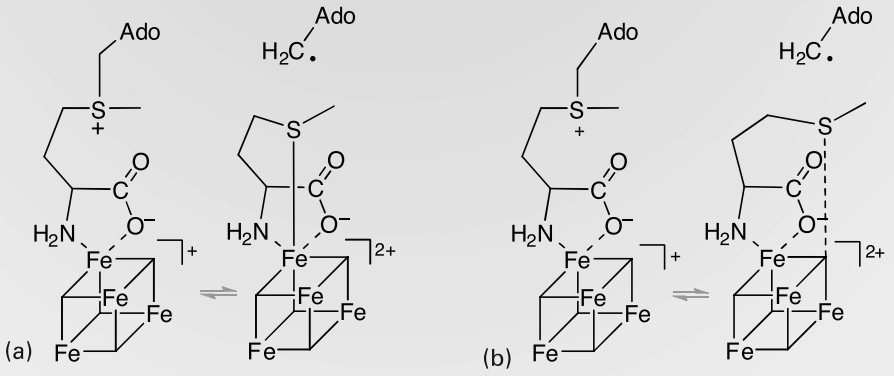



**Figure B27.2** Two possible mechanisms by which the reactive 5′-deoxyadenosyl radical is generated by the $[4Fe\text{–}4S]^{2+}$ cluster in radical SAM enzymes.

enzyme the bond is substantially weakened, resulting in homolytic cleavage of the Co—$CH_2R$ bond. This step results in five-coordinate low-spin Co(II) and a $CH_2R$ radical, which gives rise to controlled radical chemistry in the enzyme active site pocket (Fig. 27.42). Important examples are methylmalonyl CoA mutase and diol dehydratases.

In 1970, an alternative system for catalyzing radical-based rearrangements was discovered that does not depend on Co. As described in Box 27.1, the enzymes involved use instead a [4Fe—4S] cluster to generate the active deoxyadenosyl radical by reductive cleavage of its methionyl derivative.

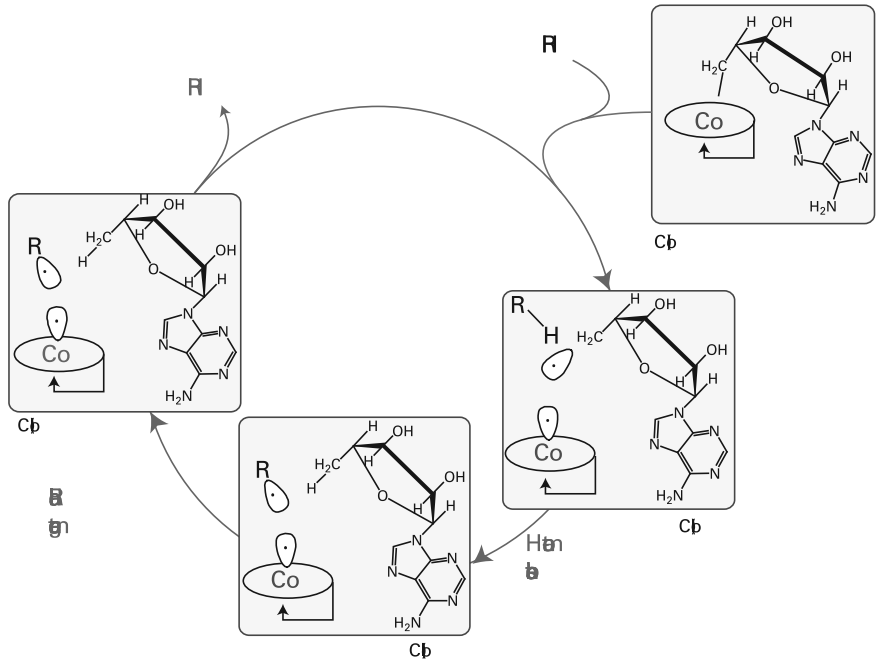



**Figure 27.42** The principle of radical-based rearrangements by coenzyme $B_{12}$. Homolytic cleavage of the Co—C bond results in low-spin Co(II) ($d_{z^2}^1$) and a carbon radical that abstracts an H atom from the substrate RH. The substrate radical is retained in the active site and undergoes rearrangement before the hydrogen atom transfers back.

**EXAMPLE 27.8** **Identifying the significance of the d-electron configuration of cobalamin**

Why is a Co-based macrocyclic complex (rather than an Fe complex like haem) well suited for radical-based rearrangements?

***Answer*** To answer this question, we need to consider the electron configuration of Co(II) complexes in which there is a strong equatorial ligand field. Radical-based rearrangements depend on homolytic cleavage of the Co—C bond, which generates the adenosine radical and leaves an electron in the $d_{z^2}$ orbital of the Co. This is the stable configuration for a low-spin Co(II) ($d^7$) complex, but for Fe, this configuration would require the oxidation state Fe(I), which is not normally encountered in coordination complexes.

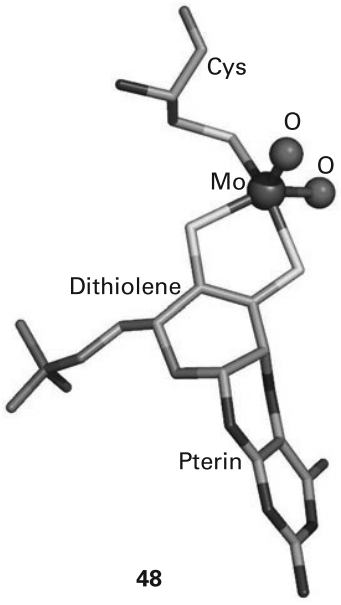
***Self-test 27.8*** Provide an explanation for why the toxicity of mercury is greatly increased by the action of enzymes containing cobalamin.

## 27.12 Oxygen atom transfer by molybdenum and tungsten enzymes

**Key points:** Mo is used to catalyse O atom transfer in which the O atom is provided by a water molecule; related chemistry, but in more reducing environments, is displayed by W.

Molybdenum and tungsten are the only heavier elements known so far to have specific functions in biology. Molybdenum is widespread across all life forms, and this section deals with its presence in enzymes other than nitrogenase (Section 27.13). By contrast, W has so far only been found in prokaryotes. Molybdenum enzymes catalyse the oxidation and reduction of small molecules, particularly inorganic species. Reactions include oxidation of sulfite, arsenite, xanthine, aldehydes, and carbon monoxide, and reduction of nitrate and dimethyl sulfoxide (DMSO).

Both Mo and W are found in combination with an unusual class of cofactors (**11**), at which the metal is coordinated by a dithiolene group. In higher organisms, Mo is coordinated by one pterin dithiolene along with other ligands that often include cysteine. This is illustrated by the active site of sulfite oxidase, where we see also how the pterin ligand is twisted (**48**). In prokaryotes, Mo enzymes have two pterin cofactors coordinated to the metal atom and although the role of such an elaborate ligand is not entirely clear, it is potentially redox active and may mediate long-range electron transfer. Coordination of the




**48**

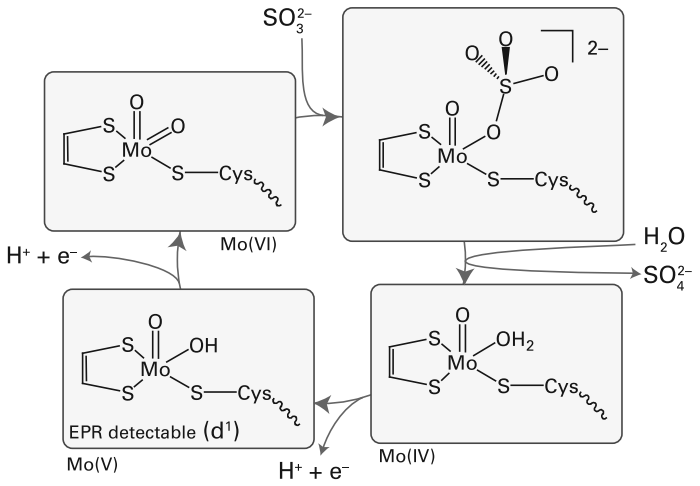



**Figure 27.43** Oxidation of sulfite to sulfate by sulfite oxidase, illustrating the direct O-atom transfer mechanism for Mo enzymes.

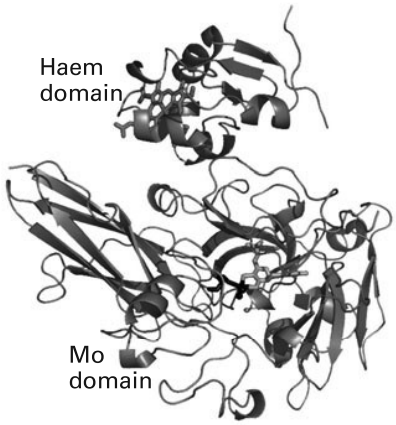



**Figure 27.44** Structure of sulfite oxidase showing the Mo and haem domains. The region of polypeptide linking the two domains is highly mobile and is not resolved by crystallography.

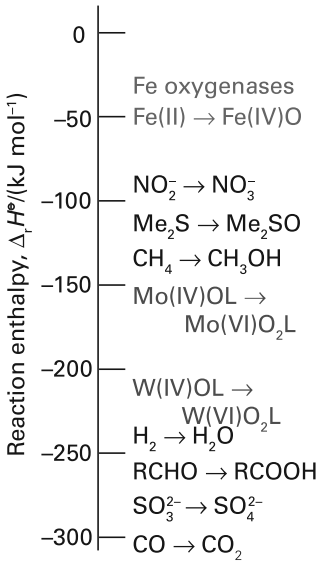


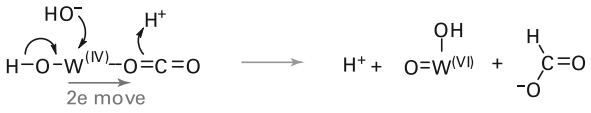

**Figure 27.45** Scale showing relative enthalpies for O-atom transfer. Fe(IV) oxido species are powerful O-atom donors, whereas Mo(IV) and W(IV) are good O-atom acceptors.

Mo is usually completed by ligands derived from $H_2O$, specifically $H_2O$ itself, $OH^-$, and $O^{2-}$. Molybdenum is suited for its role because it provides a series of three stable oxidation states, Mo(IV), Mo(V), and Mo(VI), related by one-electron transfers that are coupled to proton transfer. Typically, Mo(IV) and Mo(VI) differ in the number of oxido groups they contain, and Mo enzymes are commonly considered to couple one-electron transfer reactions with O-atom transfer. In humans and other mammals, an inability to synthesize molybdenum cofactor has serious consequences. Sulfite oxidase deficiency is a rare, inherited defect (sulfite ions are very toxic) and it is often fatal.

A mechanism often considered for Mo enzymes is direct O-atom transfer, which is illustrated in Fig. 27.43 for sulfite oxidase. The S atom of the sulfite ion attacks an electron-deficient O atom coordinated to Mo(VI), leading to Mo–O bond cleavage, formation of Mo(IV), and dissociation of $SO_4^{2-}$. Reoxidation back to Mo(VI), during which a transferable O atom is regained, occurs by two one-electron transfers from an Fe-porphyrin that is located on a mobile 'cytochrome' domain of the enzyme (Fig. 27.44). The intermediate state containing Mo(V) ($d^1$) is detectable by EPR spectroscopy.

This kind of oxygenation reaction can be distinguished from that of the Fe and Cu enzymes described previously, because with Mo enzymes the oxido group that is transferred is not derived from molecular $O_2$ but from water. The Mo(VI)=O unit can transfer an O atom, either directly (inner sphere) or indirectly to reducing (oxophilic) substrates, such as $SO_3^{2-}$ or $AsO_3^{2-}$, but cannot oxygenate C–H bonds. Figure 27.45 shows the reaction enthalpies for O-atom transfer: we see that the highly oxidizing Fe species formed by reaction with $O_2$ are able to oxygenate all substrates, whereas Mo(VI) oxo species are limited to more reducing substrates and Mo(IV) is able to extract an O atom from nitrate.

As expected from its position below Mo in Group 6, the lower oxidation states of W are less stable than those of Mo, so W(IV) species are usually potent reducing agents. This potency is illustrated by the W-containing formate dehydrogenases present in certain primitive organisms, which catalyse the reduction of $CO_2$ to formate, the first stage in non-photosynthetic carbon assimilation. This reaction does not involve O-atom insertion but rather the formation of a C–H bond. One mechanism that has been proposed is




Microbial oxidation of CO to $CO_2$ is important for removing more than 100 Mt of this toxic gas from the atmosphere every year. As shown in Box 27.2, this reaction is carried out by enzymes that contain either a Mo-pterin/Cu cofactor or an air-sensitive [Ni–4Fe–5S] cluster.

**BOX 27.2 Life on carbon monoxide**

Contrary to what is expected from its well-known toxicity, carbon monoxide is one of Nature's most essential small molecules. Even at atmospheric levels (0.05—0.35 ppm) CO is scavenged by a diverse range of microbial organisms for which it provides a source of carbon for growth and a 'fuel' for energy (CO is a stronger reducing agent than $H_2$). Two unusual enzymes, known as carbon monoxide dehydrogenases, catalyse the rapid oxidation of CO to $CO_2$. Aerobes use an enzyme containing an unusual Mo-pterin group (Fig. B27.3). A sulfido ligand on the Mo atom is shared with a Cu atom that is coordinated to one other ligand, a cysteine-S, completing the linear arrangement that is so common for Cu(I). A possible mechanism for $CO_2$ formation involves one of the oxo-groups of Mo(VI) attacking the C-atom of a CO that is coordinated to Cu(I). In contrast, certain anaerobes with the unique ability to live on CO as sole energy and carbon source use an enzyme that contains an unusual [Ni4Fe–5S] cluster. X-ray diffraction studies on crystals of the Ni enzyme incubated in the presence of $HCO_3^-$ at different potentials have revealed the structure of an intermediate showing $CO_2$ coordinated by Ni and Fe (Fig. B27.4). This intermediate supports a mechanism in which, during the conversion of CO to $CO_2$, CO binds to the Ni site (which is square-planar Ni(II)) and the C-atom is attacked by a $OH^-$ ion that was coordinated to the pendant Fe atom.

Carbon monoxide is carried in mammals by haemoglobin, and it is estimated that about 0.6 per cent of the total haemoglobin of an average healthy human is in the carbonylated form. Free CO is produced by the action of haem oxygenase, a P450-type enzyme that catalyses the first step in haem degradation. In addition to releasing Fe and CO, the breakdown of haem produces biliverdin and bilirubin, the familiar green and yellow pigments responsible for the appearance of a bruise. Like NO, CO appears to be a cell signalling agent and, in low amounts, it has important therapeutic effects, including suppression of hypertension (high blood pressure) and protection against tissue rejection following an organ transplant. There is therefore considerable interest in developing pharmaceutical agents, such as the water-soluble complex $[Ru(CO)_3Cl(glycinate)]$, to release CO slowly and supplement the action of haem oxygenase.

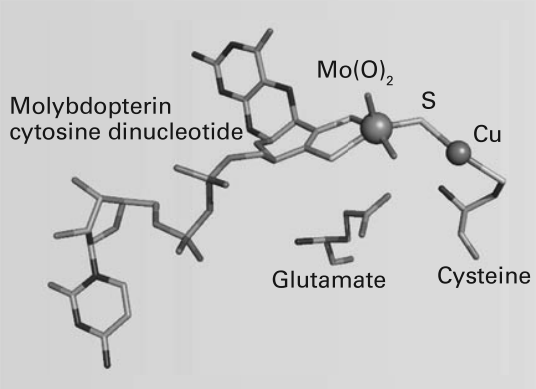



**Figure B27.3** The Mo-pterin cofactor of CO-dehydrogenases found in aerobes.

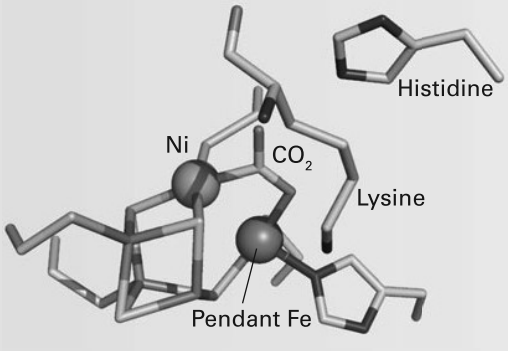



**Figure B27.4** X-ray crystallographic observation of an intermediate in the interconversion between CO and $CO_2$, catalysed by the [Ni4Fe—5S] cluster in CO-dehydrogenases from anaerobes.

## Biological cycles

Nature is extraordinarily economical and maximizes its use of elements that have been taken up from the non-biological, geological world, often with great difficulty. We have already seen how iron is assimilated by using special ligands, and how this hard-earned resource is stored in ferritin. Thus useful species are recycled rather than returned to the environment. An important example is nitrogen, which is so hard to assimilate from its unreactive gaseous source, $N_2$, and the elusive gas $H_2$, rapid cycling of which is achieved by microbes in processes analogous to electrolytic and fuel cells.

### 27.13 The nitrogen cycle

**Key points:** The nitrogen cycle involves enzymes containing Fe, Cu, and Mo, often in cofactors having very unusual structures; nitrogenase contains three different kinds of FeS cluster, one of which also contains Mo and a small interstitial atom.

The global biological nitrogen cycle involves organisms of all types and a diverse variety of metalloenzymes (Fig. 27.46 and Box 15.2). The cycle can be divided into uptake of usable nitrogen (assimilation) from nitrate or $N_2$ and denitrification (dissimilation). The nitrogen cycle involves many different organisms and a variety of metal-containing enzymes.

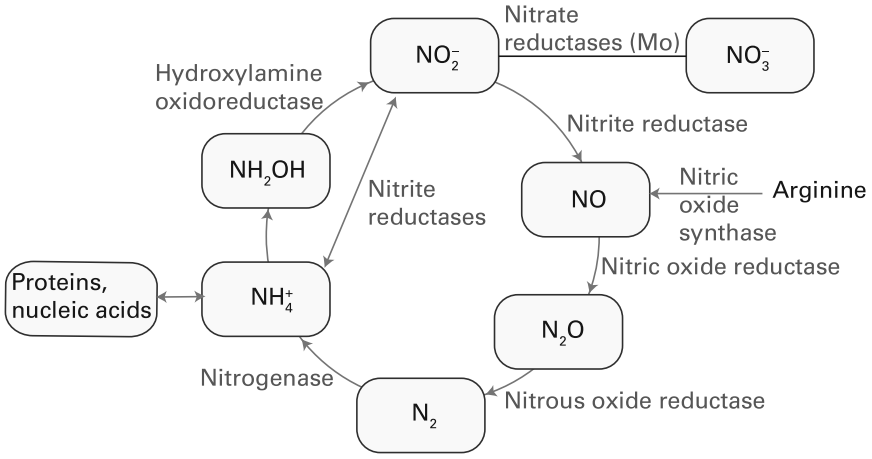
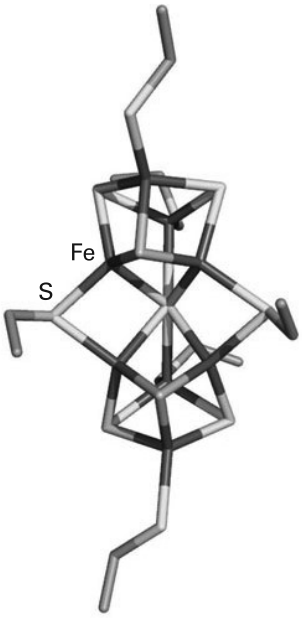
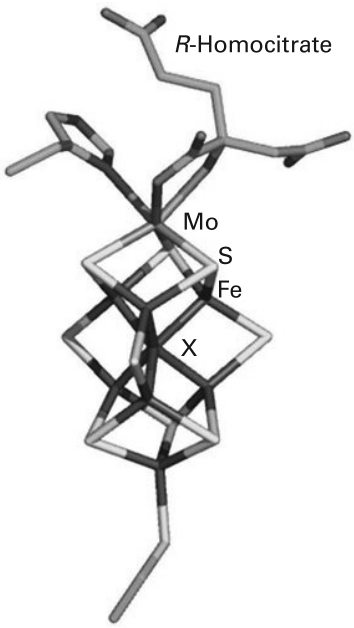



**Figure 27.46** The biological nitrogen cycle.




**49** Nitrogenase P-cluster [8Fe-7S]




**50** Nitrogenase FeMoco [Mo7Fe–8S,X]

Many of the compounds are toxic or environmentally challenging. Ammonia is a crucial compound for the biosynthesis of amino acids and $NO_3^-$ is used as an oxidant. Molecules such as NO are produced in small amounts to serve as cell signalling agents that play a crucial in physiology and health. Nitrous oxide, which is isoelectronic with $CO_2$, is a potential greenhouse gas: its release to the atmosphere depends on the balance between activities and abundancies of NO reductase and $N_2O$ reductase across the biological world.

The so-called 'nitrogen-fixing' bacteria found in soil and root nodules of certain plants contain an enzyme called nitrogenase that catalyses the reduction of $N_2$ to ammonia in a reaction that is coupled to the hydrolysis of 16 molecules of ATP and the production of $H_2$:

$$N_2 + 8H^+ + 8e^- + 16\,ATP \rightarrow 2NH_3 + H_2 + 16\,ADP + 16PO_3(OH)^{2-}$$

'Fixed' nitrogen is essential for the synthesis of amino acids and nucleic acids, so it is central to agricultural production. Industrial production of ammonia by the Haber process (Section 26.12) involves reaction of $N_2$ and $H_2$ at high pressures and high temperatures; by contrast, nitrogenase produces $NH_3$ under normal conditions, and it is small wonder that it has attracted so much attention. Indeed, the mechanism of activation of the $N_2$ molecule by nitrogenase has inspired coordination chemists for several decades. The process is very costly in terms of energy for a reaction that is not very unfavourable thermodynamically. However, as we saw in Section 15.6, $N_2$ is an unreactive molecule and energy is required to overcome the high activation barrier for its reduction.

Nitrogenase is a complex enzyme that consists of two types of protein: the larger of the two is called the 'MoFe-protein' and the smaller is the 'Fe-protein' (Fig. 27.47). The Fe-protein contains a single [4Fe–4S] cluster that is coordinated by two cysteine residues from each of its two subunits. The role of the Fe-protein is to transfer electrons to the MoFe-protein in a reaction that is far from understood: in particular, it is unclear why each electron transfer is accompanied by the hydrolysis of two ATP molecules, which are bound to the Fe-protein.

The MoFe protein is $\alpha_2\beta_2$, each $\alpha\beta$ pair of which contains two types of supercluster. The [8Fe−7S] cluster (**49**) is known as the 'P-cluster' and is thought to be an electron transfer centre, whereas the other cluster (**50**), formulated as [Mo7Fe−8S,X] and known as 'FeMoco' (FeMo cofactor), is thought to be the site at which $N_2$ is reduced to $NH_3$. The Mo is coordinated also by an imidazole-N from histidine and two O atoms from an exogenous molecule *R*-homocitrate. The mechanism of $N_2$ reduction is still unresolved, the main question being whether $N_2$ is bound and reduced at the Mo atom or at some other site. Here it is significant that nitrogenases are known in which the Mo atom is replaced by a V or Fe atom, arguing against a specific role for Mo. The cage-like cluster provided by the six central Fe atoms also coordinates a small central atom ('X'),

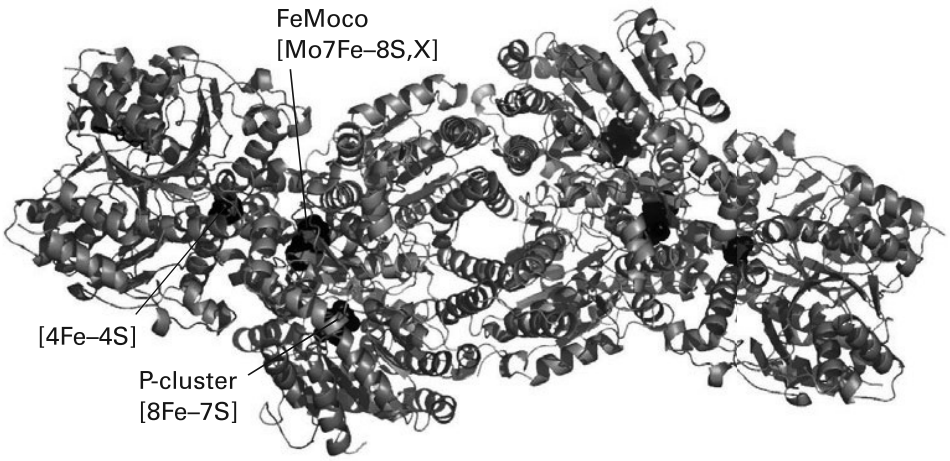


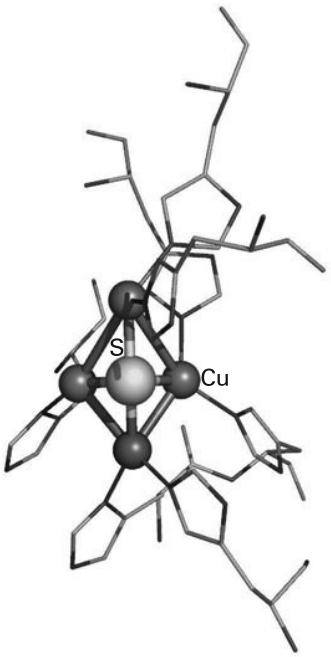

**Figure 27.47** The structure of nitrogenase showing the Fe protein and the MoFe protein complexed with each other. Positions of the metal centres (black) are indicated. The MoFe protein is an $\alpha_2\beta_2$ of different subunits (red and blue) and it contains two P-clusters and two MoFe cofactors. The Fe protein (green) has a [4Fe—4S] cluster and it is also the site of binding and hydrolysis of Mg-ATP.

which is proposed to be C, N, or O. It was nearly ten years after the determination of the structure of the MoFeS cage that this small central atom was discovered, after improvements in resolution and detailed consideration of the X-ray interference characteristics. The six Fe atoms would otherwise be three-coordinate with a flattened trigonal-pyramidal geometry.

Nitrate reductase is another example of a Mo enzyme involved in the transfer of an O atom, in this case catalysing a reduction reaction (the standard potential for the $NO_3^-/NO_2^-$ couple corrected to pH = 7 is +0.4 V, thus $NO_3^-$ is quite strongly oxidizing). The other enzymes in the nitrogen cycle contain either haem or Cu as their active sites. There are two distinct classes of nitrite reductase. One is a multi-haem enzyme that can reduce nitrite all the way to $NH_3$. The other class contains Cu and carries out one-electron transfer, producing NO: it is a trimer of identical subunits, each of which contains one 'blue' Cu (mediating long-range electron transfer to the electron donor, usually a small 'blue' Cu protein) and a Cu centre with more conventional tetragonal geometry that is thought to be the site of nitrite binding.

The nitrogen cycle is notable for using some of the most unusual redox centres yet encountered as well as some of the strangest reactions. Another unusual cofactor is a [4Cu—S] cluster, named $Cu_Z$, that is found in $N_2O$ reductase and has the structure shown in (**51**). It is puzzling how this centre is able to bind and activate $N_2O$, which is a poor ligand. Long-range electron transfer in $N_2O$ reductase is carried out by a $Cu_A$ centre, the same as found for cytochrome *c* oxidase.

Of particular importance for humans are two enzymes that manipulate NO. One of these, NO synthase, is a haem enzyme responsible for producing NO, by oxidation of L-arginine, on receipt of a signal. Its activity is controlled by calmodulin (Section 27.4). The other enzyme is guanylyl cyclase, which catalyses the formation of the important regulator cyclic guanosine monophosphate (cGMP) from guanosine triphosphate. Nitric oxide binds to the haem Fe of guanylyl cyclase, displacing a histidine ligand and activating the enzyme. Another interesting NO-binding protein is nitrophorin, which is found in some bloodsucking parasites, notably 'kissing bugs' (predacious bugs of the family *Reduviidae*). Nitrophorin binds NO tightly until it is injected into a victim, where a change in pH causes its release. The free NO causes dilation of the surrounding blood vessels, rendering the victim a more effective blood donor.




**51** $Cu_Z$ cluster [4Cu–S]

**EXAMPLE 27.9** Identifying intermediates formed during the reduction of $N_2$ to $NH_3$

Suggest likely intermediates formed during the six-electron reduction of an $N_2$ molecule to $NH_3$.

***Answer*** To identify possible intermediates we need to recall from Chapter 5 that p-block elements normally undergo two-electron transfers that are accompanied by proton transfers. Because $N_2$ has a triple bond, the

two N atoms will remain bonded together throughout most of the six-electron reduction. We would propose diazene ($N_2H_2$) and hydrazine ($N_2H_4$) along with their deprotonated conjugate bases.

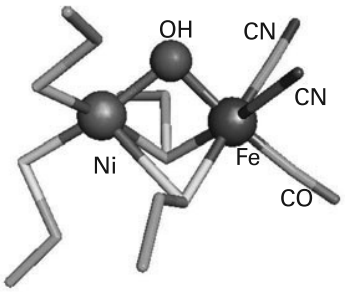
***Self-test 27.9*** The MoFe cofactor can be extracted from nitrogenase by using dimethylformamide (DMF), although it is catalytically inactive in this state. Suggest experiments that could establish if the structure of the species in DMF solution is the same as present in the enzyme.

## 27.14 The hydrogen cycle

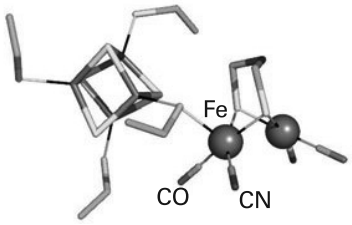
**Key point:** The active sites of hydrogenases contain Fe or Ni, along with CO and CN ligands.

It has been estimated that 99 per cent of all organisms utilize $H_2$. Even if these species are almost entirely microbes, the fact remains that almost all bacteria and archaea possess extremely active metalloenzymes, known as hydrogenases, that catalyse the interconversion of $H_2$ and $H^+$ (as water). The elusive molecule $H_2$ is produced by some organisms (it is a waste product) and used by others as a fuel, helping to explain why so little $H_2$ is in fact detected in the atmosphere (Box 10.1). Human breath contains measurable amounts of $H_2$ due to the action of bacteria in the gut. Hydrogenases are very active enzymes, with turnover frequencies (molecules of substrate transformed per second per molecule of enzyme) exceeding 10 000 $s^{-1}$. They are therefore attracting much attention for the insight they can provide regarding clean production of $H_2$ (Section 10.4, Box 10.3) and oxidation of $H_2$ in fuel cells—technology that currently depends greatly on Pt (Box 5.1).

There are three classes of hydrogenase, based on the structure of the active site. All contain Fe and some also contain Ni. The two best-characterized types are known as [NiFe]-hydrogenases and [FeFe]-hydrogenases, and structures of the active sites of two representative enzymes are shown as (**52**) and (**53**). The active sites contain at least one CO ligand, and provide further examples of biological organometallic active sites (in addition to coenzyme $B_{12}$). Further ligation is provided by $CN^-$, cysteine (and sometimes selenocysteine), and the active site of [FeFe]-hydrogenases (the site is commonly referred to as an 'H-cluster') contains a [4Fe-4S] cluster linked by a bridging cysteine thiolate, and an unusual bridging bidentate ligand that is thought to be either dithiomethylamine or dithiomethylether. These fragile active sites are buried deeply within the enzyme, thus necessitating special pores and pathways to convey $H_2$ and $H^+$, and a relay of FeS clusters for long-range electron transfer (as shown in Fig. 27.26).

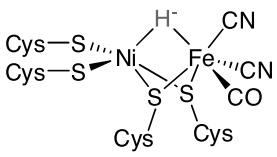



**52** [NiFe]-hydrogenase




**53** [FeFe]-hydrogenase

The [FeFe]-enzymes tend to operate in the direction of $H_2$ production; they are usually found in strictly anaerobic organisms and are very sensitive to $O_2$. The mechanism of catalysis is uncertain, but it involves the participation of unusual Fe(I) species: the form assigned as Fe(I)Fe(I) binds $H^+$ and that assigned as Fe(II)Fe(I) binds $H_2$, probably in an analogous manner to the dihydrogen complex shown (**11**) in Section 10.6. The [NiFe]-enzymes are noted for $H_2$ oxidation and a catalytically active form assigned by EPR spectroscopy as Ni(III)–$H^-$ (a hydrido complex) has been identified (**54**). Single-crystal EPR studies show that the unpaired electron is coupled strongly to an H-atom nucleus lying along an axis that points towards the Fe (that is, the H is in a bridging position). The active sites of [NiFe]- and [FeFe]-hydrogenases react with $O_2$, often irreversibly, and this sensitivity is a limiting factor in developing and exploiting renewable $H_2$ production by microorganisms (Section 10.4 and Box 10.1).




**54**

## Sensors

A number of metalloproteins are used to detect and quantify the presence of small molecules, particularly $O_2$, NO, and CO. These proteins therefore act as sensors, alerting an organism to an excess or deficit of particular species, and triggering some kind of remedial action. Special proteins are also used to sense the levels of metals such as Cu and Zn that are otherwise always strongly complexed in a cell.

## 27.15 Iron proteins as sensors

**Key point:** Organisms use sophisticated regulatory systems based on Fe-containing proteins to adapt quickly to changes in cellular concentrations of Fe and $O_2$.

We have already seen how FeS clusters are used in electron transfer and catalysis (Sections 27.8 and 27.9). The coordination of an FeS cluster ties together different parts of a protein and thus controls its tertiary structure. The sensitivity of the cluster to oxygen, electrochemical potential, or Fe and S concentrations makes it able to be an important sensory device. In the presence of $O_2$ or other potent oxidizing agents, [4Fe−4S] clusters have a tendency (controlled by the protein) to degrade, producing [3Fe−4S] and [2Fe−2S] species. The cluster may be removed completely under some conditions (Fig. 27.48). The principle behind an organism's exploitation of FeS clusters as sensors is that the presence or absence of a particular cluster (the structure of which is very sensitive to Fe or oxygen) alters the conformation of the protein and determines its ability to bind to nucleic acids.

In higher organisms, the protein responsible for regulating Fe uptake (transferrin) and storage (ferritin) is an FeS protein known as the **iron regulatory protein** (IRP), which is closely related to aconitase (Section 27.9) but is found in the cytoplasm rather than in mitochondria. It acts by binding to specific regions of messenger RNA (mRNA) that carry the genetic command (transcribed from DNA) to synthesize transferrin receptor or ferritin. A specific interaction region on the RNA is known as the **iron-responsive element** (IRE). The principle is outlined in Fig. 27.49. When Fe levels are high, a [4Fe−4S] cluster is present, and the protein does not bind to the IRE that controls translation of ferritin. In this case binding would be a 'stop' command, and the cell will respond by synthesizing ferritin. Simultaneously, binding of the [4Fe−4S]-loaded protein to the transferrin receptor IRE destabilizes the RNA, so transferrin receptor is not made. When Fe levels are high, the opposite actions occur: a [4Fe−4S] cluster is formed, ferritin synthesis is activated, and transferrin receptor synthesis is switched off (repressed).

The common gut bacterium *E. coli* derives energy either by aerobic respiration (using a terminal oxidase related to cytochrome *c* oxidase, Section 27.10) or by anaerobic respiration with an oxidant such as fumaric acid, or nitrate, using the Mo enzyme nitrate

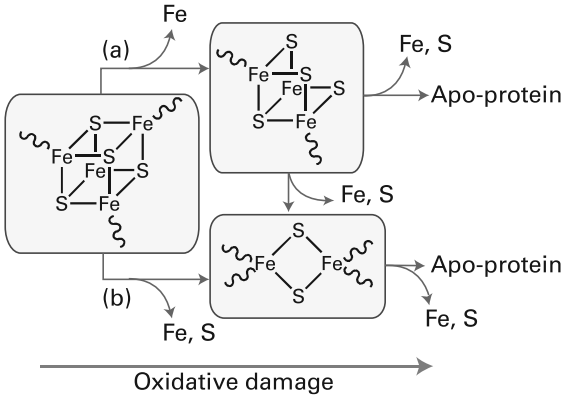



**Figure 27.48** The degradation of Fe−S clusters forms the basis for a sensory system The [4Fe−4S] cluster cannot support a state in which all Fe are Fe(III); thus severe oxidizing conditions, including exposure to $O_2$, causes their breakdown to [3Fe−4S] or [2Fe−2S] and eventually complete destruction. Degradation to [3Fe−4S] (a) requires only removal of an Fe subsite whereas degradation to [2Fe−2S] (b) may require rearrangement of the ligands (cysteine) and produce a significant protein conformational change. These processes link cluster status to availability of Fe as well as $O_2$ and other oxidants and provide the basis for sensors and feedback control.

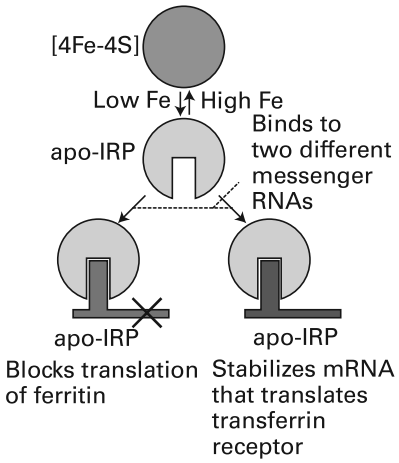



**Figure 27.49** Interactions of iron-regulatory protein with iron-responsive elements on the RNAs responsible for synthesizing ferritin or transferrin receptor depend on whether an Fe−S cluster is present and form the basis for regulation of cellular Fe levels.

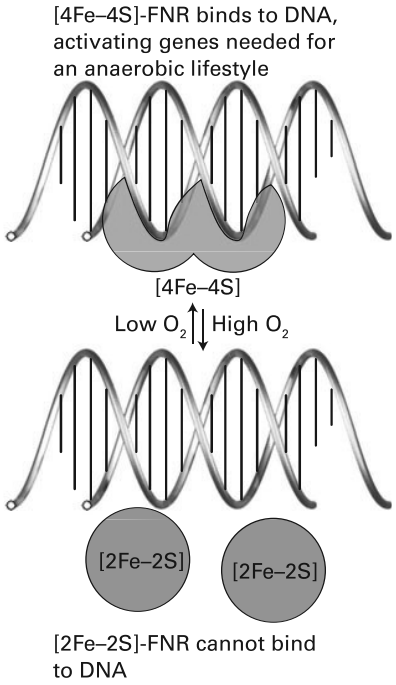



**Figure 27.50** The principle of operation of the fumarate nitrate regulatory system that controls aerobic versus anaerobic respiration in bacteria.

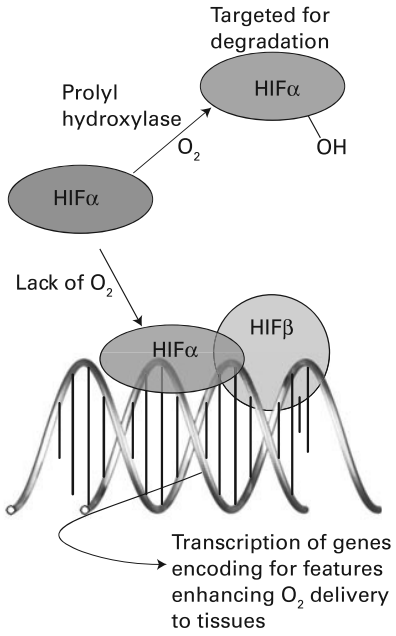



**Figure 27.51** The principle of $O_2$ sensing by prolyl oxygenases.

reductase (Sections 27.12 and 27.13). The problem the organism faces is how to sense whether $O_2$ is present at a sufficiently low level to warrant inactivating the genes functioning in aerobic respiration and activate instead the genes producing enzymes necessary for the less efficient anaerobic respiration. This detection is achieved by an FeS protein called fumarate nitrate regulator (FNR). The principle is outlined in Fig. 27.50. In the absence of $O_2$, FNR is a dimeric protein with one [4Fe–4S] cluster per subunit. In this form it binds to specific regions of DNA repressing transcription of the aerobic enzymes and activating transcription of enzymes such as nitrate reductase. When $O_2$ is present, the [4Fe−4S] cluster is degraded to a [2Fe−2S] cluster and the dimer breaks up so that it cannot bind to DNA. The genes encoding aerobic respiratory enzymes are thus able to be transcribed, whereas those for anaerobic respiration are repressed.

In higher animals, the system that regulates the ability of cells to cope with $O_2$ shortage involves an Fe oxygenase. Prolyl hydroxylases catalyse the hydroxylation of specific proline residues in proteins, thus altering their properties. In higher animals, one such target protein is a transcription factor called hypoxia inducible factor (HIF), which mediates the expression of genes responsible for adapting cells to low-$O_2$ conditions (hypoxia). We should bear in mind here that the internal environment of cells and cell compartments is usually quite reducing, equivalent to an electrode potential below –0.2 V, and even though we regard $O_2$ as essential for higher organisms, its actual levels may be fairly low. When $O_2$ levels are above a safe threshold, prolyl hydroxylases catalyse oxygenation of two conserved proline residues of HIF, causing the transcription factor to be recognized by a protein that induces its degradation by proteases. Hence genes such as those ultimately responsible for producing more red blood cells (which will help an individual to cope better when $O_2$ supply is a problem) are not activated. The principle is outlined in Fig. 27.51.

Although essential for higher organisms, $O_2$ requires stringent control of its four-electron reduction, and increasing amounts of research are being carried out to prevent and cure malfunctions of normal $O_2$ consumption. The term 'oxidative stress' is used to describe conditions in which the normal function of an organism is threatened by a build up of partially reduced $O_2$ intermediates, such as superoxides, peroxides, and hydroxyl radicals known collectively as **reactive oxygen species** (ROS). Prolonged exposure to ROS is associated with premature aging and certain cancers. To avoid or minimize oxidative stress cells must first sense ROS and then produce agents to destroy them. Both sensory and attack agents are proteins with active groups such as metal ions (particularly Fe, Cu) and exposed, redox-active cysteine thiols.

An underlying principle of haem sensors is that the small molecules being sensed are π acceptors that can bind strongly to the Fe and displace an indigenous ligand. This binding results in a change in conformation that alters catalytic activity or the ability of the protein to bind to DNA. Of the indigenous ligands, two classic examples are NO and CO; although we have long thought of these molecules as toxic to higher life forms, they are becoming well established as hormones. Indeed, there is evidence that the sensing of trace levels of CO is important for controlling circadian rhythms in mammals.

The enzyme guanyl cyclase senses NO, a molecule that is now well established as a hormone that delivers messages between cells. Guanyl cyclase catalyses the conversion of guanidine monophosphate (GMP) to cyclic GMP (cGMP), which is important for activating many cellular processes. The catalytic activity of guanyl cyclase increases greatly (by a factor of 200) when NO binds to the haem, but (as is obviously important) by a factor of only 4 when CO is bound.

An excellent, atomically defined example of CO sensing is provided by a haem-containing a transcription factor known as CooA. This protein is found in some bacteria that are able to grow on CO as their sole energy source under anaerobic conditions. Whether growth on CO takes place depends on the ambient CO level, as an organism will not waste its resources synthesizing the necessary enzymes when the essential substrate is not present. CooA is a dimer, each subunit of which contains a single *b*-type cytochrome (the sensor) and a 'helix-turn-helix' protein fold that binds to DNA (Fig. 27.52). In the absence of CO, each Fe(II) is six-coordinate and both its axial ligands are amino acids of the protein, a histidine imidazole and, unusually, the main-chain $-NH_2$ group of a proline that is also the N-terminal residue of the other subunit. In this form, CooA cannot bind to the specific DNA sequence to transcribe the genes for synthesizing the CO-oxidizing enzymes necessary for existing on CO. When CO is present it binds to the Fe, displacing the distal

proline residue and causing CooA to adopt a conformation that will bind to the DNA. The likelihood of NO binding in place of CO to cause a false transcriptional response is prevented because NO not only displaces proline but also results in dissociation of the proximal histidine; the NO complex is thus not recognized.

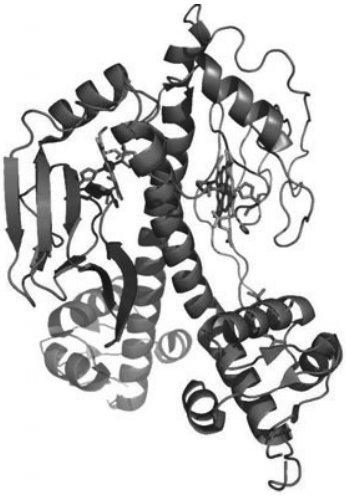


**Figure 27.52** The structure of CooA, a bacterial CO sensor and transcription factor. The molecule, a dimer of two identical subunits (represented as red and blue), has two haem-binding domains and two 'helix–turn–helix' domains that recognize a section of DNA. The protein ligands to the Fe atoms are a histidine from one subunit and an N-terminal proline from the other. The binding of CO and displacement of proline disrupts the assembly and allows CooA to bind to DNA.

### 27.16 Proteins that sense Cu and Zn levels

**Key point:** Cu and Zn are sensed by proteins with binding sites specially tailored to meet the specific coordination preferences of each metal atom.

The levels of Cu in cells are so strictly controlled that almost no uncomplexed Cu is present. An imbalance in Cu levels is associated with serious health problems such as Menkes disease (Cu deficiency) and Wilson's disease (Cu accumulation). Most of what we know about how Cu levels are sensed and converted to cell signals stems from studies on the *E. coli* system, which involves a transcription factor called CueR (Fig. 27.53). This protein binds Cu(I) with high selectivity, although it also binds Ag(I) and Au(I). Metal coordination causes a conformational change that enables CueR to bind to DNA at a receptor site that controls transcription of an enzyme known as CopA, which is an ATP-driven Cu pump. CopA is located in the cytoplasmic membrane and exports Cu into the periplasm. In CueR, the Cu(I) is coordinated by two cysteine-S atoms arranged in a linear coordination geometry. Titrations using $CN^-$ as a buffer show that $Cu^+$ is bound with a dissociation constant of approximately $10^{-21}$. As may be understood by reference to Section 7.3, this ligand environment leads to remarkably selective binding for $d^{10}$ ions, and measurements with Ag and Au show that these ions are taken up with similar affinities.

Most of our current insight about Zn sensing, as for Cu, is provided by studies of bacterial systems. The major difference with respect to Cu is that although Zn is also coordinated (mainly) by cysteine thiolates, the geometry is tetrahedral rather than linear. *E. coli* contains a $Zn^{2+}$-sensing transcription factor known as ZntR that is closely related to CueR. The factor ZntR contains two Zn-binding domains each of which coordinates a pair of Zns using cysteine and histidine ligands. The surrounding protein fold is shown in Fig. 27.53, for comparison with CueR. The extent to which these dynamic Zn-binding sites can be identified with zinc fingers remains unclear.

**EXAMPLE 27.10 Identifying links between redox chemistry and metal ion sensing**

Suggest a way in which the binding of Cu or Zn to their respective sensor proteins might be linked to the level of cellular $O_2$.

***Answer*** To address this problem we recall from Chapter 16 that strong S—S bonds arise from the combination of S atoms or radicals. A pair of cysteines that are coordinating a metal ion or are able to approach each other at close range can undergo oxidation by $O_2$ or other oxidants, resulting in the formation of a disulfide bond (cystine). This reaction prevents the cysteine S atoms from acting as ligands and provides a way in which even redox inactive metals such as Zn may be involved in sensing $O_2$.

***Self-test 27.10*** Why might Cu sensors function to bind Cu(I) rather than Cu(II)?

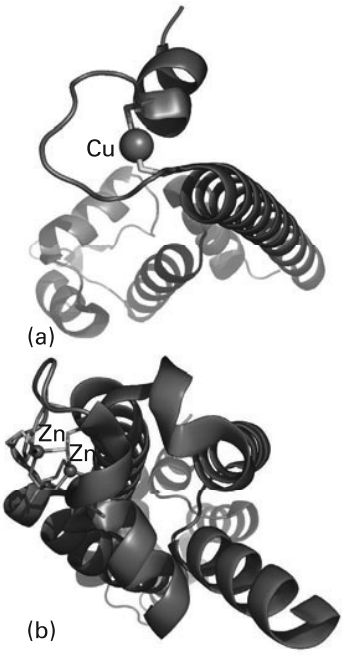



**Figure 27.53** Comparison of (a) the Cu- and (b) the Zn-binding sites in the respective transcription factors CueR and ZntR. Note how Cu(I) is recognized by a linear binding site (to two cysteines) whereas Zn is recognized by an arrangement of Cys and His ligands that binds two Zn(II) atoms together with a bridging phosphate group.

## Biomineralization

**Key points:** Calcium compounds are used in exoskeletons, bones, teeth, and other devices; some organisms use crystals of magnetite, $Fe_3O_4$, as a compass; plants produce silica-based protective devices.

Biominerals can be either infinite covalent networks or ionic. The former include the silicates, which occur extensively in the plant world. Leaves, even whole plants, are often covered with silica hairs or spines that offer protection against predatory herbivores. Ionic biominerals are mainly based on calcium salts, and exploit the high lattice energy and low solubilities of these compounds. Calcium carbonate (calcite or aragonite, Section 12.10) is the material present in sea shells and eggshells. These minerals persist long after the organism has died, indeed chalk is a biogenic mineral, a result of the process of

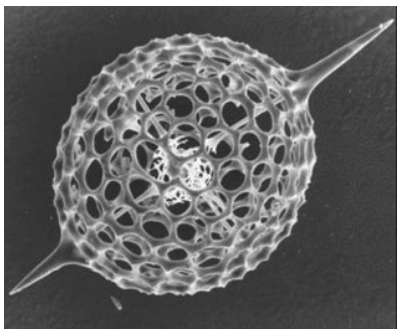


**Figure 27.54** The porous silica structure of a radiolarian microskeleton showing the large radial spines. (Photograph supplied by Professor S. Mann, University of Bristol.)

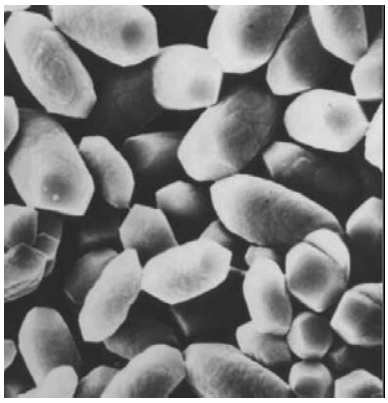


**Figure 27.55** Our gravity sensor. Crystals of biologically formed calcite that are found in the inner ear. (Photograph supplied by Professor S. Mann, University of Bristol.)

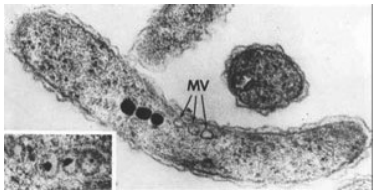



**Figure 27.56** Magnetite crystals in magnetotactic bacteria. These are tiny compasses that guide these organisms to move vertically in river bed sludge. MV indicates empty vesicles (Photograph supplied by Professor S. Mann, University of Bristol.)

**calciferation** of prehistoric organisms. Calcium phosphate (hydroxyapatite, Section 15.4) is the mineral component of bones and teeth, which are particularly good examples of how organisms fabricate 'living' composite materials. Indeed, the different properties of bone found among species (such as stiffness) are produced by varying the amount of organic component, mostly the fibrous protein collagen, with which hydroxyapatite is associated. High hydroxyapatite/collagen ratios are found for large marine animals, whereas low ratios are found for animals requiring agility and elasticity.

**Biomineralization** is crystallization under biological control. There are some striking examples that have no counterparts in the laboratory. Perhaps the most familiar example is the exoskeleton of the sea urchin, which comprises large sponge-like plates containing continuous macropores 15 μm in diameter: each of these plates is a single crystal of Mg-rich calcite. Large single crystals support other organisms, such as diatoms and radiolarians, with their skeletons of silica cages (Fig. 27.54).

Biominerals produce some intriguing gadgetry. Figure 27.55 shows crystals of calcite that are part of the gravity sensor device in the inner ear. These crystals are located on a membrane above sensory cells and any acceleration or change of posture that causes them to move results in an electrical signal to the brain. The crystals are uniform in size and spindle-shaped so that they move evenly without becoming hooked together. The same property is seen for crystals of magnetite ($Fe_3O_4$) that are found in a variety of magnetotactic bacteria (Fig. 27.56). There are considerable variations in size and shape across different species, but within any one species, the crystals, formed in magnetosome vesicles, are uniform. Magnetotactic bacteria live in fluid sediment suspensions in marine and freshwater environments, and it is thought that their microcompasses allow them to swim always in a downwards direction to maintain their chemical environment during turbulent conditions.

There is great interest in how biomaterials are formed, not least because of the inspiration they provide for nanotechnology (Chapter 25). The formation of biominerals involves the following hierarchy of control mechanisms:

1. Chemical control (solubility, supersaturation, nucleation).
2. Spatial control (confinement of crystal growth by boundaries such as cells, subcompartments, and even proteins in the case of ferritin, Section 27. 6).
3. Structural control (nucleation is favoured on a specific crystal face).
4. Morphological control (growth of the crystal is limited by boundaries imposed by organic material that grows with time).
5. Constructional control (interweaving inorganic and organic materials to form a higher-order structure, such as bone).

Bone is continually being dissolved and reformed; indeed it functions not only as a structural support but also as the central Ca store. Thus, during pregnancy, bones tend to be raided for their Ca in a process called **demineralization**, which occurs in special cells called *osteoclasts*. Depleted or damaged bones are restored by mineralization, which occurs in cells called *osteoblasts*. These processes involve phosphatases (Section 27.9).

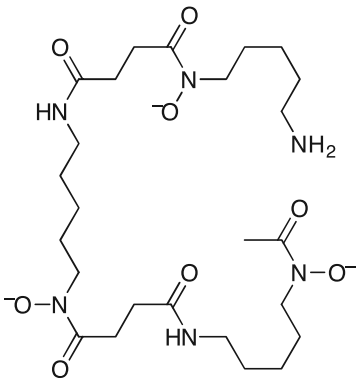
## The chemistry of elements in medicine

Serendipity has played an important role in drug discovery, with many effective treatments arising from chance discoveries. There appears to be a special role for compounds containing metals that are not otherwise present in biological systems, for example Pt, Au, Ru, and Bi. A major challenge in pharmacology is to determine the mechanism of action at the molecular level, bearing in mind that the drug that is administered is unlikely to be the molecule that reacts at the target site. This is particularly true for metal complexes, which are usually more susceptible to hydrolysis than organic molecules. In general, the mechanism of action is proposed by extrapolation of *in vitro* studies. Orally administered drugs are highly desirable because they avoid the trauma and potential hazards of injection; however, they may not pass through the gut wall or survive hydrolytic enzyme action. Inorganic compounds are also used in the diagnosis of disease or damage, a particularly interesting example being the use of radioactive technetium.

## 27.17 Chelation therapy

**Key points:** The treatment of Fe overload involves sequestration of Fe by ligands based on or inspired by siderophores.

'Iron overload' is the name given to several serious conditions that affect a large proportion of the world's population. Here we recall that, despite its great importance, Fe is potentially a highly toxic element, particularly in its ability to produce harmful radicals by reaction with $O_2$, and its levels are normally strictly controlled by regulatory systems. In many groups of people, a genetic disorder results in breakdown of this regulation. One kind of iron overload is caused by an inability for the body to produce sufficient porphyrin. Other problems are caused by faults in the regulation of Fe levels by ferritin or transferrin production. These disorders are treated by **chelation therapy**, the administration of a ligand to sequester Fe and allow it to be excreted. Desferrioxamine ('Desferral', **55**) is a ligand that is similar to the siderophores described in Section 27.6. It is a very successful agent for iron overload, apart from the trauma of its introduction into the body, which involves it being plumbed into an intravenous supply.



**55** Desferral

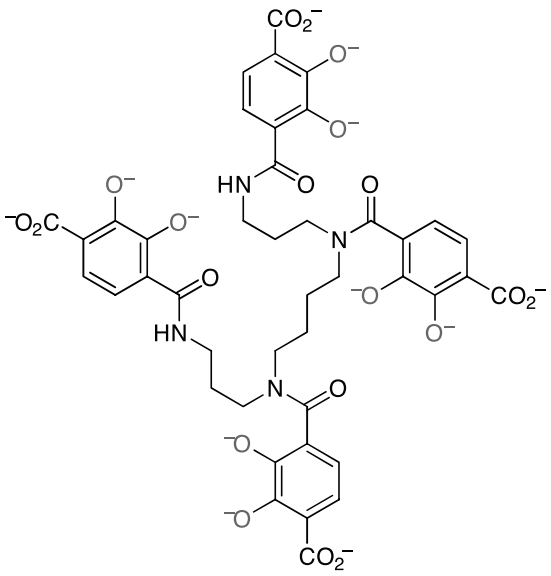
A special case of chelation therapy is the treatment of individuals who have been contaminated with Pu following exposure to nuclear weapons. In its common oxidation states, Pu(IV) and Pu(III) have similar charge densities to Fe(III) and Fe(II). Siderophore-like chelating ligands have been developed, such as 3,4,3-LIMACC (**56**), which contains four catechol groups.

## 27.18 Cancer treatment

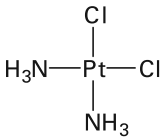
**Key points:** The complex *cis*-$[PtCl_2(NH_3)_2]$ results in the inhibition of DNA replication and prevention of cell division; other drugs cause DNA to be degraded by oxygenation.

'Cancer' is a term that covers a large number of different types of the disease, all characterized by the uncontrolled replication of transformed cells that overwhelm the normal operation of the body. The principle of treatment is to apply drugs that destroy these malignant cells selectively.

The remarkable action of the complex *cis*-$[PtCl_2(NH_3)_2]$ (**57**, known as cisplatin) was discovered in 1964 while examining the effect of an electric field on the growth of bacteria. The behaviour of a colony of bacteria suspended in solution between two platinum electrodes was observed, and it was noted that the cells continued to grow in size, forming long filaments, but stopped replicating. The effect was traced to a complex that was



**56**



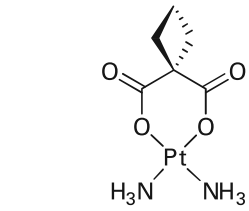
**57** Cisplatin

formed electrochemically by dissolution of Pt into the electrolyte, which contained $NH_4Cl$. Since then, cisplatin has been a successful drug for the treatment of many forms of cancer, particularly testicular cancer, for which the success rate approaches 100 per cent. The other geometric isomer, *trans*-$[PtCl_2(NH_3)_2]$, is inactive.

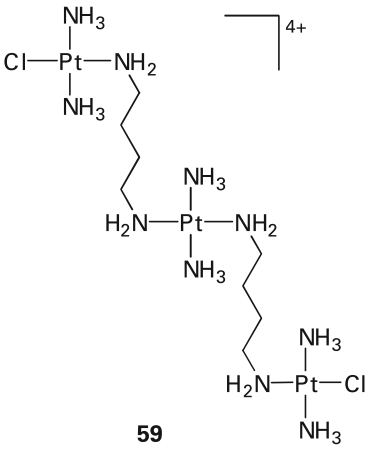
The ultimate molecular basis of the chemotherapeutic action of cisplatin and related drugs is thought to be the formation of a stable complex between Pt(II) and DNA. Cisplatin is administered into the bloodstream of the patient, where, because the plasma contains high concentrations of $Cl^-$, it tends to remain as the neutral dichlorido species. The electrical neutrality of the dichlorido complex facilitates its passage through the cell and nuclear membranes. Once it is subjected to the lower $Cl^-$ concentrations inside the cell (Table 27.1), the $Cl^-$ ligands are replaced by $H_2O$, and the resulting cationic species (with charges +1 or +2) are attracted electrostatically to DNA and form inner-sphere complexes in which the $-Pt(NH_3)_2$ fragment becomes coordinated to the N atoms of the nucleotide bases. Some classic studies have shown that the preferred target is a pair of N atoms on consecutive guanine bases in the same strand. Complexes of the $-Pt(NH_3)_2$ fragment with oligonucleotides have been studied by X-ray crystallography and $^{195}$Pt-NMR (Fig. 27.57). Complexation with Pt causes the helix to bend and partially unwind. It is thought that this distortion renders the DNA incapable of replication or repair. The distortion also makes the DNA recognizable by 'high mobility group' proteins that bind to bent DNA; the cell may thus be targeted for its own death.

Despite its efficacy, cisplatin has highly undesirable side effects, in particular it causes serious damage to the kidneys before it is eventually excreted. Great efforts have been made to find Pt complexes that are effective with fewer side effects. One example in clinical use is carboplatin (**58**). Effective drugs may also include trinuclear Pt(II) (**59**) as well as Pt(IV) complexes such as satraplatin (**60**), which can be administered orally.

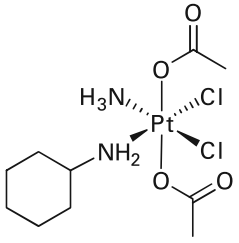
Other metal complexes are being discovered that bind by intercalation within the DNA interior and offer improved efficacy over Pt drugs. They include Ru(III) complexes such as *fac*-$[RuCl_3(NH_3)_3]$, which are believed to function by providing a source of Ru(III) that is carried to cancer cells by transferrin, and the complex (**61**), which may be activated by reduction to Ru(II) *in vivo*. There is increasing interest in organometallic compounds. The Ru(II) arene complex (**62**), which possesses high anti-cancer activity, is believed to coordinate to guanine-N in a similar way to Pt complexes but the interaction is supplemented by intercalation of the biphenyl group within the hydrophobic DNA core as well as hydrogen bonding between guanine and the $-NH_2$ groups of the en ligand. Even common Ti compounds such as $TiCp_2Cl_2$ have undergone clinical trials. Metallo-supramolecular 'cylinders' formed by placing a metal cation at either end of a bundle of ligands (**63**) have much larger dimensions that mimic those of Zn fingers. The cylinders bind in the major groove of DNA, causing it to form small coils.




**58** Carboplatin




**59**




**60**

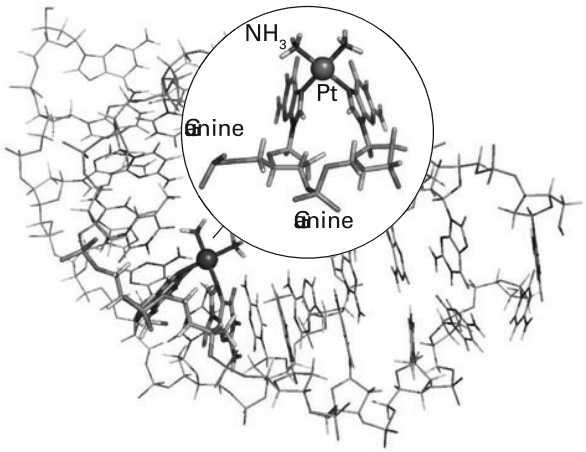
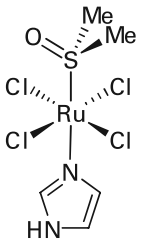
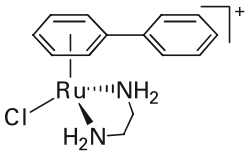


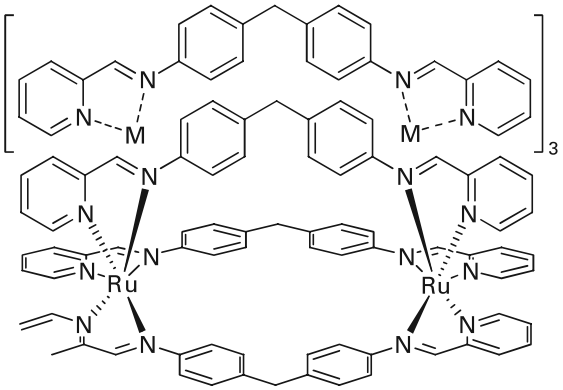

**Figure 27.57** Structure of an adduct formed between $-Pt(NH_3)_2$ and two adjacent guanine bases on an oligonucleotide. Expanded view shows the square-planar ligand arrangement around the Pt atom. Coordination of Pt causes bending of the DNA helix.



61

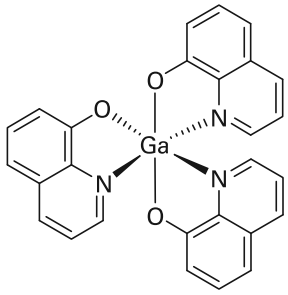


62



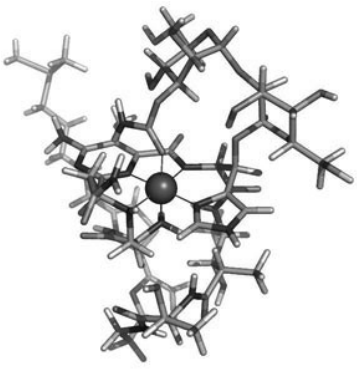
63

Compounds of Ga(III) are under investigation as anti-cancer drugs. Like Fe(III), Ga(III) is a hard Lewis acid and the two metal ions have similar radii, however Ga(III) is not easily reduced to Ga(II) and any redox or $O_2$ binding proteins that have incorporated Ga in place of Fe will be inactive. It is thought that Ga(III) enters cells using the same transport systems as Fe. The target for Ga is the Fe-containing enzyme ribonucleotide reductase, which is essential for producing the bases used in DNA. Compounds undergoing trials range from simple salts like gallium nitrate to complexes such as (**64**) that can pass through the intestinal wall.



64

The challenge with cancer chemotherapy is to identify complexes that select malignant cells and ignore healthy cells. Some Ru complexes undergo selective interaction with DNA, and are activated on irradiation, becoming potent oxidizing agents capable of carrying out cleavage of phosphodiester linkages. This method of treating cancers is known as **phototherapy**. Bleomycin (**65**) is representative of a class of drug that appears to function by binding to DNA and generating, on reaction with $O_2$, an Fe(IV) (ferryl) species that oxygenates particular sites and leads to degradation.



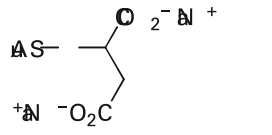
**65** Bleomycin

## 27.19 Anti-arthritis drugs

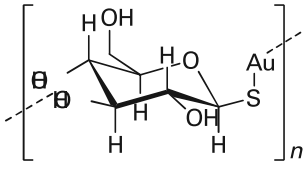
**Key point:** Complexes of Au are effective against rheumatoid arthritis.

Gold drugs are used in the treatment of rheumatoid arthritis, an inflammatory disease that affects the tissue around joints. The inflammation arises by the action of hydrolytic enzymes in cell compartments known as lysosomes that are associated with the Golgi apparatus (see Fig. 27.1). Although the mechanism of action is not established, it is known that Au accumulates in the lysosomes, and it is therefore possible that Au inhibits these hydrolytic enzymes. Another hypothesis is that Au(I) compounds deactivate singlet $O_2$, a harmful species that can be formed by oxidation of superoxide. The mechanism for this reaction might involve promotion of intersystem crossing by the high spin-orbit coupling constant of the heavy element.

Commonly administered drugs include sodium aurothiomalate ('myochrisin', **66**), sodium aurothioglucose ('solganol', **67**; the linkage between units is uncertain), and others, all of which feature Au(I) with linear coordination. Many are water-soluble polymers, but they cannot be administered orally because they undergo acid hydrolysis in the stomach. By contrast, the compound known as auranofin (**68**) can be given orally. Because Au(I) is chemically soft it is likely that it targets sulfur groups such as cysteine side chains in proteins. It is much more likely to survive in biological environments than Au(III), which is highly oxidizing. As expected, Au compounds lead to side effects, which include skin allergies as well as kidney and gastrointestinal problems.



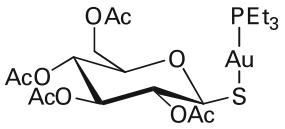
**66** Myochrisin



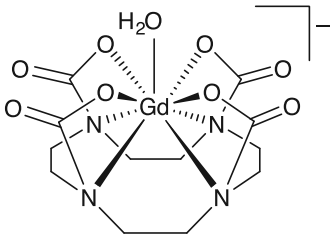
**67** Solganol

## 27.20 Imaging agents

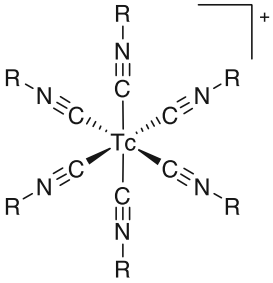
**Key point:** Particular organs and tissues are targeted according to the ligands that are present.



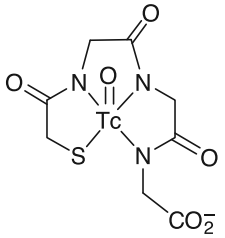
**68** Auranofin



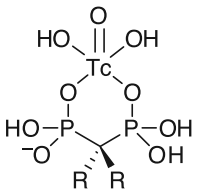
**69** Dotarem



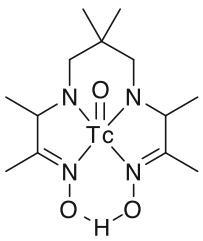
**70** Cardiolyte



**71** Tc-MAG-3



**72**



**73** Ceretec

Complexes of gadolinium(III) ($f^7$) are used in magnetic resonance imaging (MRI), which has become an important technique in medical diagnosis. Through their effect on the relaxation time of $^1$H-NMR spectroscopic resonances, Gd(III) complexes are able to enhance the contrasts between different tissues and highlight details such as the abnormalities of the blood–brain barrier. A number of Gd(III) complexes are approved for clinical use, each exhibiting different degrees of rejection or retention by certain tissues, as well as stability, rates of water exchange, and magnitude of relaxation parameters. All are based on chelating ligands, particularly those having multiple carboxylate groups. One example is the complex (**69**) formed with the macrocyclic aminocarboxylate ligand DOTA, which is known as dotarem.

Technetium is an artificial element that is produced by a nuclear reaction, but it has found an important use as an imaging agent. The active radionuclide is $^{99m}$Tc (m for metastable), which decays by γ-emission and has a half-life of 6 h. Production of $^{99m}$Tc involves bombarding $^{98}$Mo with neutrons and separating it as it is formed from the unstable product $^{99}$Mo:

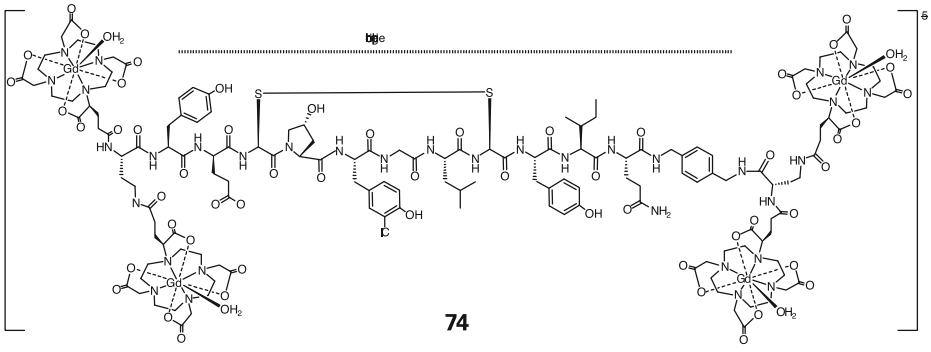
$$^{98}\text{Mo} \xrightarrow{\text{neutron capture}} {}^{99}\text{Mo} \xrightarrow{\beta\text{-decay, 90h}} {}^{99\text{m}}\text{Tc} \xrightarrow{\gamma\text{-emission, 6 h}}$$
$$^{99}\text{Tc} \xrightarrow[\beta\text{-decay, 200ka}]{} {}^{99}\text{Ru}$$

High-energy γ-rays are less harmful to tissue than α- or β-particles. The chemistry of technetium resembles manganese except that higher oxidation states are much less oxidizing.

A variety of substitution-inert Tc complexes can be made that, when injected into the patient, target particular tissues and report on their status. Complexes have been developed that target specific organs such as the heart (revealing tissue damage due to a heart attack), kidney (imaging renal function), or bone (revealing abnormalities and fracture lines). A good basis for organ targeting appears to be the charge on the complex: cationic complexes target the heart, neutral complexes target the brain, and anionic complexes target the bone and kidney. Of the different imaging agents, $[Tc(CNR)_6]^+$ (**70**) is the best established: known as cardiolyte, it is widely used as a heart imaging agent. The compound of Tc(V) with mercaptoacetyltriglycine (**71**), known as Tc-MAG-3, is used to image kidneys because of its rapid excretion. For imaging bone, complexes of Tc(VII) with hard diphosphonate ligands (**72**) are effective. Brain imaging is carried out with compounds such as ceretec (**73**).

To produce Tc tracers, radioactive $^{99}MoO_4^{2-}$ is passed onto an anion exchange column, where it binds tightly until it decays to the pertechnate ion $^{99m}TcO_4^-$ and the lower charge causes it to be eluted. The eluate is treated with a reducing agent, usually Sn(II), and the ligands required to convert it into the desired imaging agent. The resulting compound is then administered to the patient.

Tracers are being developed that are much more specific for their targets. These tracers contain the metal in a stable coordination sphere that is covalently linked to a biologically active fragment. An example is the Gd contrast agent EP-210R (**74**), which contains four $Gd^{3+}$ complexes linked to a peptide that recognizes and binds to fibrin, a molecule produced by thrombi (blood clots).



**74**

## Perspectives

In this final section, we stand back from the material in the chapter and review it from a variety of different perspectives, from the point of view of individual elements and from the point of view of the contribution of bioinorganic chemistry to urgent social problems.

## 27.21 The contributions of individual elements

**Key point:** Elements are selected by Nature for their inherent useful properties and their availability.

In this section we summarize the major roles of each element and correlate what we have discussed with emphasis on the element rather than the type of reaction that is involved.

**Na, K, and Li** The ions of these elements are characterized by weak binding to hard ligands and their specificity is based on size and hydrophobicity that arises from a lower charge density. Compared to $Na^+$, $K^+$ is more likely to be found coordinated within a protein and is more easily dehydrated. Both $Na^+$ and $K^+$ are important agents in controlling cell structure through osmotic pressure, but whereas $Na^+$ is ejected from cells, $K^+$ is accumulated, contributing to a sizeable potential difference across the cell membrane. This differential is maintained by ion pumps, in particular the $Na^+$,$K^+$-ATPase, also known as the Na-pump. The electrical energy is released by specific gated ion channels, of which the $K^+$ channel (Section 27.3) has been studied the most.

An important related issue is the widespread use of simple Li compounds (particularly $Li_2CO_3$) as psychotherapeutic agents in the treatment of mental disorders, notably bipolar disorder (manic depression). One possibility is that hydrated $Li^+$ binds tightly in the $Na^+$ or $K^+$ channels in place of the dehydrated ions that are normally transported selectively in the filter region of these proteins. As $Li^+$ ions are highly labile, we can be confident that the aqua ion itself, or a complex with an abundant ligand, is the active species. As a simple aqua ion, $Li^+$ is strongly solvated, in fact the solvated radius is greater than that of $Na^+$.

**Mg** Magnesium ions are the dominant 2+ ion in cytoplasm and the only ones to occur above millimolar levels in the free, uncomplexed state. The energy currency for enzyme catalysis, ATP, is always present as its $Mg^{2+}$ complex. Magnesium has a special role in the light-harvesting molecule chlorophyll because it is a small 2+ cation that is able to adopt octahedral geometry and can stabilize a structure without promoting energy loss by fluorescence. The $Mg^{2+}$ ion is a weak acid catalyst and is the active metal ion in rubisco, the highly abundant enzyme responsible for removing from the atmosphere some 100 Gt of $CO_2$ per year. Rubisco is activated by weak binding of $Mg^{2+}$ to two carboxylates and a special carbamate ligand, leaving three exchangeable water molecules.

**Ca** Calcium ions are important only in eukaryotes. The bulk of biological Ca is used for structural support and devices such as teeth. The selection of Ca for this function is due to the insolubility of Ca carbonate and phosphate salts. However, a tiny amount of Ca is used as the basis of a sophisticated intracellular signalling system. The principle of this process is that Ca is suited for rapid coordination to hard acid ligands, especially carboxylates from protein side chains, and has no preference for any particular coordination geometry.

**Mn** Manganese has several oxidation states, most of which are very oxidizing. It is well suited as a redox catalyst for reactions involving positive reduction potentials. One reaction in particular, in which $H_2O$ is used as the electron donor in photosynthesis, is responsible for producing almost all the $O_2$ in the Earth's atmosphere. This reaction involves a special $Mn_4Ca$ cluster. Manganese(II) is also used as a weak acid–base catalyst in some enzymes. Spectroscopic detectability varies depending on oxidation state: EPR has been useful for Mn(II) and for particular states of the Mn cluster that constitutes the catalyst for the evolution of $O_2$.

**Fe** Versatile Fe is probably essential to all organisms and was certainly a very early element in biology. Three oxidation states are important, namely Fe(II), Fe(III), and Fe(IV). Active sites based on Fe catalyse a great variety of redox reactions ranging from electron transfer to oxygenation, as well as acid–base reactions that include reversible $O_2$ binding, dehydration/hydration, and ester hydrolysis. Iron-containing active sites feature ligands ranging from soft donors such as sulfide (as in FeS clusters) to hard donors such as carboxylate. The porphyrin macrocycle is particularly important as a ligand. Iron(II) in various coordination environments is used to bind $O_2$, either reversibly or as a prerequisite for activation. Iron(III) is a good Lewis acid, whereas the Fe(IV)=O (ferryl) group may be considered as Nature's way of managing a reactive O atom for insertion into C–H bonds. Cells contain very little uncomplexed Fe(II) and extremely low levels of Fe(III). These ions are toxic, particularly in terms of their reaction with peroxides, which generates the hydroxyl radical. Primary uptake into organisms from minerals poses problems because Fe is found predominantly as Fe(III), salts of which are insoluble at neutral pH (see Fig. 5.12). Iron uptake, delivery, and storage are controlled by sophisticated transport systems, including a special storage protein known as ferritin. Iron porphyrins (as found

in cytochromes) show intense UV–visible absorption bands and most active sites with unpaired electrons give rise to characteristic EPR spectra.

**Co** Cobalt and nickel are among the most ancient biocatalysts. Cobalt is processed only by microorganisms and higher organisms must ingest it as vitamin $B_{12}$ in which Co is complexed by a special macrocycle called corrin. Complexes in which the fifth ligand is a benzimidazole that is covalently linked to the corrin ring are known as cobalamins. Cobalamins are cofactors in enzymes that catalyse alkyl transfer reactions and many radical-based rearrangements. Alkyl transfer reactions exploit the high nucleophilicity of Co(I). In the special cofactor known as coenzyme $B_{12}$, the sixth ligand to Co(III) is a carbanion donor atom from deoxyadenosine. Radical-based rearrangements involve the ability of coenzyme $B_{12}$ to undergo facile homolytic cleavage of the Co–C bond, producing stable low-spin Co(II) and a carbon radical that can abstract a hydrogen atom from substrates. Cobalamin-containing enzymes show strong UV–visible absorption bands; EPR spectra are observed for Co(II).

**Ni** Nickel is important in bacterial enzymes, notably hydrogenases, where it also uses the +3 and +1 oxidation states, which are rare in conventional chemistry. A particularly remarkable enzyme, coenzyme A synthase, uses Ni to produce CO and then react it with $CH_3-$ (provided by a cobalamin enzyme) to produce a C–C bond in the form of an acetyl ester. Nickel is also found in plants as the active site of urease. Urease was the first enzyme to be crystallized (in 1926), yet it was not until 1976 that it was discovered to contain Ni.

**Cu** Unlike Fe, copper probably became important only after $O_2$ had become established in the Earth's atmosphere and it became available as soluble Cu(II) salts rather than insoluble sulfides ($Cu_2S$). The main role of Cu is in electron transfer reactions at the higher end of the potential scale and catalysis of redox reactions involving $O_2$. It is also used for reversible $O_2$ binding. Both Cu(II) and Cu(I) are strongly bound to biological ligands, particularly soft bases. Free Cu ions are highly toxic and almost absent from cells.

**Zn** Zinc is an excellent Lewis acid, forming stable complexes with ligands such as N and S donors, and catalysing reactions such as ester and peptide hydrolysis. The biological importance of Zn stems largely from its lack of redox chemistry, although its common adoption of di-, tri-, and tetrathiolate ligation provides a link to the redox chemistry of cysteine/cystine interconversions. Zinc is used as a structure former in enzymes and proteins that bind to DNA. A major problem has been the lack of good spectroscopic methods for studying this $d^{10}$ ion. In some cases, Zn enzymes have been studied by EPR, after substituting the Zn by Co(II).

**Mo and W** Molybdenum is an abundant element that is probably used by all organisms as a redox catalyst for the transfer of O atoms derived from $H_2O$. In these oxo-transfer enzymes the Mo is always part of a larger pterin-containing cofactor in which it is coordinated by a special dithiolene ligand. Interconversion between Mo(IV) and Mo(VI) usually results in a change in the number of terminal oxo ligands, and recovery of the starting material occurs by single-electron transfer reactions with Mo(V) as an intermediate. Aside from oxo-transfer and related reactions, Mo has another intriguing role, that of nitrogen fixation, in which it is part of a special FeS cluster. Use of W is confined to prokaryotes, where it is also used as a redox catalyst, but in reactions where a stronger reducing agent is required.

**Si** Silicon is often neglected among biological elements, yet its turnover in some organisms is comparable to that of carbon. Silica is an important material for the fabrication of the exoskeleton and of prickly defensive armour in plants.

**Pt, Au, Bi, and Ru** These elements have no known deliberate biological functions and are foreign agents to biological systems, acting under normal conditions as poisons. However, used in controlled procedures, and dressed up by complexation to target a particular site, they are potent drugs, active against a range of diseases and disorders.

## 27.22 Future directions

**Key points:** Biological metals and metalloproteins have important futures in medicine, energy production, green synthesis, and nanotechnology.

The pioneering studies of the structures and mechanism of ion channels mentioned in Section 27.3 are providing important new leads in neurophysiology, including the rational design of drugs that can block or modify their action in some way. New functions for Ca

are continually emerging, and one intriguing aspect is its role in determining the left–right asymmetry of higher organisms, a prime example being the specific placements of heart and liver in the body cavity. The so-called **Notch signalling pathway** in embryonic cells depends on transient extracellular bursts of $Ca^{2+}$ that are dependent in some way on the activity of an $H^+/K^+$-ATPase. There is also a growing awareness of the role of Zn and Zn transport proteins in control of cellular activity, and also of neural transmission. Indeed, the term **metalloneurochemistry** has been coined to describe the study of metal-ion function in the brain and nervous system at the molecular level. An important challenge is to map out the distribution and flow of Zn in tissue such as brain, and advances are being made in the design of fluorescent ligands that will bind Zn selectively at cellular levels and report on its transport across different zones, for example the synaptic junctions. Metal ions are involved in protein folding, and it is believed that Cu, in particular, may have an important role in fatal neurodegenerative disorders. These roles include controlling the behaviour of prions involved in transmittable diseases such as spongiform encephalopathy (Creuzfeldt–Jakob disease, the human form of 'mad cow' disease) as well as amyloid peptides that are implicated in Alzheimer's disease.

In many regions of the world, rice is the staple food but this commodity is low in Fe. Thus transgenic techniques are being used to improve Fe content. The object is to produce better plant siderophores and improve Fe storage (by enhanced expression of the ferritin gene).

Enzymes tend to show much higher catalytic rates and far higher selectivity than synthetic catalysts, leading naturally to greater efficiency and lower energy costs. The principal disadvantages of using enzymes as industrial catalysts are their lower thermal stability, limitations on solvent and pH conditions, and a large mass per active unit. There is much interest in achieving enzyme-like catalytic performance with small synthetic molecules, a concept that is known as 'bioinspired catalysis'. The idea is to reproduce, using all the tools of synthetic chemistry, the properties of an enzyme trimmed down to its smallest fully functional component. Examples of bioinspired catalysts have already been described: areas of particular interest for industrial production are the conversion of methane to methanol (Section 27.10), activation of $N_2$ to produce cheap fertilizers, and production of hydrogen.

In the not so distant future, when fossil fuels have been depleted, $H_2$ will become an important energy carrier, used either directly or indirectly (after conversion into fuels such as alcohol) to power vehicles of all kinds. One of the scientific challenges is how to obtain efficient electrolytic production of $H_2$ from water, given that electricity will be widely available from a variety of sources. This process requires demanding conditions of temperature and overpotential (Section 10.4), or catalysts that are currently based on Pt and other precious metals. However, Nature has already shown us that rapid hydrogen cycling is possible under mild conditions by using just the common metals Fe and Ni. A related challenge is the synthesis of efficient electrocatalysts that can convert water to $O_2$ without requiring a large overpotential, not because there is a need for $O_2$ itself, but because it is an essential byproduct of electrolytic or photolytic $H_2$ production (see Box 10.3). Once again, we can turn to the biosphere for inspiration because by elucidating the mechanism of the Mn catalyst, we might synthesize new catalysts that are both cheap and durable.

We have seen the exquisite structures of materials that are produced by organisms. This understanding is now leading to new directions in nanotechnology (Chapter 25). For example, sponge-like single crystals of calcite, having intricate morphological features, have been produced on polymer membranes formed by templating the skeletal plates of the sea urchin. Another recent development is the production of Pd nanoclusters by hydrogen-oxidizing bacteria, the action of hydrogenases making available controlled electron flow to effect the electroplating of Pd on to microscopic sites.

## FURTHER READING

J.J.R. Frausto da Silva and R.J.P. Williams, *The biological chemistry of the elements*. Oxford University Press (2001). An excellent, detailed book that looks at the broader picture of the relationship between elements and life.

L. Que Jr. and W.B. Tolman, *Bio-coordination chemistry. Comprehensive coordination chemistry, Vol. 8*. Elsevier (2004). A text providing particularly detailed insight into model compounds.

R.R. Crichton, F. Lallemand, I.S.M. Psalti, and R.J. Ward, *Biological inorganic chemistry*. Elsevier (2007). A modern introduction to biological inorganic chemistry.

E. Gouaux and R. MacKinnon, Principles of selective ion transport in channels and pumps. *Science*, 2005, **310**, 1461. An article linking detailed three-dimensional structural data of giant proteins with physiological function and the chemistry of Group 1 and 2 metal ions and $Cl^-$.

R.K.O. Sigel and A.M. Pyle, Alternative roles for metal ions in enzyme catalysis and the implications for ribozyme chemistry. *Chem. Rev.*, 2007, **107**, 97. A review describing the role of metal ions, particularly Mg, as active centres in catalysts based on RNA instead of proteins.

E. Kimura, Model studies for molecular recognition of carbonic anhydrase and carboxypeptidase. *Acc. Chem. Res.*, 2001, **34**, 171. This review describes the acid–base and catalytic properties of small Zn complexes in an effort to understand how Zn enzymes function.

K.N. Ferreira, T.M. Iverson, K. Maghlaoui, J. Barber, and S. Iwata, Architecture of the photosynthetic oxygen-evolving center. *Science*, 2004, **303**, 1831. A seminal article on the structure of the catalyst responsible for $O_2$ in the atmosphere.

L. Que, Jr., The road to non-heme oxoferryls and beyond. *Acc. Chem. Res.*, 2007, **40**, 493. A stimulating account of efforts to understand the chemistry of Fe(IV) species and produce new catalysts for organic oxygenation reactions.

E.A. Lewis and W.B. Tolman, Reactivity of dioxygen–copper systems. *Chem. Rev.*, 2004, **104**, 1047. A review of small molecule analogues of Cu-containing enzymes.

S.C. Wang and P.A. Frey, S-adenosylmethionine as an oxidant: the radical SAM superfamily. *Trends in Biochemical Sciences*, 2007, **32**, 101. An authoritative account of the discovery of radical SAM enzymes. It categorises the different classes of enzymes and describes the mechanisms by which the [4Fe–4S] cluster cleaves SAM to initiate radical reactions.

H.B. Gray, B.G. Malmström, and R.J.P. Williams, Copper coordination in blue proteins. *J. Biol. Inorg. Chem.*, 2000, **5**, 551. An article that draws together and summarizes the theories spanning more than 30 years of research on blue Cu centres.

P.J. Kiley and H. Beinert, The role of Fe–S proteins in sensing and regulation in bacteria. *Curr. Opin. Chem. Biol.*, 2003, **6**, 182. A review describing how Fe–S clusters are involved in sensing.

J. Green and M.S. Paget, Bacterial redox centres. *Nature Reviews*, 2004, **2**, 954. A general review on the mechanisms by which reactive oxygen species are sensed in cells.

S. Mann, *Biomineralisation: principles and concepts in bioinorganic materials chemistry*. Oxford University Press (2001).

M.D. Archer and J. Barber (ed.), *Molecular to global photosynthesis*. Imperial College Press (2004).

C.W. Cady, R.H. Crabtree, and G.W. Brudvig, Functional models for the oxygen-evolving complex of photosystem II. *Coord. Chem. Rev.*, 2008, **252**, 444. A review of recent efforts to understand and mimic the chemistry of the $Mn_4Ca$ cluster that converts water to $O_2$.

M.J. Hannon, Supramolecular DNA recognition. *Chem. Soc. Rev.*, 2007, **36**, 280. An account of efforts to make large metal complexes that can recognise certain DNA sequences.

M.A. Jakupec, M. Galanski, V.B. Arion, C.G. Hartinger, and B. Keppler, Antitumour metal compounds: more than theme and variations. *Dalton Transactions*, 2008, **2**,183.

P.C.A. Bruijnincx and P. J Sadler, New trends for metal complexes with anticancer activity. *Curr. Opin. Chem. Biol.*, 2008, **12**, 197. Two complementary reviews of recent developments in anti-cancer drugs based on metal complexes.

P. Caravan, Strategies for increasing the sensitivity of gadolinium-based MRI contrast agents. *Chem. Soc. Rev.*, 2006, **35**, 512. A review of developments in producing selective Gd-based magnetic resonance imaging reagents.

B.E. Mann and R. Motterlini, CO and NO in medicine. *Chem. Commun.*, 2007, 4197. A review of the roles of NO and CO in biology and medicine.

## EXERCISES

**27.1** Calcium-binding proteins can be studied by using lanthanoid ions ($Ln^{3+}$). Compare and contrast the coordination preferences of the two types of metal ion and suggest techniques in which lanthanoid ions would be useful.

**27.2** In zinc enzymes, 'spectroscopically silent' Zn(II) can often be replaced by Co(II) with high retention of activity. Explain the principles by which this substitution can be exploited to obtain structural and mechanistic information.

**27.3** Compare and contrast the acid–base catalytic activities of Zn(II), Fe(III), and Mg(II).

**27.4** Propose physical methods that would allow you to determine whether a reactive intermediate isolated by rapid freeze quenching contains Fe(V).

**27.5** Figure 27.58 shows Mössbauer spectra of a sample of ferredoxin from chloroplasts at 77 K. With reference to Section 8.7, interpret the data with regard to the oxidation states and spin states of the two Fe atoms and comment on the electron delocalization at this temperature.

**27.6** The structure of the P-cluster in nitrogenase differs significantly between oxidized and reduced states. Comment on this observation in the light of proposals that it participates in long-range electron transfer.

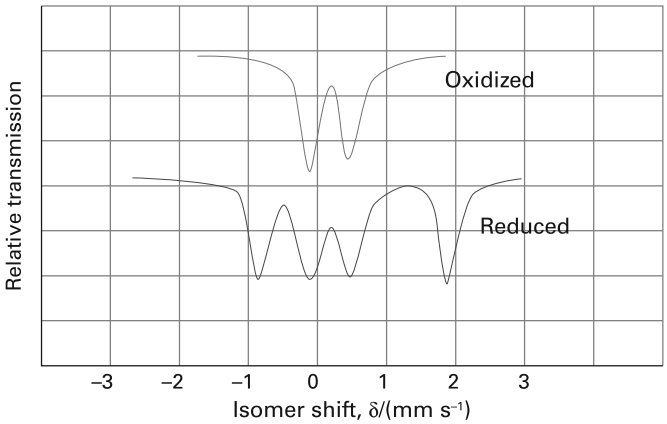



**Figure 27.58** Mössbauer spectra of a sample of ferredoxin from chloroplasts at 77 K.

**27.7** Microorganisms can synthesize the acetyl group ($CH_3CO-$) by direct combination of methyl groups with CO. Make some predictions about the metals that are involved.

## PROBLEMS

**27.1** With reference to details that have been discussed in Section 27.3 for the $K^+$ channel, predict the properties of $Na^+$, $Ca^{2+}$, and $Cl^-$ binding sites that would be important for providing selectivity in their respective transmembrane ion transporters.

**27.2** Comment on the implications of discovering, by microwave detection, substantial levels of $O_2$ on a planet in another solar system.

**27.3** Apart from direct O-atom transfer (Fig. 27.43), another mechanism proposed for Mo enzymes is indirect O-atom transfer, also known as *coupled electron–proton transfer*. In this mechanism, as shown in Fig. 27.59 for sulfite oxidase, the O atom that is transferred originates instead from an uncoordinated $H_2O$ molecule. Propose a way to distinguish between direct and indirect O-atom transfer mechanisms.

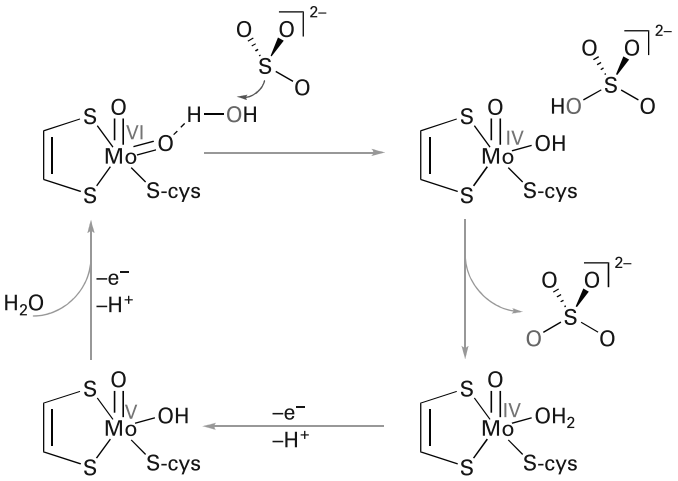



**Figure 27.59** The mechanism referred to in Problem 27.3.

**27.4** In justifying research into small molecule catalysts for producing $NH_3$ from $N_2$, it is sometimes stated that nitrogenase is an 'efficient' enzyme: how true is this statement? Comment critically on the argument that 'knowing the three-dimensional structure of nitrogenase has not enlightened us as to its mechanism of action' and discuss how this view might be valid more generally for enzymes for which a structure is known.

**27.5** 'In Mo enzymes, the bond between a terminal oxo anion and Mo(VI) is usually written as a double bond, whereas it is more correctly assigned as a triple bond.' Discuss this statement. Suggest how a terminal oxido ligand influences the reactivity of other coordination sites on the Mo atom and explain how a terminal sulfido ligand (as occurs in xanthine oxidase) would alter the properties of the active site.

*This page intentionally left blank*