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Review

Iron in evolution ☆

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ABSTRACT

Iron chemistry in the environment and in organisms is entwined. The iron surface minerals in solution for the first billion years of the planet were ferrous compounds. This ion became and has remained a major participant in organisms. The evolution of iron was due to its oxidation to insoluble ferric ions by oxygen released from organisms. The evolution of cellular iron chemistry then required uptake from this oxidised state. Use was expanded from the mainly electron transfer properties in the original reductive cell interior to employment in external oxidative chemistry. The environment/organisms evolution is that of one predictable chemical system.

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1. Introduction

Antonio came to me at the behest of a friend and scientific collaborator of mine, John Fraù'sto da Silva, and from the beginning he showed himself to be one of my best pupils. He worked at first in a group of us who were studying the confirmation of AMP, adenosine monophosphate, by NMR. The work was amongst the first using NMR lanthanide probes [1]. The molecule is mobile internally so our analysis was computer programmed for generating a multitude of similar structures letting the final picture appear as that structure which had the most very similar structures close to it, as is much used in protein structural work. Later Antonio joined a group of mine examining by NMR the structure of cytochrome-c. We showed a little later that the iron of multi-heme proteins had histidine both as the fifth and sixth ligand to the central iron [2]. It was from this starting point that Antonio began his long analysis of especially multi-heme proteins. I shall make the central ion of the heme, iron, the subject of this paper.

2. The beginnings of iron chemistry on Earth

Iron is an extremely abundant element in the universe due to its nuclear stability and the freezing of the nuclear kinetics of element formation. It is close in abundance to oxygen on Earth though most of it is in the central metallic core. From the beginning of the condensed state of Earth it was bound also in oxides, silicates and sulfides and probably sometime later in carbonates. Many of these minerals were too deep in Earth's mantle to be of great interest but one of them, $MgFeSiO_4$, olivine, was on the surface of the seabed and resulted in the high initial availability of iron in the sea and later in the content of organisms [3]. It is also similar to much iron in organisms since iron there is Fe^{2+} not Fe^{3+}. The parallel extends to the sulfides where common sulfides, FeS and particularly FeS_2, can be compared with a fair part of iron as Fe^{2+} in Fe/S proteins. The suggestion is that the redox potential of the early sea and the cytoplasm of the earliest cells were at about −0.2 V.

3. Cellular early reductive chemistry

Life is centred on the reduction of the oxides of carbon and as a consequence of this reaction, inside a near spherical lipid membrane, wider organic chemistry was initiated. Such synthesis generated free oxidising agents too but they had to be released outside the cell. It was then an essential feature of life that oxidising equivalents were exported to the environment, or that sources of reducing equivalents were imported from extracellular compounds which became oxidised. Today both are observed as oxygen diffuses from mitochondria to the environment while sources of electrons such as H_2S, H_2O and H_2 donate electrons to the chloroplasts inside. In the second case electrons must cross the containing membrane and it is generally considered that the

Abbreviation: Ga, billion of years ago

☆ This article is dedicated to the memory of Antonio Xavier.

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first carriers were Fe/S clusters held in the membrane by very early proteins. (Antonio introduced a group of his pupils to the study of these Fe/S proteins [4].) The release of oxygen was also dependent on iron electron chains in membranes. Of course the Fe/S group of proteins included many in the cytoplasm for electron transfer but to my knowledge none occurred outside the cell membrane. A very confusing feature of their history was that an EPR signal of $g = 1.94$ from their proteins was observed. The nature of the signal was shown to be due to coupling of spins between the two iron ions. The structure was discovered by a physicist, Thornley, to whom the problem was introduced by me but solved with the help of three other colleagues, Whatley, Hall and Gibson [5] Amongst the equipment put together by Antonio in what had been a backward chemistry laboratory in Lisbon were both NMR and EPR spectrometers, the latter for the study of this EPR signal especially by his students [4].

Returning to the function of Fe/S centres they were found at the low potential ends of oxidative and photochemical phosphorylation, giving ATP. They were also involved in low potential reactions of hydrogenase and nitrogenase as well as in a number of other reductive enzymes. They are still rarely used as centres of electron transfer at redox potentials above +0.2 V. The Fe/S proteins required an import of iron. Quite intriguingly their basic synthesis today is by a complex iron uptake system followed by reactions in the outer membrane of mitochondria in eukaryotes. How were they formed in the most primitive anaerobic prokaryotes?

Sometime shortly after life appeared, thought to be close to 3.5 Ga (billions of years ago), the porphyrin complexes of Fe, Co, Ni and Mg were synthesised. This is a complex synthesis of the porphyrin ring perhaps starting from cyanide. Note again the final step of iron insertion in the ring came to be placed later in the outer zone of mitochondria. The appearance of these porphyrins must have been when the redox potential of cells was still below −0.2 V for this is close to the value of the cytoplasm which has been maintained ever since and is needed to reduce cyanide. They must have been used at first at this low redox potential and many of the iron cytochromes so formed have negative redox potentials, see below, but many today have more positive potentials since unlike Fe/S centres they are not so sensitive to oxidation.

4. Oxidation of iron

The history of iron in the sea changed from that of Fe^{2+} to that of Fe^{3+} and much then was precipitated as hydroxide and is now seen in Banded Iron Formations (BIF) formed around 3.0 Ga, Fig. 1. The BIF precipitated, altered from Fe_2O_3 plus F_3O_4 at first to virtually all Fe_2O_3 at later times through the rise of redox potential (due to oxygen release). Now there was a period from around 2.5 to 1.0 Ga when BIF is not observed only for it to reappear as Fe_2O_3 in smaller quantity around 0.9 Ga [6]. The explanation given is dependent on two observations: the appearance of some pyrite, FeS_2, and of the oxidation of sulfides to give increasing amounts of sulfate in the deeper regions of the sea. Note that the Fe^{2+}/Fe^{3+} oxidation is of similar potential to that of HS^-/SO_4^{2+}. The sulfate became extremely useful as an oxidising agent giving energy to the so-called sulfur-bacteria which reduced it back to sulfide. The sulfide was then in part reoxidised by Fe^{3+} generating Fe^{2+} and FeS_2, precipitates. While these reactions of sulfur and iron occurred in the moderately deep to deep ocean, the surface layer which contained photosynthesising bacteria, converted H_2O to oxygen which oxidised Fe^{2+} to Fe^{3+} and sulfide to sulfate. The surface layer was then at a redox potential closer to +0.2 V while lower regions were still at −0.2 V. Mixing was poor [3]. The function of iron had changed from simple engagement in electron transfer reduction to a catalyst of the reactions of oxidation especially in the organisms in or nearer the top layer of the sea. Examples are the complex oxidases such as cytochrome P-450.

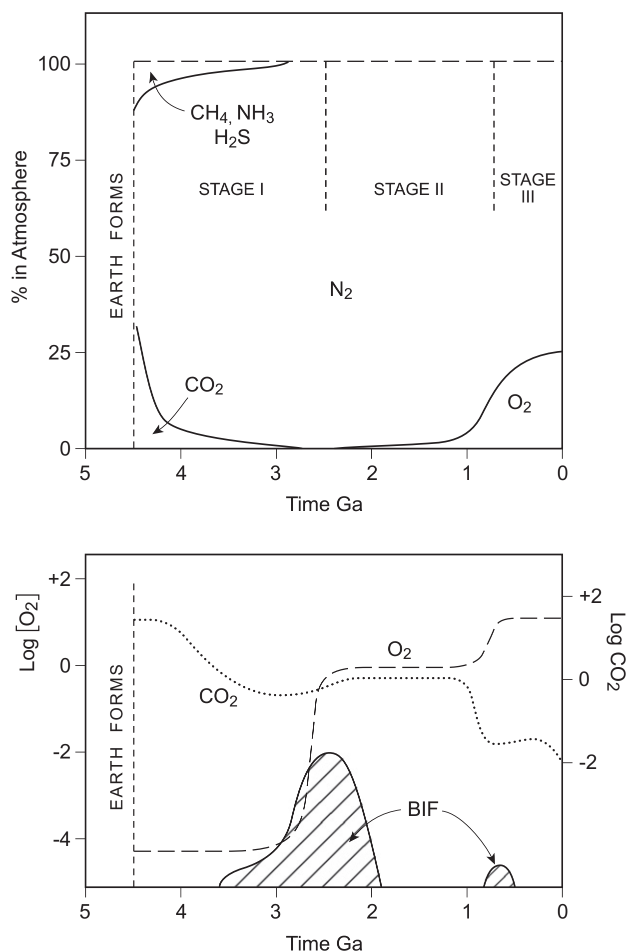

Fig. 1. The times of banded iron oxide formation (BIF) together with the increase in oxygen in the atmosphere.

By about 1.0 Ga the rate of O_2 production for oxidation overwhelmed that of sulfate production. Subsequently energy for organisms was obtained by oxidation of organic molecules by oxygen itself, that is today's oxidative phosphorylation. From then on to today this is the major route of animal energy production. The oxygen now oxidised the remaining iron which precipitated giving the last small burst of BIF production at 0.9 Ga, Fig. 1. The dates of evolution can be followed by noting the oxidation states of trace elements in this BIF and other sediments.

The role of iron had also developed in energy production and in photochemistry as the Fe/S electron transfer system had become extended, using cytochromes, to long chains of reaction in membranes. In all the bioenergy paths the first step was the generation of a proton gradient and then ATP [7,8]. The question arose as to whether there was the possibility of converting hydrogen into H^+ with oxidising equivalents in a particle to generate energy. This idea was championed by Antonio in a series of papers devoted to the redox behaviour of multi-cytochrome proteins [9]. There is no doubt that this exacting study revealed much of the redox potentials and the exchange of electrons in these proteins but the coupling to energy rather than the simpler use of them in redox reactions remains uncertain.

I return to my theme of the historical development of iron on Earth. The very fact that the original iron concentration in the sea changed from about 10^{-6} M Fe^{2+} to form 10^{-10} M total Fe^{3+} in

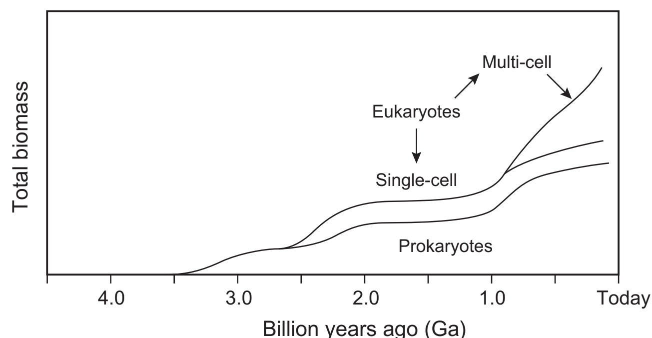

Fig. 2. The times of evolution of single-cell and then multicell eukaryotes. Notice the close correspondence with the two quicker rises (steps) in oxygen in the atmospheres, Fig. 1.

complexes and to much lower free Fe^{3+} at 10^{-17} M, made iron availability very low. The demand for iron by organisms remained since it is the only available metal ion which had redox potentials as low as –0.2 but above –0.5, when water would give hydrogen freely, and as high as +0.5 V. The difficulties were overcome by devising transport siderophores, especially "designed" organic chelating agents for uptake which had to bind Fe^{3+} but not Al^{3+}, which is of similar concentration in the sea. We return to this problem of iron distribution after introducing a striking unexplained event in evolution – the coming of eukaryotes apparently from two prokaryotes at a particular time, around 2.5 Ga, Fig. 2, with the first rapid oxygen rise, Fig. 1.

The single-cell eukaryote is a cell containing several compartments or vesicles but most strikingly two descendants of earlier prokaryotes for energy generation – mitochondria in both plant and animal cells and the chloroplast in plants alone. Later unicellular eukaryote cells of both kinds came together to form multicellular organisms, plants and animals. Not only the uptake and distribution of iron for them increased in difficulty but the difficulty in use of many substrates and ions, notably oxygen and metal ions also increased greatly. These ions which were used with oxygen in catalysed reactions, required extracellular circulation later in the more advanced multicellular animals. One carrier for oxygen is hemoglobin with an iron porphyrin while the second, hemerythrin, was based on two iron atoms, while iron transport was by a protein, transferrin. Before and during these developments of carriers there was a considerable array of oxidative enzymes. Most notable are the P.450 cytochromes with an iron porphyrin, and the non-heme iron oxidases which have a simple Fe^{2+} centre coupled to 2-oxoglutarate. Both of these enzymes can be used in hydroxylation of sterols, of long-chain lipids, and several transmitters [6].

One other requirement in cellular iron chemistry is control of its concentration. Inspection shows that to today the cytoplasm is maintained as buffered Fe^{2+} at 10^{-6}–10^{-7} M. A considerable set of iron, Fe^{+}, enzymes as well as controls of iron input to cells are restricted in concentration by transcription factors. These factors switch off the production of proteins of iron uptake, such as transferring, and iron storage proteins such as ferritin in both prokaryotes and eukaryotes, once enough iron has been obtained. The homeostatic (buffered) Fe^{2+} concentration is then linked not only to iron enzymes for oxidation and production of transmitters but to synthesis of all kinds of simple iron proteins. The iron concentration controls such reaction pathways as the Krebs cycle. The iron is bound to many transcription factors so that it is also prominent in the switch by facultative bacteria from anaerobic to aerobic metabolism. The Fe^{2+} is still held in the cytoplasm at the same concentration as it was in the sea as long ago as 3.5 Ga. Notice that the implication is that many iron proteins, transcription factors and

Table 1
Common inorganic element changes in eukaryotes of all groups.

Element	Change or increase of use
Zinc	Digestive functions
	Increased metalloproteases
	Protection from superoxide
	Zing fingers, messenger
Copper	Oxidative reactions
	Protection from superoxide
Calcium	Signalling, vesicle stores
	Biominerals (animals)
Silica	Biominerals
Cobalt, Nickel	Reduced use (vitamin B_{12})
Iron	Fe/S proteins maintained
	Heme oxidases
	Non-heme non-Fe/S oxidases
Selenium	Anti-oxidation and reducing catalysts
Manganese	Glycosylation
	O_2-formation maintained
Molybdenum	Reductive enzymes at first Oxidative enzymes later

enzymes all have similar binding constants of about 10^{6} M^{-1}. Iron is very rarely (if ever) held in other internal compartments of the cell. In the cell heme protein synthesis is also controlled at 10^{-6} M by the iron chelatases, now called chaperones. However once the porphyrin is made the iron no longer exchanges with free iron and can be used even outside the cytoplasm at higher redox potential, for example in cytochrome c.

Given the ever-increasing role of metal ion chemistry in organisms, Table 1 [6], we have to inquire into the manner in which the genetic code was expanded to give the possibility of production of novel iron proteins from time to time [10]. Their earliest use in reduction, the formulation of which is a mystery, was clearly greatly complicated by their later employment in oxidation requiring novel proteins.

The evolution of iron biochemistry which is greatly involved in this evolution, can be taken as an example which raises the whole question of how evolution progresses. The two possibilities are (1) the conventional and well-established random changes in genes by mutation to match changes in the environment, here of iron and oxygen, and (2) by *genetic change caused directly by environmental change* which has been conventionally dismissed. Much recent work has however revived interest in this second possibility [10]. The conventional methods of study of the first possibility are (1) to date as far as is possible the appearance of biological organisms; this gave rise to the conventional Darwinian "tree" of evolution; (2) to use present-day knowledge of DNA or RNA sequences to follow the essence of their changes so as to form a lineage. The lineage can then be extrapolated by calculation employing mathematical equations of rates of evolution based on known mutational rates. Both these two methods have as a source of primary data the ages of fossils. The two procedures have given an agreed firm base for evolution from the Cambrian Explosion, 0.54 Ga, to today. Both of them have led to the suggestions that evolution proceeds by small steps as the organisms improve fitness for the environment and its changes. There are some remaining questions concerning extinctions due to relatively rapid change of climate and of meteorite impacts after 0.54 Ga. Now before this time, 0.54 billion years ago (Ga), the fossil record is poor, the nature of living organisms is not very clear, and the rate of genetic extrapolation becomes uncertain as the rate of mutation is not known.

Our approach to evolution has been quite different and is based on chemical information from sediments beginning 4.5 Ga years ago and showing considerable changes until 0.54 Ga. We consider that, since sediments have little changed after about 0.5 Ga the interlocked chemistry of organisms and the environment have changed little since then and diversity is then understandable under the above two classical descriptions. Before that time the

chemical developments in sediments are very clear and required rapid gene changes, spurts. The trace element in sediments arose from the chemistry of organisms, especially their production of oxygen. These elements in the sea back-reacted with organisms making evolution a combined inevitability.

The method we use is dependent firstly on analysis of the quantity of an element and the ratio of its isotopes in sediments. This treatment has led geochemists to consider that there were two rapid environmental changes around 2.5 Ga and after 1.0 Ga with a chemical buffered region between them as described already, Fig. 1. We can correlate these relatively fast environmental changes as they follow rises of oxygen in the atmosphere and of redox potential of the sea with the known evolution of elements in different classes of organisms. In the period between the two rapid oxygen rises organisms changed less rapidly. We know that the changes in organisms around 2.5 Ga were the appearance of micro-aerobes dependent on sulfate from oxygen reactions, mentioned above and some aerobes dependent directly on oxygen. Between 2.5 and 2.0 Ga single-cell eukaryotes also evolved, Fig. 2, and then around 1.0–0.54 Ga multicellular organism fossils are found [3,6]. We wish to consider the way in which DNA changes in the whole period from 3.5 Ga, as deduced from the nature of fossils and comparable organisms of today, were connected to these environmental changes. The study of DNA/RNA and proteins shows that they did not change by mutation alone but there are many examples of local gene duplication.

A very intriguing approach then is to use bio-informatics to uncover not just DNA or RNA sequences but to reveal the number of proteins which have been not just mutated but duplicated. Single mutations of a given protein are sufficient to explain the evolution of a given organisms and the source of diversity of organisms but do not explain dramatic evolutionary changes, "jumps" [10]. Duplication followed by mutations is clearly a way of maintaining the properties of a given protein while searching for a new function within the duplicates, that is maintaining the original activity while making new related proteins. Our approach has been to look at metalloprotein duplication as organisms increased in complexity since their sequences are easily followed from metal ion binding sites. Moreover changes in metal ions are the easiest environmental labels to follow. I have worked with the groups of Professor Bertini [11] and with Professor Dupont [12] to use duplication to throw light on the evolution of iron proteins as one example and the subject of this article. It is clear that there are very many duplicates of iron proteins in many genomes, amounting to several hundred each, of, for example Fe/S, cytochrome P-450, and the 2-oxo-glutarate iron proteins, Table 2 [13,14]. There are very few duplicates of cytochrome oxidase. The conclusion is that duplication is local, much though whole genomes may also be duplicated as seen in multiple chromosomes. There are also progressively more duplications of iron oxidases or we go from simple to more complex organisms. Again plants have many more iron oxidases than animals of similar genome size, Table 2. Plants are more open to oxidative damage. We wish to understand how they have evolved [13,14]. It is apparent

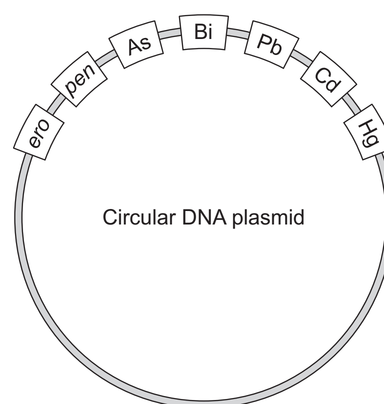

Fig. 3. A DNA map of the resistance proteins on a plasmid. The deduction made from it is that novel proteins are generated in the same way from both drug, organic, and metal ion, inorganic poisons. The origin of DNA due to a new drug in an organisms is relatively well understood.

that duplications arose with the environmental changes and as organisms became more increasingly complex.

"Oxidases" may have been originally protective to remove certain organic poisons. This alone requires many proteins of similar function but with somewhat different specificity as the environment contains many poisonous organic chemicals changing over time. Later these enzymes have become those for synthesis of organic messengers and hormones by oxidation. The supposition is that the expansion of their action is managed by gene duplication. A typical example is cytochrome P-450 which is found in bacteria as a detoxifying enzyme and in multicellular organisms in synthesis of steroid hormones. The substrates are usually rather water-insoluble in contrast with those of the 2-oxoglutarate-dependent examples. The multiplicity of these iron proteins definitely reflects the stages of evolution matching the rise in oxygen. Another example is the multiplicity of Fe/S proteins which were required to generate more electron-transfer pathways starting from the primitive hydrogenase-like proteins. The diversification extends from Fe_2S_2 to Fe_4S_4 proteins.

A source of thinking about gene multiplication is provided by the immune system in which duplication plus mutation is common and fast [10]. A similar fast rate of evolution is known in resistance to drugs such as methatrexate where it is again known that gene multiplication occurs. Clearly these fast multiplications must arise locally from an interruption of DNA as it is reproduced. Mutation is at an increased rate within the duplicates. In the immune system the duplication and protection occur in special cells and they are not inherited but drug resistance is reproducible. How??

Now there is a parallel set of observations in resistance of bacteria to various poisons. The new DNA appears in satellite, plasmid, DNA, Fig. 3. When this is mapped it contains resistance genes to modern poisonous metal ions in the same plasmid as that for drug resistance. This parallel suggests that novel metal ions have the

Table 2
Local DNA (gene) duplication.

Organism	Numbers of very similar proteins					
	Peroxidase	P450 Heme	Cu oxidases EC:1	Fe oxidases 2OG-FeII	Zn fingers	Calmodulin
Eubacteria	1	1	3	1?	5	0
Yeast	1	3	9	1	373	4
Arabidopsis	95	268	111	116	1631	80
Homo sapiens	0	70	21	9	2984	160

Note: Proteome size from top ~3500, 6000, 32 615, 37 742.

Fig. 4. An illustration of the completely interactive system of the environment and organisms and of organisms amongst themselves. It is suggested that the left-hand side before later organisms, labelled "man", that is before 0.54 Ga, replaces the Darwin idea of a tree which becomes more clearly valid in the stages leading to higher plants and animals that is from 0.54 Ga to today (Ga = a billion years ago).

same effect as other poisons on the DNA. Now the metal ions, including iron, are the main chemicals in the environment which were oxidised chiefly in the steps of oxygen increase. We are led to suggest that the major pathway to evolution of not only iron but many other proteins is by local, that is directed duplication, and not by random searching.

There is a way novel metal ions or organic chemicals could bring about duplication of given local stretches of DNA. Let us consider an enzyme which binds to either of these poisons. The relief of the stress in the cell requires it to increase concentration of the protein which is reflected in an increase of its RNA production. This need will be reflected further to the local sequence of the related DNA to the RNA when this sequence will remain exposed for a longer time so that it becomes open to enzyme action by, say, methylation of histones or conversion of thymidine to uridine by the action of thymine deaminase. It is known that disruption in reading causes duplication. This is nothing more than a suggestion but it does give a way in which quite novel metallo-proteins may arise to overcome environmental stress in a non-random way. Once the mechanism of relief of stress from a poison has evolved in one organism it can be transferred to other organisms. The interaction between organisms is now known to be quite intense by gene transfer and symbiosis. The idea of a tree of evolution by chance response along the branches of a tree must be replaced by a network in which the environment change, generating duplication, and gene transfer play roles as well as mutation. We have attempted to picture this progression of life in which iron is deeply involved, Fig 4 [3].

While writing this paper I have had in mind the interest and possibly the involvement in it by Antonio Xavier, since he had a strong concern with the biological chemistry of iron.

Acknowledgments

This article is in large part taken from a much wider account of evolution based on the chemistry of all the elements of the Periodic Table and as many organisms as possible. The account has been submitted as a book, entitled "Evolution's Destiny: Co-Evolution of the Environment and Life", by myself and Dr Rosalind Rickaby of the Earth Sciences Department, University of Oxford [3].

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