16 Physiology and Chemistry of Edible Muscle Tissues

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CONTENTS

16.1 INTRODUCTION

Archeological evidence indicates that humans have utilized animal products, including meat, as sources of food for thousands of years. This fact was dramatically illustrated following the discovery by mountain hikers in 1991 of Eis Mann (Ice Man), the frozen remains of a man found in a glacier high in the Italian Alps. Ötzi, as he affectionately came to be known by the area residents, had apparently died approximately 5100–5300 years ago. Various lines of evidence suggest that he was a hunter who may have died of arrow wounds as a result of a rivalry with another hunter or hunting groups [1]. His body was so well preserved by the glacial ice that it was possible for scientists to use recombinant DNA methodology to analyze the contents of his gastrointestinal tract in order to determine what he had eaten in his last two meals. The penultimate meal prior to his death consisted of meat from an ibex (a type of wild goat once found in the Alps) as well as cereal grains and other types of plant food. His final meal included red deer meat and possibly cereal grains.

Over the course of history, some cultures have eaten meat as a matter of choice, while others have done so as a matter of necessity. Likewise, religious and cultural factors have had considerable influence in defining meat that is considered acceptable for consumption vs. meat that is considered unacceptable. For example, some religious groups prescribe avoidance of pork whereas others mandate avoidance of beef. In the United States, eating horse meat is culturally considered taboo, while it is acceptable in other countries. Still other individuals avoid meat completely for ethical reasons or presumptive health concerns. Nevertheless, it is also evident that as economies of nations grow, particularly in developing countries, there is a parallel growth in demand for meat products.

The term "meat" in colloquial use sometimes connotes red muscle food (beef, pork, lamb), while poultry and fish muscle foods are in classes by themselves. In this chapter, we utilize the term "meat" more broadly to refer to skeletal muscle tissue from a mammal, bird, reptile, amphibian, or fish that has undergone a specific series of transformative biochemical reactions following death of the animal. This term specifically excludes other organ meats such as liver, thymus, and kidney, but does include heart and tongue as unique muscle tissues. The purpose of this chapter is to provide the reader with an essential understanding of muscle physiology and biochemistry, which in turn lays the foundation for understanding the biochemical events in conversion of muscle to meat and of subsequent processing factors related to the functional properties of meat components.

16.2 NUTRITIVE VALUE

The positive sensory appeal of fresh and processed meat products, as well as the feeling of satiety following consumption of a meal including meat, have combined to make muscle food products staples of human diets around the world. The high nutrient density of meat, that is, the concentrations of nutrients per kilocalorie, the variety of nutrients present in the tissues, and the excellent bioavailability of nutrients combine to make meat a significant source of nutrition for consumers.

The composition of meat is quite variable. Species, breed, sex, age, nutritional status, and activity level of the animal are major factors affecting the gross composition of meat [2]. Moreover, even for a given animal, the anatomical location of the retail cut, postslaughter processing, storage, and of course, cooking, contribute significantly to the variability of meat composition. It is beyond the scope of this chapter to examine these factors beyond a few generalizations discussed below. Detailed information on composition of meat products by species, retail cut, degree of trim, raw vs. cooked product, and so forth is available from the USDA and is periodically updated [3]. However, even these values must be regarded as approximations, given the many sources of variation.

The proximate composition of separable lean tissue (skeletal muscle trimmed of external fat) is somewhat variable, but in general, water accounts for about 70% of the weight of fresh, lean muscle (Table 16.1). The water is largely trapped within or between the muscle cells, with lesser amounts bound in varying degrees to proteins. Variation in water content is generally offset by changes in

TABLE 16.1
Proximate Composition of Meat from Various Sources[a]

	Red Meat			Poultry		Fish	
	Beef	**Pork**	**Lamb**	**Chicken**	**Turkey**	**Cod**	**Tuna**
Water	70.62	72.34	73.42	74.76	74.12	81.22	68.09
Protein	20.78	21.07	20.29	23.09	24.60	17.81	23.33
Lipid	6.16	5.88	5.25	1.24	0.65	0.67	4.90
Ash	1.02	1.04	1.06	1.02	1.02	1.16	1.18

[a] Percent by weight of edible portion.

Source: Compiled from U.S. Department of Agriculture, Agricultural Research Service (2005). *Composition of Foods Raw, Processed, Prepared.* USDA National Nutrient Database for Standard Reference, Release 18. Nutrient Date Laboratory Home Page (http://www.nal.usda.gov/fnic/foodcomp/).

TABLE 16.2
Lipid Content of Various Meats

		Content[a]		
Species	**Muscle or Type**	**Lipid (%)**	**Neutral Lipids (%)**	**Phospholipids (%)**
Chicken	White	1.0	52	48
	Red	2.5	79	21
Turkey	White	1.0	29	71
	Red	3.5	74	26
Fish (sucker)	White	1.5	76	2
	Red	6.2	93	7
Beef	Lattisimus dorsi	2.6	78	22
		7.7	92	8
		12.7	95	5
Pork	Lattisimus dorsi	4.6	79	21
	Psoas major	3.1	63	37
Lamb	Lattissimus dorsi	5.7	83	10
	Semitendinosus	3.8	79	17

[a] Percentage of gross muscle composition.

Source: Allen, C.E. and E.A. Foegeding (1981). *Food Technol*. 35:253–257.

lipid composition, while protein composition ranges from 18% to 23%, and ash or mineral content is approximately 1–1.2% (Table 16.1).

The lipid content and composition are the most variable of the four primary components of meat. Since the lipid fractions associated with muscle tissue and adipose tissue vary in quantity and composition, the amount of adipose tissue present in a meat product profoundly affects the proximate composition of the product [4]. Moreover, as the adipose tissue content of meat decreases, the percentage of phospholipid contribution to the total increases (Table 16.2). Most of the lipid in meat consists of neutral triacylglycerols, lesser amounts of phospholipids that comprise cellular membranes, and a small amount of cholesterol found primarily in the muscle plasma membrane and nervous tissue. Fatty acids in the neutral fat fraction tend to be more highly saturated than those of the

TABLE 16.3
Fatty Acid Composition of Meat from Various Sources[a]

	Red Meat			Poultry		Fish	
	Beef[b]	Pork[c]	Lamb[d]	Chicken[e]	Turkey[f]	Cod[g]	Tuna[h]
Total saturated	37.66	34.52	35.81	26.61	32.31	19.55	25.65
14:0	2.76	1.19	2.67	0.81	0.00	1.34	2.84
16:0	22.73	21.94	19.43	16.94	13.85	13.58	16.53
18:0	11.85	10.88	11.81	8.06	9.23	4.48	6.27
Total monounsaturated	42.37	45.24	40.19	24.19	16.92	14.03	32.65
16:1	3.73	3.23	3.05	2.42	1.54	2.39	3.31
18:1	38.31	41.16	36.38	20.16	13.85	9.10	18.86
Total polyunsaturated	3.90	10.71	9.14	22.58	26.15	34.48	29.24
18:2	3.08	8.67	6.86	13.71	16.92	0.75	1.08
18:3	0.32	0.34	1.33	0.81	0.00	0.15	0.00
20:4	0.49	1.19	0.95	3.23	4.62	3.28	0.88

[a] Percent of total fat in lean compiled from all retail cuts for red meat and in breast for poultry meat and in whole fish. Calculated based on the information compiled from U.S. Department of Agriculture, Agricultural Research Service (2005). *Composition of Foods Raw, Processed, Prepared*. USDA National Nutrient Database for Standard Reference, Release 18. Nutrient Date Laboratory Home Page (http://www.nal.usda.gov/fnic/foodcomp/).

[b] Beef, composite of trimmed retail cuts, separable lean only, trimmed to 1/4" fat, all grades, raw.

[c] Pork, fresh, composite of trimmed retail cuts (leg, loin, shoulder), separable lean only, raw.

[d] Lamb, domestic, composite of trimmed retail cut, separable lean only, trimmed to 1/4" fat, choice, raw.

[e] Chicken, broilers or fryers, breast, meat only, raw.

[f] Turkey, fryer-roasters, breast, meat only, raw.

[g] Cod, Atlantic, raw.

[h] Tuna, bluefin, raw.

phospholipid fraction, which is not surprising from a functional standpoint. The necessity of a fluid cell membrane at physiological temperatures requires that phospholipids have a higher percentage of unsaturated fatty acids, while a high fraction of saturated fatty acids with their higher melting points is required to maintain the integrity of adipose tissue. Despite the smaller contribution of phospholipid fraction to the total lipid composition, the polyunsaturated nature of the phospholipids together with the high surface to volume ratio makes this lipid highly susceptible to oxidative reactions that contribute to deterioration of flavor and color of meat [5].

Lipid composition varies by species, with the highest levels of polyunsaturated fatty acids found in fish, and lowest amounts in beef and mutton (Table 16.3). Lipid composition also varies from muscle to muscle within a species, particularly when comparing muscles in which most of the fibers rely on oxidative metabolism (red muscles) to muscles that generally rely on glycolytic metabolism (white muscles).

The protein content of meat is typically derived from analysis of total nitrogen content of the product, multiplied by 6.25, a factor based on the average nitrogen content of meat protein. However, this approach overestimates the amount of protein, because as much of 10% of muscle nitrogen comes from nonprotein sources including amino acids, peptides, creatine, nucleic acids, and other nitrogen-containing molecules.

Meat is an excellent source of dietary protein because the amino acid composition closely parallels human dietary amino acid requirements. The high quality together with the relative abundance of

TABLE 16.4
Mineral and Vitamin Composition of Meat from Various Sources[a]

	Red Meat			Poultry		Fish		DRI[b]
	Beef	**Pork**	**Lamb**	**Chicken**	**Turkey**	**Cod**	**Tuna**	**RDA or AI***
Minerals								
Potassium	356	380	280	255	293	413	252	4,700/4,700*
Phosphorus	199	211	189	196	206	203	254	700/700
Sodium	63	57	66	65	49	54	39.0	1,500/1,500*
Magnesium	22	23	26	28	28	32	50.0	420/320[c]
Calcium	6	16	10	11	10	16	8.0	1,000/1,000*
Zinc	4.33	2.08	4.06	0.8	1.24	0.45	0.60	11/8
Iron	2.13	0.91	1.77	0.72	1.17	0.38	1.02	8/18
Vitamins								
Thiamin	110	966	130	70	41	76	241	1,200/1,100
Riboflavin	180	273	230	92	118	65	251	1,300/1,100
Niacin	3,590	4,829	6,000	11,194	6,255	2,063	8,654	16,000/14,000
Pantothenic Acid	360	788	700	819	717	153	1,054	5,000/5,000*
B_6	440	500	160	550	580	245	455	1,300/1,300
Folate	7.0	5.0	23.0	4.0	8.0	7	2.0	400/400
B_{12}	3.25	0.67	2.62	0.38	0.47	0.91	9.43	2.4/2.4

[a] Values are expressed as mg/100 g and μg/100 g for minerals and vitamins, respectively. *Source:* Compiled from U.S. Department of Agriculture, Agricultural Research Service (2005). *Composition of Foods Raw, Processed, Prepared*. USDA National Nutrient Database for Standard Reference, Release 18. Nutrient Date Laboratory Home Page (http://www.nal.usda.gov/fnic/foodcomp/).

[b] DRI (Dietary Reference Intakes) values are expressed as RDA (Recommended Dietary Allowances) or AI* (Adequate Intake) for male/female adults (age 19–50). *Source:* Compiled from Food and Nutrition Board, Institute of Medicine, National Academy of Sciences. (1997). Dietary Reference Intakes for calcium, phosphorus, magnesium, vitamin D and floride; Food and Nutrition Board, Institute of Medicine, National Academy of Sciences. (1998). Dietary Reference Intakes for thiamin, riboflavin, niacin, vitamin B_6, folate, vitamin B_{12}, pantothenic acid, biotin, and choline; Food and Nutrition Board, Institute of Medicine, National Academy of Sciences. (2000). Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc; and Food and Nutrition Board, Institute of Medicine, National Academy of Sciences. (2004). Dietary Reference Intakes for water, potassium, sodium, chloride, and sulfate.

[c] Age 31–50.

protein in meat means that a single 85-g serving of meat may provide 50–100% of the daily protein intake recommended for maintenance of growth and health [6]. Moreover, the complete amino acid composition of meat enables complementation of other dietary sources of protein. For example, even a small amount of meat added to a cereal- or legume-based diet, which would be deficient in lysine and sulfur amino acids, respectively, dramatically improves the nutritional value of the plant-derived protein.

Muscle tissue is an excellent source of many water-soluble vitamins including thiamin, riboflavin, niacin, B_6, and B_{12} (Table 16.4). However, as with other nutrients, the vitamin content is strongly influenced by species, age, sex, and nutritional status of the animal. Most noteworthy are the very high levels of thiamin and the low levels of B_{12} in pork compared with beef and lamb. Vitamins C, D, E, and K tend to be low in all muscle foods. However, studies have indicated that levels of vitamin E in meat can be significantly elevated through increased dietary supplementation. Because vitamin E functions as an antioxidant, its presence at elevated levels may have a significant beneficial effect in stabilizing meat color, reducing lipid oxidation, and enhancing human health [7].

Red meats are particularly good sources of iron because of their high myoglobin content; however, even white muscle of poultry and fish can be significant sources of iron (Table 16.4). Moreover, the heme form of the iron imparts a high degree of bioavailability compared with most inorganic sources of iron. Potassium, phosphorus, and magnesium are relatively abundant in meat. Calcium, despite its importance in regulating muscle contraction, is present in muscle at very low levels relative to dietary requirements. In mechanically separated meats, calcium may be present at higher levels because of the presence of small amounts of microscopic bone fragments present in the final product [8].

Carbohydrates make only a small contribution to the composition of fresh meat (<1%). The major source of carbohydrate in muscle is glycogen, with minor amounts of monosaccharides and glycolytic metabolites. During conversion of muscle to meat, glycogen is largely converted to lactate by anaerobic glycolysis, thus making lactate the primary carbohydrate in meat [9].

16.3 STRUCTURE AND FUNCTION OF MUSCLE

16.3.1 Structure of Skeletal Muscle

Individual skeletal muscles vary greatly in size and morphology. In general, they consist of a parallel arrangement of elongated, multinucleated cells called myofibers or muscle fibers. Individual myofibers range from 10 to 100 μM in width, and from a few millimeters to several centimeters long, sometimes spanning the entire length of a muscle. Myofibers are arranged in hierarchical fashion with associated blood, circulatory, nerve, and blood tissues forming the whole muscle organ (Figure 16.1). Each myofiber is encased in a layer of connective tissue called the endomysium. Groups of myofibers are organized into primary and secondary bundles or fascicles that are segregated by another layer of connective tissue called the perimysium. A final layer of heavy connective tissue sheaths, the epimysium, surrounds the whole muscle. These sheaths merge with the connective tissue tendons to link the muscle to the bones. The molecular and structural properties of connective tissue are described in Section 16.3.4.1.

Muscle is infiltrated by a complex system of nerves involved in the regulation of muscle contraction and maintenance of muscle tone, as well as a vascular system through which blood provides oxygen and nutrients while removing metabolic end products (Figure 16.1). The perimysium and endomysium combine to provide the necessary framework for maintaining the structural integrity of these tissues within the muscle at rest, and more importantly during the mechanical stress of contraction. Adipose tissue may also be found embedded in the perimysial layer and is visible in red meats as white flecks of fat (marbling) in contrast to the red background of myofibers. The abundance of marbling is often used as an indicator in visual appraisal of meat quality [10].

The unique structure of muscle cells enables translation of electrochemical impulses, triggered by neural stimulation, into increased intracellular calcium concentrations that in turn trigger muscle contraction. Like all cells, a myofiber is bounded by a plasma membrane, referred to as the sarcolemma (SL) in a muscle (Figure 16.2). However, the skeletal muscle SL is distinguished from the plasmalemma of other cells by periodic invaginations of the membrane into the interior of the muscle cell, much like fingers poking into the skin of a balloon. These inward extensions of the SL, referred to as transverse tubules or T-tubules, transmit the action potential or depolarization signal for contraction from the neuromuscular junction to the interior of the myofiber. The T-tubules are in physical contact at periodic intervals with an extensive, highly developed intracellular membrane network called the sarcoplasmic reticulum (SR), which is the muscle equivalent of the endoplasmic reticulum (Figure 16.2). The SR network encircles the contractile organelles (myofibrils) and functions as a reservoir of calcium ions that serve as the trigger for muscle contraction. Numerous proteins embedded in the SR are responsible for specific functions related to calcium regulation. Some proteins in the interior (lumen) of the SR bind calcium ions while the muscle is at rest [11]. Other proteins form channels that open in response to the depolarization signal, thereby allowing diffusion of calcium ions from within the SR to the sarcoplasm; this process triggers muscle contraction [12]. Another SR

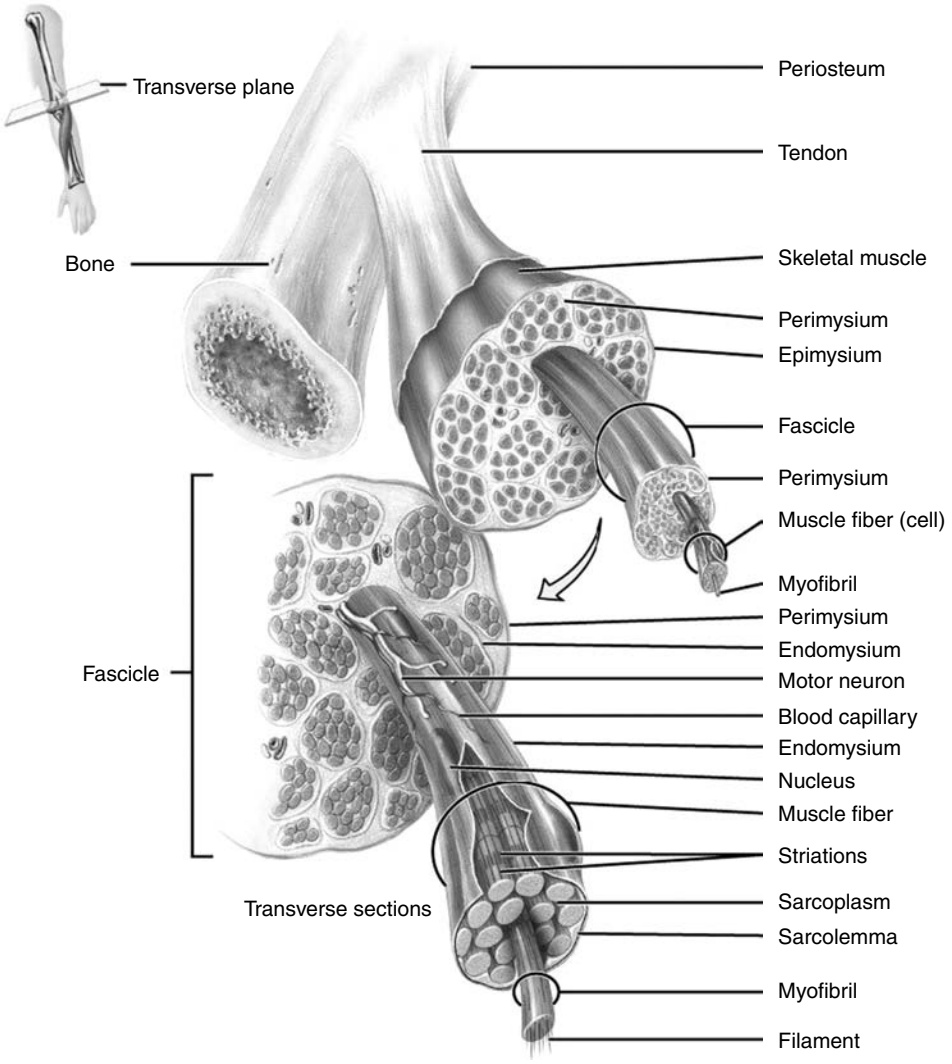


FIGURE 16.1 Diagrammatic representation of the structural organization of muscle from subcellular myofibrils to whole organ. Individual muscle cells (fibers) are surrounded by a layer of connective tissue (endomysium), which, in turn, are organized into bundles (fascicles), separated by another layer of connective tissue called the perimysium. Blood vessels and nerves penetrate the perimysium as supporting tissues for muscle function. (Reprinted from Tortora, G.J. and B. Derrickson (2006). *Principles of Anatomy and Physiology*. John Wiley and Sons, Inc., Hoboken, NJ.)

protein pumps calcium back into the lumen of SR during relaxation. These proteins are discussed in greater detail in Section 16.3.4.6.

Muscle cells possess other organelles typical of all cells. Because of the developmental path by which skeletal muscle cells develop, myofibers are typically multinucleated (Figure 16.2). The nuclei are usually dispersed to the periphery of the cell, and are typically found immediately beneath the SL. Mitochondria serve as energy transducers for the myofiber and are found throughout the cell in close association with myofibrils. Lysosomes serve as a major reservoir for a family of proteolytic enzymes known as cathepsins, which play a catabolic function in protein turnover.

The sarcoplasm (cytoplasm) of muscle may contain glycogen particles and lipid droplets, the quantity of which depend on the type of muscle fiber (oxidative or nonoxidative), and the nutritional

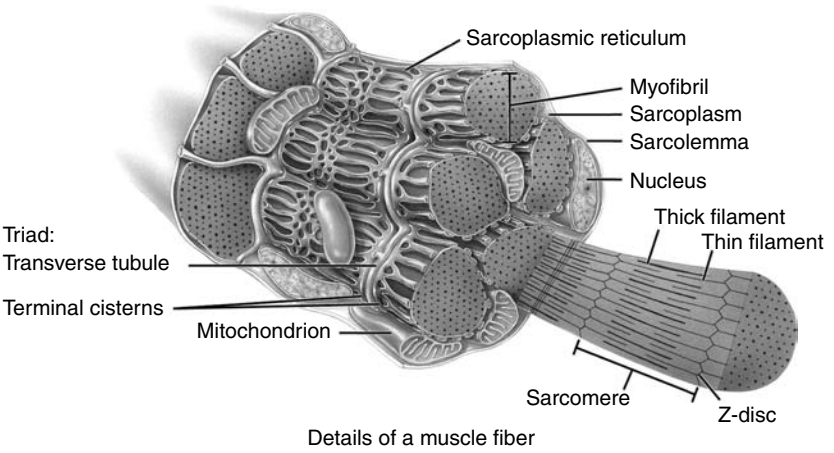


FIGURE 16.2 Schematic representation of the structural organization of a muscle fiber. Multinucleated muscle fibers are encased by the sarcolemma. Invaginations of the sarcolemma into the center of the muscle fiber form structures called transverse tubules (T-tubules). Each T-tubule is connected with two terminal cisternae of the SR, forming a structure called a triad. The SR is an organelle that envelopes the myofibrils, storing calcium ions when muscle is at rest, and releasing calcium ions to the sarcoplasm during muscle contraction. (Reprinted from Tortora, G.J. and B. Derrickson (2006). *Principles of Anatomy and Physiology*. John Wiley and Sons, Inc., Hoboken, NJ.)

and exercise/resting state of the organism. The oxygen-storage protein myoglobin is found to varying degrees within the sarcoplasm, as are various enzymes, metabolic intermediates, and other compounds such as nucleotides, amino acids, and so forth.

Muscle contraction is effected through the action of specialized proteins that are organized into parallel, interdigitating thin and thick filaments (myofilaments) that comprise 80–90% of the volume of the myofiber. Myofilaments are grouped into myofibrils that function in coordinated fashion as the contractile organelles of a muscle cell. The high degree of structural organization of the myofibrils is evident when thin longitudinal sections of skeletal muscle are viewed with a microscope. One sees a pattern of alternating light and dark bands that result from longitudinal repetition of the fundamental structural unit of muscle contraction known as the sarcomere (Figure 16.3a). When viewed with polarized light, the dark bands are anisotropic and are thus referred to as "A-bands." The lighter bands, "I-bands," are isotropic in polarized light. The boundaries of the sarcomere are defined by structures known as Z-discs, which are narrow, dark, electron-dense bands of proteins in the center of the I-band. The term Z-disc is derived from the German *zwischen* meaning "between," indicating its position at the center of the I-band. The matrix of proteins that constitutes the Z-disc serves as the anchoring structure for the proteins of the thin filaments that emanate from both sides of the Z-disc.

The sarcomere consists of alternately placed thin and thick filaments. The I-band consists of thin filaments, while the A-band consists of overlapping thin and thick filaments. The center of the A-band is slightly less dense than the distal regions and therefore appears brighter because this zone consists only of thick filaments with no overlapping thin filaments. This band is called the H-zone, derived from the German *helle* meaning bright. At the center of the H-zone is a dark zone analogous to the Z-disc. This structure, called the M-line, consists of proteins that maintain the structural arrangement of the thick filament proteins and serve as an anchoring point for the protein titin that spans from the M-line to the Z-disc (see Section 16.3.4.5).

In 1954, Huxley and Hanson proposed a theory of muscle contraction, called the sliding filament theory, which has survived largely intact to the present era [13]. The theory is based on the observation that lengths of both thin and thick filaments remain constant, independent of whether the muscle is stretched, contracting, or in the resting state. In contrast, sarcomere lengths, defined as the distance between adjacent Z-discs, vary depending on the state of contraction or stretch force applied to

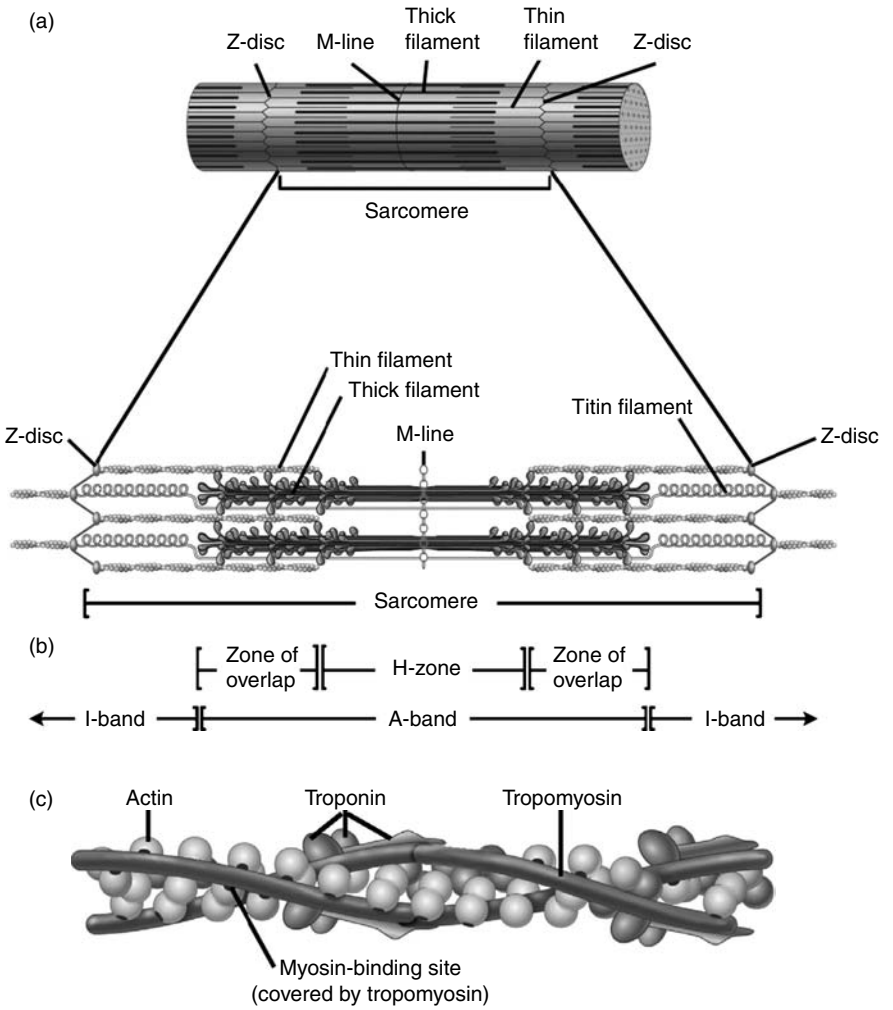


FIGURE 16.3 Structural arrangement of the sarcomere. (a) The sarcomeric unit of a myofibril begins at one Z-disc and extends to the next Z-disc. At the center of each sarcomere is an arrangement of proteins that form the M-line. (b) The major components of the sarcomere are thin filaments, which are anchored at the Z-disc, thick filaments in the central region of the sarcomere that partially overlap with the thin filaments, and titin filaments that span from the Z-disk to the M-line. (c) Actin monomers polymerize to form a double-stranded coiled coil that constitutes the backbone of the thin filament. Tropomyosin polymerizes in "head-to-tail" fashion and lies near the groove of the actin double helix, covering myosin-binding sites on the actin backbone. One tropomyosin molecule spans seven actin monomers. One troponin molecule (consisting of three subunits) binds to one tropomyosin molecule via the asymmetrically shaped troponin-T subunit. (Reprinted from Tortora, G.J. and B. Derrickson (2006). *Principles of Anatomy and Physiology*. John Wiley and Sons, Inc., Hoboken, NJ.)

the fiber. In addition, electron micrographs of muscle cross sections indicated that the thin and thick filaments interdigitate in such a way that a thick filament is surrounded by an array of six thin filaments. Huxley and Hanson proposed that when contraction takes place, the thin and thick filaments slide past each other such that the thin filaments at opposite ends of a sarcomere move toward each other. This results in shortening of the sarcomere length (Figure 16.4). Conversely, stretching results from an increase in Z-disc separation, again accomplished by sliding of thin filaments of a sarcomere away from each other as they move along the A-band. The extent to which thin and thick filaments overlap has great practical significance with respect to meat tenderness. As we shall see

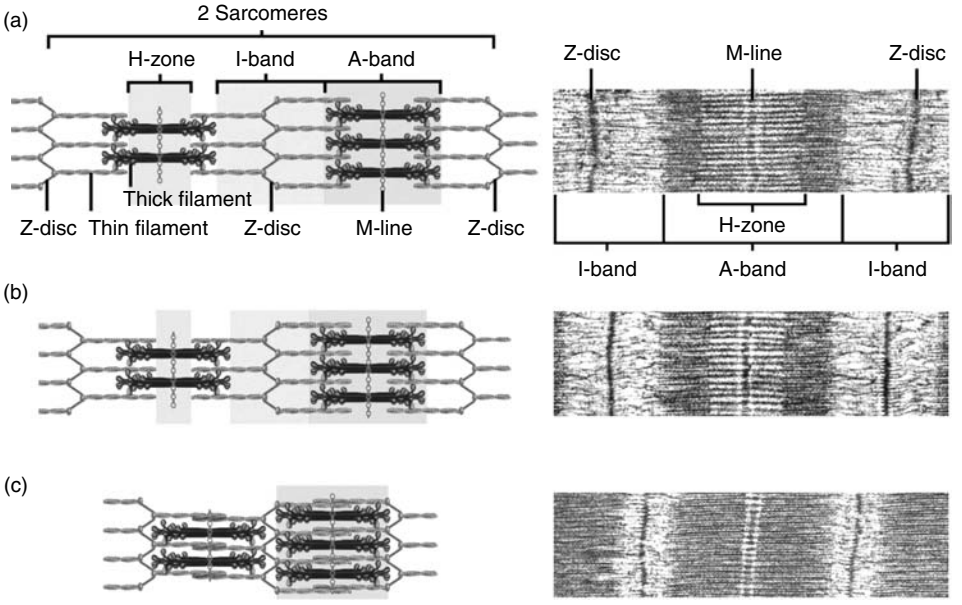


FIGURE 16.4 Illustration of the sliding filament theory: (a) Relaxed; (b) Partially contracted and (c) Maximally contracted. Thin filaments and thick filaments interdigitate allowing for sliding of filaments past each other. Note the shortening of the distance between Z-discs as the muscle undergoes contraction, as well as the increasing overlap of thin and thick filaments as muscle shortens. (Reprinted from Tortora, G.J. and B. Derrickson (2006). *Principles of Anatomy and Physiology*. John Wiley and Sons, Inc., Hoboken, NJ.)

later (Section 16.5.3), there is generally a negative correlation between sarcomere length and meat toughness. When muscle is maximally contracted, the sarcomeres are at their shortest, and the high degree of overlap between filaments together with the large number of rigor bonds formed between the two types of filaments result in increased toughness. The molecular details of how the sliding of filaments results in muscle contraction will be discussed in Section 16.3.5.

16.3.2 Structure of Cardiac Muscle

Heart muscle may be used as a food directly, or more commonly may be minced and incorporated into processed meat products such as sausages. Like skeletal muscle, cardiac muscle is striated, which suggests an arrangement of the contractile proteins of cardiac muscle which is similar to that of skeletal muscle. From an anatomical point of view, the fiber arrangement is somewhat less regular than skeletal muscle fibers, and in contrast to the multinucleated skeletal muscle fibers, cardiac muscle typically has only 1–2 nuclei that are centrally located. Although the proteins comprising the cardiac contractile machinery are the same as skeletal, the isoforms are often specific to cardiac muscle. The differences in amino acid sequence between the skeletal and cardiac isoforms impart differences in protein functionality between proteins from these tissues. In addition, the signaling mechanism for excitation of cardiac muscle fibers and the mechanism of calcium release differ from that of skeletal muscle. All of these factors have important implications with respect to postmortem metabolism of cardiac muscle and utilization of cardiac muscle as a food. A detailed comparison is beyond the scope of this chapter. The reader is advised to consult other references for more details on cardiac muscle biology [14,15].

16.3.3 Structure of Smooth Muscle

Some smooth muscles (e.g., gizzard, stomach, and intestine) are consumed as specialized organ foods. Unlike skeletal and cardiac muscles, smooth muscles do not exhibit the pattern of striation

when longitudinal sections are viewed under the microscope; hence, the term "smooth muscle." This results from a relatively unstructured arrangement of contractile proteins within smooth muscle. Many of the same proteins are involved in smooth muscle contraction as in skeletal muscle, but some are notably absent, and there exist multiple mechanisms of regulation of muscle contraction among different smooth muscles as well as among species. As with cardiac muscle, even when the same proteins are present (e.g., myosin, actin, tropomyosin), the isoforms expressed are tissue-specific and differ sufficiently from the skeletal isoforms such that their functional properties in processed meat products may differ substantially from that of skeletal muscle. The reader is referred to other references for more details on the physiology of smooth muscle [16].

16.3.4 Proteins of the Muscle Tissue

Proteins in skeletal muscle have been categorized according to solubility or biological function. Categories related to biological function generally refer to a protein's contribution to muscle structure, contraction, and metabolism, among others. The solubility category is classically based on differential solubilization of muscle proteins at varying salt concentrations that yields three primary classes of proteins. These classes generally correlate with cellular localization and are identified as (1) sarcoplasmic proteins, (2) myofibrillar proteins, and (3) stromal proteins.

As the name implies, sarcoplasmic proteins comprise proteins found in the sarcoplasm of the myofiber including the glycolytic enzymes, myoglobin, and other enzymes involved in metabolism. These proteins are sometimes termed "water-soluble" proteins because they can be dissolved at low ionic strength ($>$0.3 mM). This fraction constitutes about 30% of the total muscle protein content [17].

The myofibrillar class of proteins constitutes the largest fraction (50–60%) of muscle protein. These proteins require high salt concentrations (e.g., $>$0.3 M NaCl) for solubilization; thus, they are sometimes referred to as the "salt-soluble fraction" of muscle proteins. In muscle tissue, the physiological salt concentration is approximately 0.15 M. This concentration is sufficiently low to prevent these proteins from dissolving in the sarcoplasm, thereby maintaining the complex quaternary structure of the myofilaments.

Myosin and actin, the primary constituents of the thick and thin filaments, respectively, comprise about 65% of the total myofibrillar protein content and about 40% of the total muscle protein content [18] (Figure 16.3a). On the basis of abundance, the chemical behavior of these two proteins in salt solutions accounts for the solubility properties of this group as well as for the development of processed meat products. It must be noted that although the myofibrillar proteins are generally equated with high salt solubility, this is a broad generalization. For example, in some cases complete solubilization of cod myofibrillar proteins has been observed at very low ionic strength ($<$0.0002) [19]. Other myofibrillar proteins such as the troponin complex, when purified, are also soluble at very low ionic strength.

The quality characteristics of fresh meat products are also highly dependent on the abundance and composition of the stromal proteins, which constitute 10–20% of total muscle protein content. The content of stromal proteins varies with species, age, and muscle [20]. These proteins are generally insoluble under the usual conditions of extraction: near-neutral pH, low-salt or high-salt concentrations, and cold temperatures. Collagen, the most abundant protein in the body, is the dominant protein in the stromal fraction. The stromal proteins form the connective tissue layers described previously that strengthen and protect muscles; thus, there may be some correlation of collagen quantity and quality with meat toughness [21]. In addition, collagen molecules are covalently cross-linked (see below), and the number of cross-links increases with age of the animal [22]. The significance of collagen to meat quality is highlighted by the fact that various processing and cookery methods for meat are designed to disrupt and partially solubilize collagen fibers, thereby enhancing meat tenderness [23].

16.3.4.1 Connective Tissue Proteins and Matrix

As noted above, connective tissues permeate the muscle in the form of epimysium, perimysium, and endomysium, and these structures give rise to the strong matrix necessary to support muscle function while maintaining a degree of elasticity [24]. Connective tissue consists of a variety of cell types, including fibroblasts that synthesize collagen, macrophages, lymphoid cells, mast cells, and eosinophils. There is also an extracellular matrix that is composed of fibrous and non-fibrous proteins including members of the collagen family, elastin, and the proteoglycans. Until rather recently, the primary role of extracellular matrix was thought to be maintenance of the structural integrity of the muscle. Since the mid-1990s, it has become apparent that the extracellular matrix plays key roles in regulating the behavior of surrounding cells [25]. The extracellular matrix communicates information to cells via integrin receptors embedded in plasma membranes (Figure 16.5). Integrins serve as linkers between the intracellular cytoskeletal network and the extracellular matrix [26]. Through the integrins, the extracellular matrix regulates and modulates various activities including gene expression, migration, adhesion, proliferation, and differentiation. Differentiation of skeletal muscle is absolutely dependent on proteoglycan synthesis [27], and the types of proteoglycans expressed change as development proceeds [28]. Moreover, it has been suggested that reduced expression level of extracellular matrix proteins [29] as well as postmortem degradation of integrins may contribute to the excessive drip loss associated with poor meat quality [30].

The dominant protein of connective tissue is collagen. The term "collagen" actually refers to a family of at least 27 different protein isoforms found in connective tissues throughout the body including bone, tendon, cartilage, blood vessels, skin, teeth, as well as muscle [31]. Collagen contributes significantly to toughness of mammalian muscle, and in semipure form, it is an important functional ingredient in various food products such as gelatin. Skeletal muscles from small animals and fish have fewer requirements for weight-bearing strength, and tend to be composed of lesser

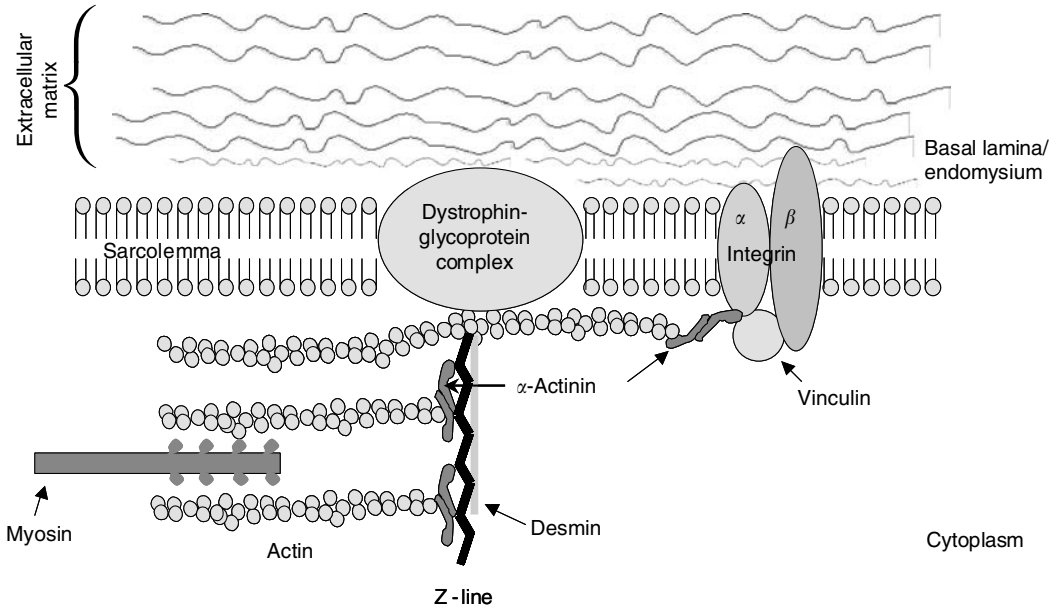


FIGURE 16.5 Diagrammatic representation of the localization and the interaction of extracellular matrix proteins with the intracellular cytoskeletal protein network. Extracellular matrix proteins connect to the intracellular cytoskeleton via the integrin complex embedded in the sarcolemma. (Modified from Lewis, M.P., J.R.A. Machell, N.P. Hunt, A.C.M. Sinanan, and H.L. Tippett (2001). *Eur J Oral Sci* 109:209–221.)

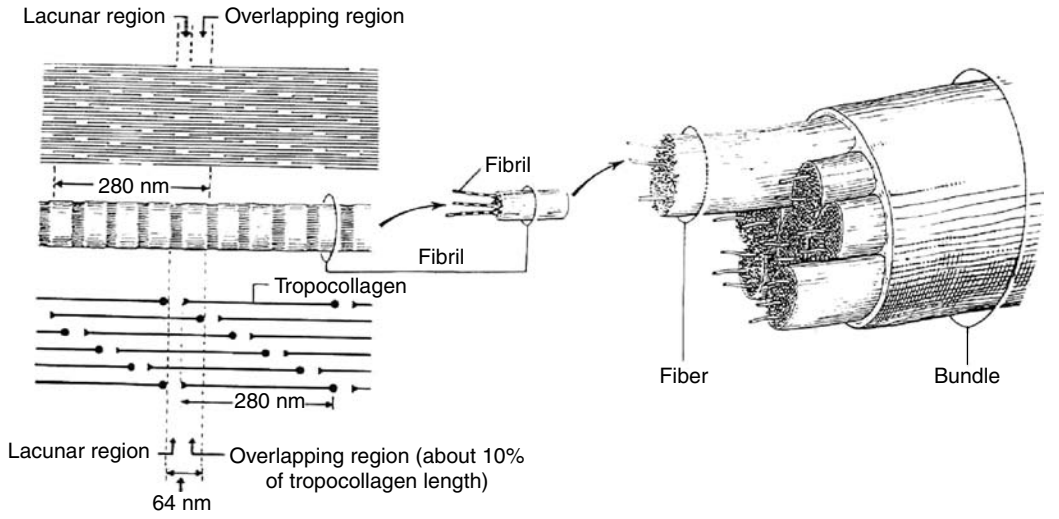


FIGURE 16.6 Formation of collagen fibrils. Tropocollagen units assemble in staggered, side-by-side arrays with head–tail overlap. Gaps and overlaps created in the staggered arrangement of tropocollagen units give rise to the appearance of light zones (lacunar regions) and dark zones (overlapping regions). Collagen fibrils encase muscle fibers, fiber bundles, and whole muscle. (Reprinted from Junqueira, L.C., J. Carneiro, and R.O. Kelley (1989). *Basic Histology*. Appleton & Lange, Norwalk, CT.)

levels of collagen and of lesser cross-linking relative to collagen in larger land animals. This accounts for collagen contributing less to tough texture in meat from small animals and fish.

The collagens are grouped according to the supramolecular structures that they form. These groups include (1) striated, fibrous; (2) nonfibrous, network forming; (3) microfibrillar or filamentous; and (4) fibril-associated. A few examples of types of collagen associated with muscle follow.

Types I, III, and V collagens are members of the striated collagen family. Examination of these collagens by electron microscopy reveals repeating bands every 64–70 nm, resulting from staggered side-by-side arrays of collagen molecules (Figure 16.6). These three collagen types are associated with epimysium that consists of only type I, and perimysium, which includes both types I and III with some type V [32]. Some of the older muscle literature refer to a heat-resistant, stromal protein fraction called reticulin that forms fibers associated with the perimysium. It is now clear that these fibers are type III collagen fibers, the abundance of which is correlated with meat toughness [33].The endomysium consists primarily of type IV collagen, which belongs to the network-forming collagen group [32]. Unlike the striated, fibrous collagens, type IV collagen forms a sheet-like appearance that resembles the structure of a chain-link fence. This structure arises from differences in amino acid sequence that prevent the side-by-side association observed in the striated collagen family. Small amounts of types I, III, and V are also associated with the endomysium.

The basic unit of collagen is called tropocollagen; it consists of three polypeptide chains that entwine around each other in a coiled-coil, superhelical fashion forming a linear molecule about 280 nm long and 1.4–1.5 nm wide (Figure 16.7). The chains may be identical or they may differ in amino acid sequence depending on the type of collagen. For example, type I collagen consists of two identical polypeptide chains termed α1(I) and one chain with a different amino acid sequence termed α2(I). Type III collagen consists of three identical chains: α1(III). By convention, the Arabic numbers are used to identify different collagen chains within a given type, whereas the Roman numerals refer to the collagen type. Accordingly, α1 chains from type I collagen differ from α1 chains from type III collagen.

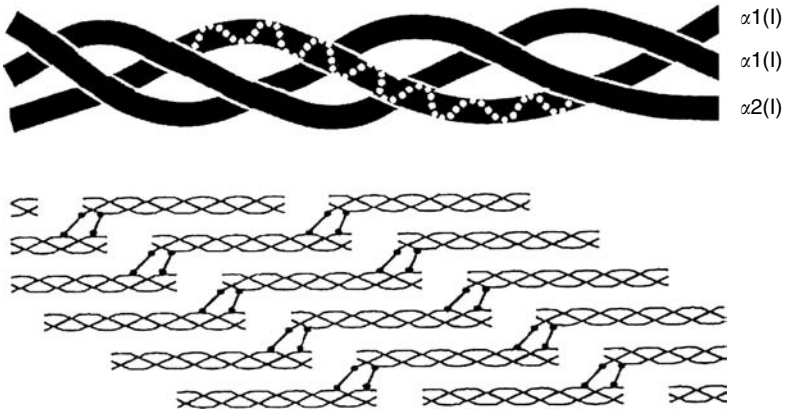


FIGURE 16.7 Schematic representation of the tropocollagen triple helix and cross-links between tropocollagen molecules in a collagen fibril. (Reprinted from Chiang, W., G.M. Strasburg, and T.M. Byrem (2007). In *Food Chemistry: Principles and Applications*, 2nd edn. Y.H. Hui (Ed.), Science Technology System, West Sacramento, CA.)

In type I collagen, an average polypeptide chain consists of approximately 1000 amino acid residues, with a characteristic repeating sequence throughout most of the chain of $(Gly–X–Y)_n$. Residue X in this sequence is often proline, and Y is often hydroxyproline or hydroxylysine. The latter amino acids are formed by posttranslational hydroxylation of proline and lysine by prolyl hydroxylase and lysyl hydroxylase, respectively. Overall, collagens generally contain approximately 33% glycine, 12% proline, 11% alanine, 10% hydroxyproline, 1% hydroxylysine, and small amounts of polar and charged amino acids. Tryptophan is notably absent from collagen; in fact, the absence of tryptophan in collagen preparations is sometimes used as a criterion for the purity of collagen preparations. The dominance of the amino acids identified above and the notable absence of most essential amino acids makes collagen a poor protein source in the human diet.

The characteristic amino acid sequence of collagen dictates the folding and assembly of the collagen family of proteins. The presence of glycine at the beginning of each triplet followed frequently by a proline residue gives rise to a highly extended polypeptide α-chain that forms an unique, shallow, left-handed helix. In type I collagen, three α-chains form a right-handed triple-helical coiled coil that constitutes the tropocollagen molecule (Figure 16.7). Structural studies indicate that the side chain of each glycine residue, that is, a hydrogen atom, is directed toward the center of the coiled-coil helix. Owing to the small size of the hydrogen atom compared with other amino acid side chains, glycine is the only amino acid whose side chain could be accommodated in such a structure. Moreover, each chain is slightly staggered with respect to the other two. This enables hydrogen bonding between the polypeptide amide hydrogen of a glycine residue with the carbonyl oxygen of the adjacent X residue on another chain. The presence of proline and hydroxyproline at frequent intervals along the sequence prevent the chain from adopting a classical α-helix because of constraints of allowable ϕ, ψ angles of these residues. Moreover, they lack amide hydrogen atoms that are characteristically involved in stabilization of α-helices. The hydroxyl groups of hydroxyproline and hydroxylysine are also thought to be stabilized by interchain hydrogen bonds. Thus, the secondary structure of collagen is an unusually extended, relatively rigid, distinctive helix among the proteins [34].

Collagen polypeptides are synthesized as precursors, termed pro-α chains. These precursor polypeptides include an N-terminal signal sequence that directs the polypeptide to the lumen of the endoplasmic reticulum of the fibroblast. Following the signal sequence at the N-terminal end as well as at the C-terminus is a series of additional residues collectively termed propeptides. Upon entry of the polypeptide into the lumen of the endoplasmic reticulum, selected proline and lysine residues are hydroxylated, and a few hydroxylysine residues are glycosylated. The pro-α chains then combine

to form the triple-stranded procollagen molecule. The propeptide sequences are believed to initiate formation of the procollagen molecule and they prevent the formation of large fibrils within the cell, which could not easily be secreted [25].

Procollagen is secreted to the extracellular matrix where proteinases cleave the propeptides from both ends of the molecule, forming tropocollagen which then begins self-assembly with other tropocollagen molecules to form fibrils. Tropocollagen molecules assemble in a staggered array through side-by-side associations stabilized primarily through hydrophobic and electrostatic interactions. The N-terminal 14 and C-terminal 10 residues of tropocollagen do not display the characteristic Gly–X–Y sequence found throughout most of the tropocollagen molecule; hence, these "telopeptide" regions do not form the characteristic collagen helix.

The telopeptide regions are involved in the formation of covalent intermolecular covalent cross-links between the individual chains (Figure 16.7). These cross-links provide critically needed stability and tensile strength to the supramolecular structure. There are four key residues involved in the initial cross-linking of tropocollagen chains: two lysine or hydroxylysine residues of the N-terminal telopeptides, and two lysine or hydroxylysine residues of the C-terminal telopeptides. The head-to-tail staggered arrangement of the tropocollagen molecules enables interactions of the N-terminal telopeptides with the adjacent C-terminal telopeptides.

The prerequisite step preceding cross-linking is oxidative deamination of the lysine or hydroxylysine residues to form allysine or hydroxyallysine, respectively, through the action of the enzyme lysyl oxidase. The subsequent cross-linking reactions occur spontaneously through aldol condensation or through the formation of Schiff base intermediates resulting from the condensation of an amino group from lysine or hydroxylysine with an aldehyde from allysine or hydroxyallysine. Several examples of these cross-linking reactions are shown in Figure 16.8. The divalent cross-links formed via these pathways are reducible by borohydride, and are thus termed "reducible cross-links."

As an animal matures, these divalent, reducible cross-links are converted to more stable, nonreducible, trivalent cross-links. Two types of mature cross-links have been characterized: hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP) (Figure 16.9), the latter being present in only negligible amounts in muscle. HP is probably formed via condensation of two ketoamine cross-links (Figure 16.8), as evidenced by concurrent stoichiometric disappearance of reducible cross-links with appearance of the nonreducible forms [21]. In contrast to the reducible cross-links, nonreducible cross-links are very heat-stable, which has important implications for tenderness of meat. Moreover, additional cross-links that spontaneously form during maturation of the animal contributes to the increase in meat toughness, which is often found in older animals [21,35].

The cross-link content varies not only with age but also with muscle function (e.g., postural vs. locomotor), species, exercise, and treatment with growth promoters. Moreover, while both collagen content and degree of cross-linking are casually correlated with toughness of meat, it is evident that there is an additive effect of the two components. Less tender muscles, such as bovine biceps femoris, show high collagen concentrations as well as high concentrations of HP. In contrast, a relatively tender muscle such as longissimus dorsi possesses only half to two-thirds of the total amount of collagen and HP as the former muscle. Moreover, gluteus medius is high in collagen and low in HP content, whereas pectoralis major has low abundance of collagen but has high levels of HP. Both muscles are relatively tender. Thus, it appears that toughness is more strongly correlated with an additive effect of both collagen parameters [21].

16.3.4.2 Sarcoplasmic Proteins

Sarcoplasmic proteins are present in high abundance (25–30% of total muscle protein), and as the name implies, they are located in the sarcoplasmic (cytoplasmic) fraction of the muscle cell [17]. Many, but not all, of the proteins are enzymes involved in glycolysis, glycogen synthesis, and glycogenolysis (Table 16.5). One enzyme, glyceraldehyde phosphate dehydrogenase, constitutes as much as 20% of the sarcoplasmic fraction. Together, the next four or five most abundant glycolytic

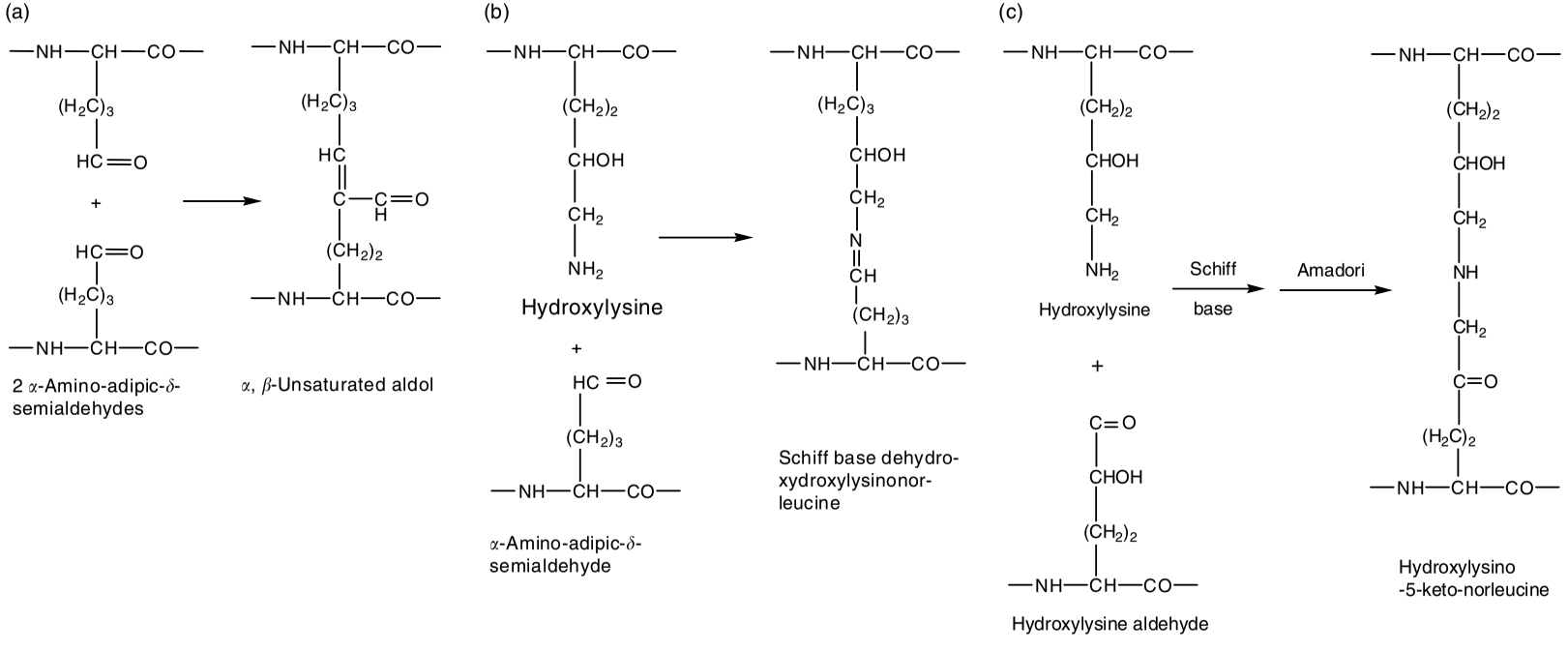


FIGURE 16.8 Mechanisms by which collagen side chains may form cross-links. (a) Aldol condensation of two allysine residues followed by loss of a water molecule; (b) Schiff base formation following condensation of hydroxylysine with allysine; and (c) Schiff base formation following condensation of hydroxylysine and hydroxyallysine. The Schiff base intermediate undergoes Amadori rearrangement to form hydroxylysino-5-keto-norleucine. (Reprinted from Foegeding, E.A., T.C. Lanier, and H.O. Hultin (1996). In *Food Chemistry*, 3rd edn. (O.R. Fennema, ed.), Marcel Dekker, Inc., NY.)

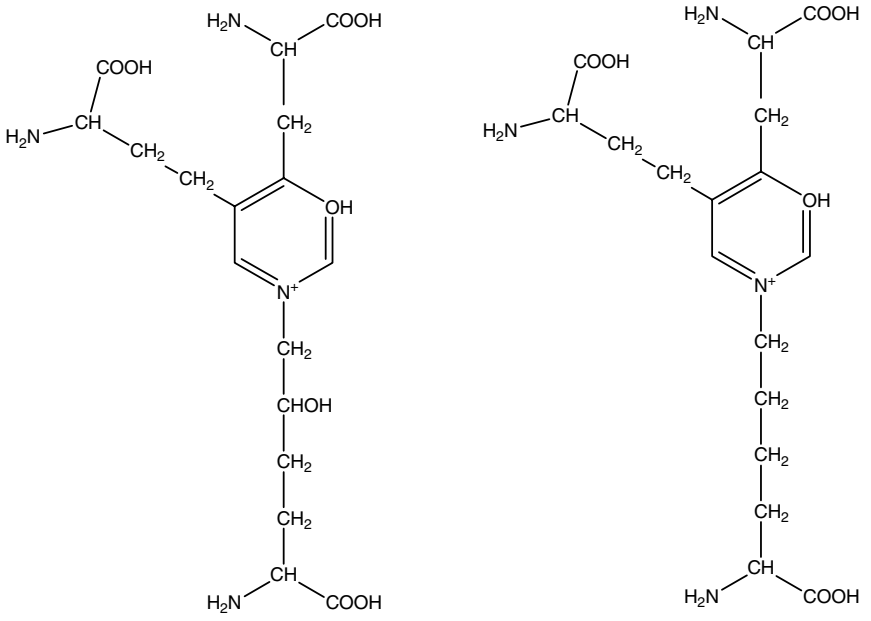


FIGURE 16.9 Structural formulas of hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP) collagen cross-links.

TABLE 16.5
Abundance of Sarcoplasmic Proteins in Muscle

Protein	mg/g
Phosphorylase B	2.5
Phosphoglucomutase	1.5
Phosphoglucose isomerase	1.0
Phosphofructokinase	1.0
Aldolase	6.0
Triose phosphate isomerase	2.0
$\alpha-$Glycerophosphate dehydrogenase	0.5
Glyceraldehyde phosphate dehydrogenase	12
Phosphoglycerate kinase	1.2
Phosphoglycerate mutase	1.0
Enolase	5
Pyruvate kinase	3
Lactate dehydrogenase	4
Creatine kinase	5
Adenylate kinase	0.5
AMP deaminase	0.2
Myoglobin	0.5–2.0

Source: Compiled from Scopes, R.K. (1970). Characterization and study of sarcoplasmic proteins (Ch. 22). In *The Physiology and Biochemistry of Muscle as a Food*, 2nd edn., (E.J. Briskey, R.G. Cassens, and B.B. Marsh, eds.), The University of Wisconsin Press, Madison, WI, pp. 471–492.

enzymes comprise over half of the total sarcoplasmic protein content. Other proteins include enzymes of the pentose shunt and auxiliary enzymes such as creatine kinase (the soluble fraction), AMP deaminase, the calpains, the oxygen storage protein, and myoglobin.

The abundance of some proteins found in the sarcoplasm may vary substantially depending on species, breed, muscle fiber type, age of the animal, and individual genetics. For example, myoglobin tends to be lower in abundance in young animals; thus, the pale color of veal compared to adult beef. Poultry breast muscles and many fish muscles have little redness because of the low levels of myoglobin, whereas poultry leg and thigh muscles are redder than breast muscles because of higher levels of myoglobin. At the other extreme, whale muscle contains the highest known levels of myoglobin; as much as 70% of the sarcoplasmic protein content of some whale muscles is myoglobin [17].

Another enzyme of great significance both in live muscle as well as in postmortem conversion of muscle to meat is creatine kinase. This enzyme is found both in the soluble sarcoplasmic protein fraction and as a component of the M-line protein matrix in the myofibril. Creatine kinase maintains stable levels of ATP for use by the muscle when subjected to intensive energy demands such as sprinting or lifting a heavy object [36]. ATP would quickly be depleted before glycolysis and oxidative metabolism could replenish the loss. Creatine phosphate (CrP) serves as a high energy reservoir compound that can donate its phosphate to ADP in the following reaction catalyzed by creatine kinase:

$$\mathrm{CrP} + \mathrm{MgADP} \rightarrow \mathrm{MgATP} + \mathrm{Cr}$$

In resting muscle, as glycolysis and oxidative metabolism restore ATP levels, the above process is reversed and some of the excess metabolic energy is converted from ATP to the reservoir form of CrP.

Two other proteins of note in the sarcoplasmic fraction are adenylate kinase and AMP deaminase. As ATP is utilized to meet energy demands, ADP is converted back to ATP by glycolysis, oxidative phosphorylation, and creatine kinase. Adenylate kinase is another enzyme that supports energy demands by synthesis of ATP through the following reaction:

$$2\mathrm{ADP} \rightarrow \mathrm{ATP} + \mathrm{AMP}$$

During periods of intense energy demands on muscle, as well as during the early phase of postmortem conversion of muscle to meat, ATP levels decline precipitously. This reaction becomes especially important when other sources of ATP generation become depleted. The other product, AMP, is deaminated by the action of AMP deaminase to inosine monophosphate (IMP) by the following reaction:

$$\mathrm{AMP} \rightarrow \mathrm{IMP} + \mathrm{NH_3}$$

IMP is further degraded to hypoxanthine that has a bitter off-flavor. Hypoxanthine has long been regarded as biochemical indicator of the postmortem age of fresh fish muscle.

Finally, several proteinases that are likely involved in muscle growth, maintenance, and postmortem muscle protein degradation exist in the muscle sarcoplasm. The role of these enzymes in aging and tenderization of meat will be described in Section 16.4.1. Another group of proteins is not soluble within the muscle sarcoplasm, but may be readily solubilized using various extraction methodologies. These proteins include some members of the intermediate and microfilaments comprising the cytoskeletal protein network in muscle.

16.3.4.3 Contractile Proteins

16.3.4.3.1 Myosin

The protein myosin serves as the molecular motor for muscle contraction. It is the dominant protein of the A-band, and at 45% of the myofibrillar protein content, it is the most abundant skeletal muscle

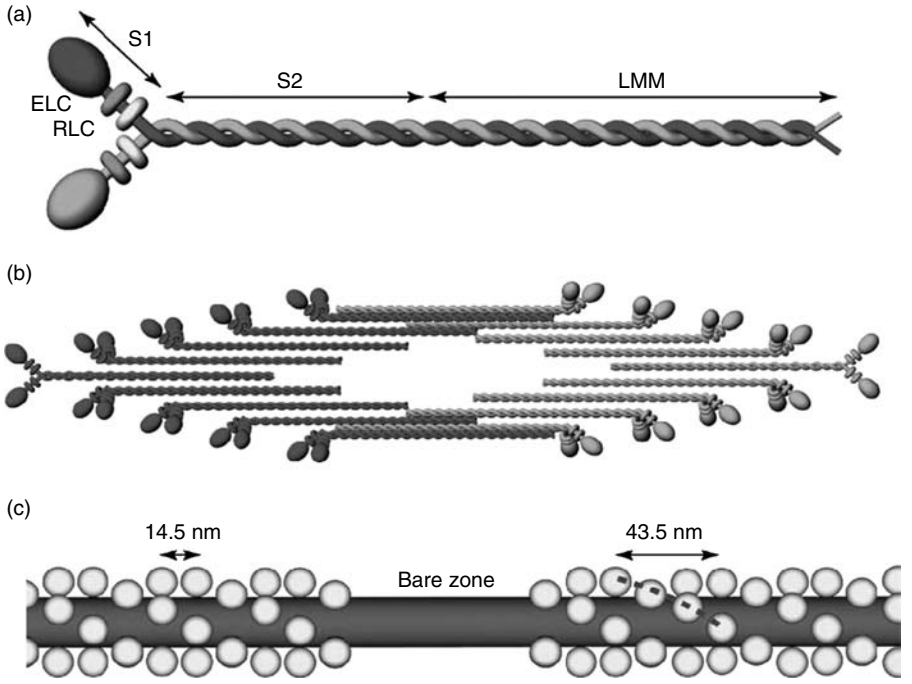


FIGURE 16.10 Schematic representation of the structural features of the myosin molecule and its assembly into thick filaments. (a) A functional myosin molecule consists of two heavy chains, each of which forms a globular head domain, and a rod-like α-helix. The helices of the two heavy chains form a rod-like coiled coil. Two light chains bind to the neck region of each heavy chain: the essential light chains (ELC) known as light chains 1 and 3, and the regulatory light chain (RLC), also known as light chain 2. Limited proteolysis of myosin yields heavy meromyosin (HMM) and light meromyosin (LMM). Limited proteolysis may also yield two subfragments of HMM: subfragment 1 (S1) consists of the head and neck domain of HMM, and subfragment 2 (S2) serves as a linker between the myosin head and the portion of the rod that is involved in filament formation. (b) Myosin molecules assemble in bipolar, staggered arrays to form thick filaments. (c) Myosin heads radiate from the thick filament shaft in a spiraling fashion. Parallel, adjacent myosin molecules are staggered by a distance of 14.5 nm; myosin heads are separated by a translational distance of 43.5 nm. (Reprinted from Craig, R. and J.L. Woodhead (2006). *Curr Opin Struct Biol* 16:204–212.)

protein [18]. A myosin molecule consists of six subunits: two "heavy chains" of $M_r \sim 220,000$ and four "light chains" that vary in mass from about 16,000 to 20,000 [37]. These six subunits combine to form a quaternary structure with a molecular mass of ~500,000 with the general appearance of two globular heads projecting from the end of a stick (Figure 16.10a). The N-terminal end of each heavy chain folds into the structure referred to as the myosin head, while the C-terminal portion (60% of the residues) consists of a sequence motif that gives rise to a long α-helix. The helices of two heavy chains intertwine to form a rod-like coiled coil. Two myosin light chains bind to each myosin head. The light chains of myosin consist of two alkali-extractable polypeptides, referred to as light chain 1, LC1 ($M_r \sim 20,900$), and light chain 3, LC3 ($M_r \sim 16,600$), respectively. Myosin light chain 2 is sometimes referred to as the (5,5′-dithiobis)-2-nitrobenzoic acid (DTNB)-light chain because treatment of myosin with DTNB removes this protein ($M_r \sim 18,000$) from the myosin head. Each myosin head contains one of the alkali-extractable light chains and one DTNB light chain. The DTNB light chain is sometimes called the regulatory light chain, because it is required for myosin-based regulation of muscle contraction in molluscan muscles and smooth muscles of higher organisms. Likewise, LC1 and LC3 are sometimes referred to as essential light chains.

The head and rod portions have distinctly different, important functions. Under physiological conditions, myosin molecules coalesce through staggered, side-by-side associations of the rod portions of each molecule (Figure 16.10b). Thus, the myosin rod domain provides the structural basis for the formation of thick filament. The thick filaments are arranged in bipolar fashion such that the origin of the filament is the M-line, and myosin molecules proceed in opposite directions away from the M-line. The myosin heads project radially from each thick filament shaft, and are directed toward the thin filaments (Figure 16.10c). The top of the myosin head binds to actin, the backbone protein of the thin filament. The myosin head also contains an ATP-binding site which serves as the molecular motor that drives muscle contraction.

Study of the functional nature of the different myosin protein domains was facilitated by the discovery that brief proteolytic treatment of myosin with enzymes such as trypsin or papain resulted in fragments of the protein that were more suitable for study under physiological conditions [38]. Two primary products of this limited proteolysis were obtained. One fragment, called heavy meromyosin (HMM), consisted of two myosin heads plus a short portion of the myosin rod emanating from the head. HMM maintained the actin-binding and ATP-hydrolytic activities of the parent myosin molecule. The remaining rod portion was called light meromyosin (LMM); it retained the molecular assembly determinants that enable the assembly of individual myosin molecules into thick filaments. Subsequently it was shown that further proteolytic treatment of HMM resulted in cleavage at the head–tail junction, thus yielding two products. The product consisting of the myosin head was called subfragment 1 (S1), and the short tail portion was called subfragment 2 (S2) (Figure 16.10a). Subfragment 2 possessed neither the self-assembly property nor the molecular motor activity of the parent molecule. Thus, it appeared to serve as a linker segment that separates the myosin head from the thick filament shaft [39].

Myosin subfragment 1 has been crystallized and its molecular structure has been determined at high resolution [40]. Among the many features observable in the structure of S1 are two prominent clefts. One pocket serves as the ATP-binding site, and the other cleft is the actin-binding site (Figure 16.11). These two functions constitute the essential features of the molecular motor activity of the myosin molecule. In addition, the structure shows a long α-helical segment that connects the myosin head (S1) to the myosin rod (S2). This helix serves as a lever arm at which the conformational change associated with myosin head movement takes place during muscle contraction.

16.3.4.3.2 Actin

At approximately 20% of the myofibrillar protein content, actin is the second most abundant protein in muscle. The actin monomer, called globular actin or G-actin, is a single polypeptide chain with a molecular mass of 42,000 and a single adenine- nucleotide-binding site. The term G-actin is rooted in the historical view that the monomer was approximately spherical. When the high-resolution crystal structure of G-actin was determined, it became clear that molecule is actually shaped rather like a peanut shell with two large domains subdivided into two additional sub-domains [41]. Nevertheless, the term G-actin persists to describe the actin monomer.

G-actin remains monomeric at very low ionic strength. However, under conditions approaching physiological ionic strength coupled with the presence of MgATP, actin polymerizes in "head-to-tail" fashion to form double-stranded, coiled-coil, thin filaments called filamentous actin or F-actin [42]. One end of each filament is anchored in the Z-disc, and the filaments project toward the M-line located at the center of the sarcomere (Figure 16.3a). Because of the directional nature of the thin filaments, actin filaments on opposite sides of the M-line are directed toward each other like arrows within the sarcomere. The lengths of the thin filaments are remarkably precise at approximately 1 μM. It is believed that other thin filament proteins including nebulin, tropomodulin, and CapZ protein play key roles in controlling the size of individual thin filaments [37].

Actin plays a dual role in the myofibril. It binds myosin during muscle contraction, forming actomyosin crossbridges between the two filaments. Binding of actin to myosin activates the ATPase activity of myosin, causing myosin to act as a molecular motor that pulls thin filaments past the thick

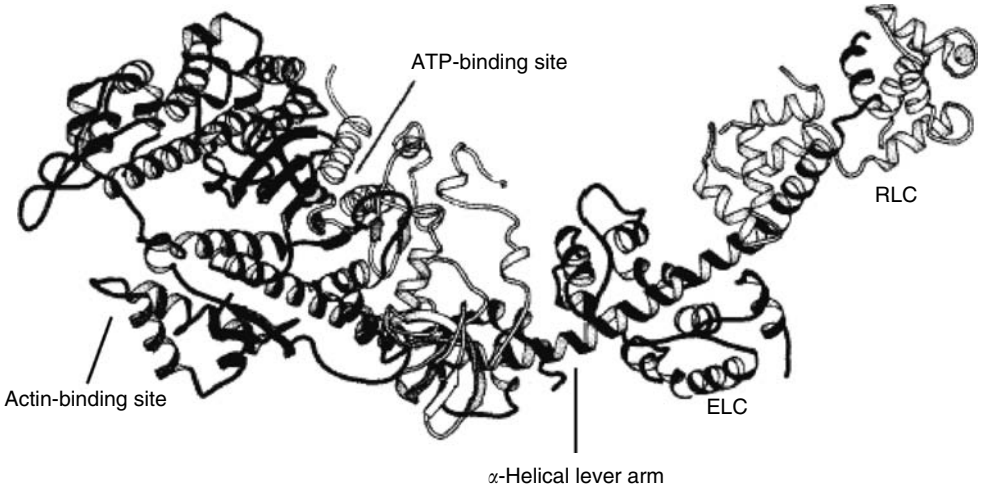


FIGURE 16.11 Representation of the three-dimensional structure of myosin subfragment 1. Two clefts are prominent in the myosin head: an actin-binding site that serves as the domain for crossbridge formation, and a nucleotide-binding site where ATP undergoes hydrolysis to yield mechanical energy to support muscle contraction. The structure also indicates a long α-helix that serves as a binding domain for the light chains, and as a lever arm on which myosin conformational changes occur during contraction and relaxation. Abbreviations: RLC, Regulatory light chain; ELC, Essential light chain. (Reprinted from Rayment I., C. Smith, and R.G. Yount (1996). *Annu Rev Physiol* 58:671–702.)

filaments to shorten the sarcomere. Second, actin forms the backbone for binding tropomyosin and troponin, two proteins that act in concert to regulate actin–myosin interaction in response to changes in calcium levels.

16.3.4.4 Regulatory Proteins

16.3.4.4.1 Tropomyosin

Tropomyosin together with troponin constitute the regulatory switch that turns muscle contraction on or off within the sarcomere [43]. Tropomyosin consists of two α-helical subunits ($M_r \sim 37,000$ Da) that intertwine to form a long, rod-like, coiled-coil protein with a molecular mass of ~74,000 Da. Two different tropomyosin isoform subunits, termed α- and β-tropomyosin, are found in skeletal muscle. These subunits may combine in various ways to form homodimers ($\alpha\alpha$ or $\beta\beta$) or heterodimers ($\alpha\beta$) [44]. The actual amounts of each isoform expressed in myofibers depend on muscle fiber type (fast vs. slow), muscle type (i.e., skeletal, cardiac, or smooth), stage of development, and species. Moreover, various alternative splicing patterns of tropomyosin generate several additional isoforms that presumably provide an additional level of modulation of contractile properties of different types of myofibers [44].

Like actin, tropomyosin polymerizes at physiological ionic strength in head-to-tail fashion, with a small overlap of about 8–11 amino acids at each end of a tropomyosin molecule. An individual tropomyosin molecule is approximately 42 nm long and spans seven actin monomeric units (Figure 16.3c). The filamentous strand of tropomyosin binds to the actin backbone at specific sites along each actin monomer, near the grooves of the thin filament double helix. In this position, tropomyosin blocks the myosin-binding sites on the outer domain of the actin filament when muscle is at rest [44].

16.3.4.4.2 Troponin

The troponin complex, which constitutes 5% of the myofibrillar protein, is a heterotrimer that binds to tropomyosin (Figure 16.12). Working in concert with tropomyosin, troponin responds to changes

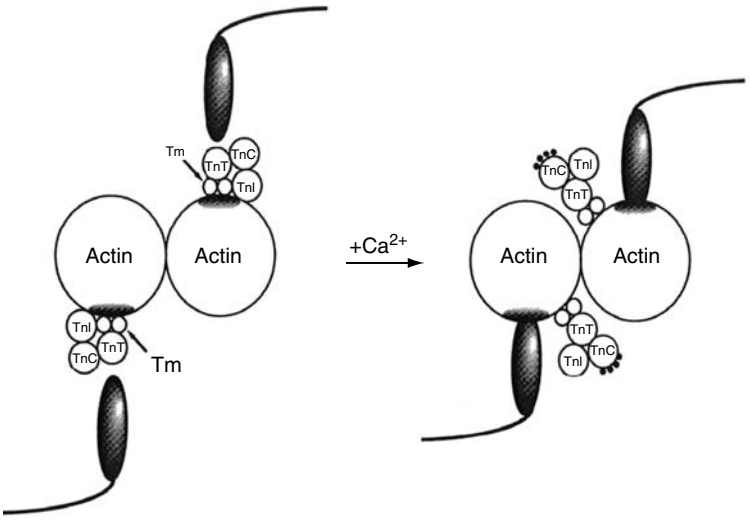


FIGURE 16.12 Regulation of contraction and relaxation by the tropomyosin–troponin complex. When muscle is in the resting state, tropomyosin sterically blocks myosin heads from binding to the myosin-binding site (shaded region) on the actin filament. When calcium binds to troponin-C, the troponin complex undergoes a conformational change that causes tropomyosin to shift deeper into the actin groove, exposing myosin-binding sites and enabling myosin crossbridge formation with actin. (Reprinted from Chiang, W., G.M. Strasburg, and T.M. Byrem (2007). In *Food Chemistry: Principles and Applications*, 2nd edn. Y.H. Hui (Ed.), Science Technology System, West Sacramento, CA.)

in calcium ion concentration to control actin–myosin interaction, and thus, contraction or relaxation of muscle [43].

Troponin-C (TnC) has an M_r of ~18,000 and is the calcium-binding subunit of troponin [45]. TnC consists of four Ca^{2+}-binding sites; two are high affinity Ca^{2+}-binding sites ($K_d \sim 10^{-9}$ M) that also bind Mg^{2+}. These two binding sites are located in the C-terminal half of the molecule. Two lower affinity calcium-binding sites ($K_d \sim 10^{-6}$ M) are in the N-terminal half of the molecule. These two sites are specific for binding Ca^{2+}. In resting muscle, the two low-affinity binding sites are believed to be empty because of the low [Ca^{2+}] ($<10^{-7}$ M), whereas the high-affinity sites are likely to be occupied by Mg^{2+} because of its high-intracellular concentration relative to Ca^{2+} [46]. When a motor neuron initiates muscle contraction, [Ca^{2+}] concentrations rise to $>10^{-5}$ M; some of the calcium ions bind to low-affinity sites of TnC, triggering a conformational change in the protein that is transmitted through the other troponin subunits to tropomyosin.

Troponin-T binds the troponin complex to tropomyosin, and troponin-I inhibits actomyosin ATPase activity. The troponin complex binds near the head–tail junction of a tropomyosin filament at a ratio of one troponin per tropomyosin molecule [43].

16.3.4.5 Structural Proteins

16.3.4.5.1 Titin

Titin (also known as connectin) is the third most abundant protein of muscle myofibrils. With over 38,000 amino acid residues and an M_r of ~4,200,000, titin is the largest single polypeptide chain known. Yet despite titin's abundance in muscle, it was discovered relatively recently by K. Wang and coworkers in 1979 [47]. Ironically, this protein escaped notice because it is so large: titin's extremely high molecular mass limited its mobility on conventional polyacrylamide gel systems used to characterize muscle proteins. Use of low-acrylamide concentrations in sodium dodecyl sulphate–polyacrylamide gels created much larger pore sizes in gels that enabled titin to

migrate into the gels, thus leading to the discovery of this protein as well as another large protein, nebulin.

Titin is a flexible, elastic filamentous protein of about 1 μM in length that spans one-half of a sarcomeric unit (Figure 16.3a). The N-terminus of titin is located in the Z-disc, and the C-terminus is anchored in the M-line. The titin domain in the I-band region functions as a molecular spring that maintains the precise structural arrangement of thick and thin filaments, and gives rise to passive muscle stiffness. The extensible region of titin in the skeletal muscle is composed of tandemly arranged immunoglobulin-like domains, and a PEVK-segment that is abundant in proline (P), glutamate (E), valine (V), and lysine (K) [48]. In addition to these segments, the extensible region of cardiac titin also contains a unique 572-residue sequence that is part of the cardiac-specific N2B element [49]. These segments have distinct bending rigidities and as a result, stretching of slack sarcomeres initially gives rise to extension of tandem Ig segments followed by extension of PEVK segment. When muscle relaxes, titin restores muscle to its resting sarcomere length. Titin may also serve as a template upon which myosin molecules form thick filaments because titin is one of the earliest genes expressed in muscle protein synthesis as muscle is formed during embryogenesis [50].

16.3.4.5.2 Nebulin

Like titin, nebulin was discovered rather recently because of its unusually large size ($M_r \sim 800,000$). It is present in amounts comparable to that of tropomyosin and troponin. Nebulin is associated with the thin filaments of skeletal muscle, with the C-terminal end partially inserted into the Z-discs, and the N-terminal end extending to the pointed ends of the thin filaments. There is also an amino acid sequence super-repeat in the nebulin sequence, analogous to that of titin. However, nebulin is inextensible (unlike titin), and its unique structural properties have made it a prime candidate to act as a molecular ruler for specifying the precise lengths of the thin filaments [51]. It may also be involved in signal transduction, contractile regulation, and myofibril force generation [52].

16.3.4.5.3 α-Actinin

A major component of the Z-disc is α-actinin ($M_r \sim 97,000$) which functions as an actin-binding protein. α-Actinin contains three major domains: a globular N-terminal actin-binding domain, a central rod domain, and a C-terminal domain that bears similarity to the calcium-binding protein calmodulin [53]. The rod domains of α-actinin monomers interact to establish antiparallel dimers that are capable of cross-linking actin and titin filaments from neighboring sarcomeres. In fact, α-actinin has the capacity to associate with numerous protein partners at the Z-disc. These protein interactions provide tensile integrity to the Z-disc, and may also serve as an additional docking site for other Z-disc-associated proteins (Figure 16.5).

16.3.4.5.4 CapZ and Tropomodulin

Actin filament formation is a dynamic process, and regulation of this process during myofilaments assembly is a key factor in maintaining their uniform filament lengths. Proteins that bind to and cap the thin filaments block filament elongation and shortening. CapZ, also known as β-actinin, is a capping protein involved in nucleation and stabilization of actin filaments. It is a heterodimer containing α ($M_r \sim 36,000$) and β ($M_r \sim 32,000$) subunits, both of which are required for capping [37,54]. In striated muscle, CapZ is localized to Z-discs where it binds α-actinin, and likely forms an anchoring complex for the thin filaments. Tropomodulin ($M_r \sim 40,000$) caps the pointed ends of the thin filaments which, in turn, modulate thin filament lengths [37].

16.3.4.5.5 Desmin

Desmin ($M_r \sim 55,000$) is the dominant protein of the intermediate filaments (10 nm diameter) that maintains the structural integrity of most cells. In muscle, desmin filaments are found at the periphery of Z-discs, and it appears that these filaments serve to cross-link adjacent myofibrils and to link the myofibrillar Z-discs to the SL [55] (Figure 16.5).

16.3.4.5.6 Filamin

Three filamins (α, β, and γ) ($M_r \sim 300,000$) have been identified in muscle. They share a similar molecular structure: an N-terminal actin-binding head followed by 24 immunoglobulin-like domains. γ-Filamin (also known as ABP-280) is the muscle-specific filamin isoform. Filamin is localized at the periphery of the Z-disc and provides a critical link between the SL and sarcomeric cytoskeleton. Since γ-filamin exists at the very early stages of developing Z-discs, it is suggested that this filamin isoform might be involved in the formation of sarcomeric Z-discs [56].

16.3.4.5.7 C-protein and H-protein

C-protein ($M_r \sim 140,000$) and H-protein ($M_r \sim 58,000$) are myosin-binding proteins. They are both distributed in the C-zone (middle third of each half of the A-band) of the thick filament, forming a series of transverse stripes spaced 43 nm apart, although H-protein is also found outside this zone [57]. C-protein is found in approximately a 1:8 molar ratio with myosin heavy chains and interacts with both the myosin and titin filaments. C-protein and H-protein may function to link and/or align the thick filaments in the A-band [58].

16.3.4.5.8 Myomesin and M-line proteins

The main protein of the M-line is myomesin, a polypeptide of about 185,000 Da [37]. In addition to serving as scaffolding for binding of titin and myosin, it may be involved in maintaining structural integrity of thick filaments. Another component of the M-line is creatine kinase, an enzyme involved in regeneration of ATP in muscle as described in Section 16.3.4.2.

16.3.4.6 Proteins of the Sarcoplasmic Reticulum and Sarcolemma

The membranes of the various organelles of muscle—nuclei, mitochondria, SL, and so forth—contain proteins that serve functions relevant to the specific needs of the organelles and of the cells. The proteins of the SR merit special attention because of their role in storage, transport, and release of calcium ions in the myofiber. Because calcium ions trigger muscle contraction and regulate key glycolytic steps, these proteins play key roles in muscle activity of the live organism. The proteins of the SR may also serve as key determinants of meat quality, depending on the extent to which they control sarcoplasmic calcium concentrations in the initial time period postmortem. Although there are many proteins that are found in the SR and SL, our focus here is on three proteins. Two of these proteins, the ryanodine receptor (RyR) and the dihydropyridine receptor (DHPR), comprise the essential elements of the calcium-release mechanism responsible for initiating muscle contraction. The third protein, the calcium pump, is involved in relaxation of muscle.

The DHPR is an intrinsic membrane protein complex embedded in the T-tubules that serves as a sensor for the change in voltage across the membrane when muscle contraction is initiated [59]. The DHPR consists of four different subunits, referred to as α_1, β, γ, and $\alpha_2\delta$ subunits. The α_1 subunit forms a calcium channel, which is responsible for the voltage-sensor function, and contains the binding site for a class of pharmaceutical compounds called dihydropyridines that modulate the protein's function. The roles of the other protein subunits are less clear, but there is evidence supporting their role in modulating the process of excitation–contraction coupling [60]. The function of DHPRs in various tissues depends in large part on the isoform expressed in the tissues. For example, the cardiac-muscle-specific α_1 isoform allows inward diffusion of calcium ions from the extracellular milieu, whereas the skeletal muscle isoform is largely inactive as a channel.

A portion of the SR called the terminal cisternae is closely apposed to the T-tubule. This portion of the SR is enriched in a protein called the SR calcium-release channel or RyR, so named because it binds a toxic plant alkaloid (ryanodine) very specifically and with high affinity. The RyR family consists of a series of homologous proteins sometimes referred to as RyR1, RyR2, and RyR3. In mammals, RyR1 is the skeletal muscle isoform, while RyR2 is the dominant form of cardiac muscle.

RyR2 is also found in other tissues including brain. RyR3 is found in various mammalian nonmuscle tissues and in trace amounts in skeletal muscle. In avian, piscine, and amphibian species, RyR3 is found in most skeletal muscles in approximately equal abundance with that of RyR1 [61]. RyRs function as channels or transmembrane conduits for diffusion of calcium ions from within the lumen of the SR, where the concentration is several millimolar, to the sarcoplasm where the resting muscle calcium concentration is $<10^{-7}$ M.

The RyR is an extremely large protein that consists of four identical subunits, each with a molecular weight of approximately 550,000, resulting in a functional ion channel with a molecular mass of ~2,200 kDa. Several other proteins are tightly associated with the RyR and modulate its function [11]. More than 80% of the RyR mass is on the cytoplasmic side of the SR membrane, forming a structure sometimes referred to as junctional foot that spans the gap between the terminal cisternae of the SR and the T-tubule. There are numerous lines of evidence suggesting skeletal muscle DHPRs and RyRs are in physical association [12]. Electron micrographs show that DHPRs assemble into groups of four units called a "tetrad." Overlays of DHPR tetrads in the T-tubule with SR junctional foot proteins (RyRs) suggest that every other RyR interacts with a tetrad; conversely, every other RyR not coupled with a DHPR (Figure 16.13) [62]. Recent evidence suggests that in birds, fish, and amphibians, RyR3, which is generally found in equal abundance with RyR1, is confined to the periphery of the SR/T-tubule junction and, thus, does not interact with the T-tubule or with the DHPR tetrad [63].

The DHPR and RyR work in concert with their associated regulatory proteins to affect Ca^{2+}-release into the sarcoplasm that triggers muscle contraction. When the neuromuscular contraction signal ceases, these proteins return to their "resting" state and the sarcoplasmic calcium concentration is restored by the action of the calcium pump protein. This protein is located primarily in the longitudinal SR, that is, the portion of the SR distal to the terminal cisternae. Using ATP as an energy

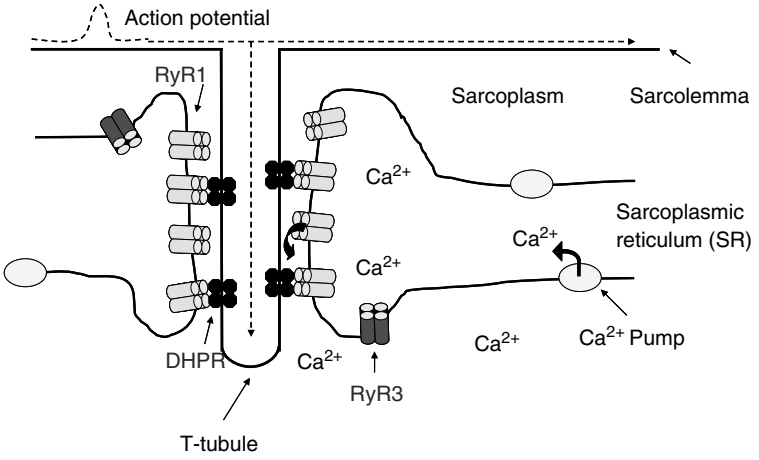


FIGURE 16.13 Schematic representation of the calcium release and uptake mechanisms in skeletal muscle. Dihydropyridine receptors (DHPRs) embedded in the T-tubule membrane serve as sensors of the action potential initiated by a motor neuron. Ryanodine receptors (RyR1 and RyR3) located in the SR serve as calcium channel proteins that open to allow calcium ions to diffuse from the SR to the sarcoplasm during contraction. Calcium pump proteins lower the sarcoplasmic calcium ion concentration by translocating calcium into the SR after RyR pores close. In mammalian skeletal muscle, every other RyR1 is coupled to a DHPR tetrad. RyR3 is localized in the peripheral region of the T-tubule, and does not associate with the DHPR. RyR3 is present in very low abundance in mammalian skeletal muscle, but is approximately equal to that of RyR1 in most avian, piscine, and amphibian muscles.

source, the calcium pump protein transports two Ca^{2+} ions against the concentration gradient across the SR membrane and into the lumen of the SR for every ATP molecule hydrolyzed [64].

16.3.5 Excitation–Contraction Coupling

A detailed understanding of the mechanism of muscle contraction may, at first glance, seem irrelevant to meat quality. The difference between live muscle tissue and meat is quite literally the difference between life and death. However, upon slaughter of an animal, muscle tissue goes through a series of transitional reactions (described in Section 16.4) known as conversion of muscle to meat. The biochemical events associated with this transition involve exactly the same mechanisms of muscle contraction and the supporting physiological and biochemical reactions of live muscle, all of which are significant contributing determinants of meat quality.

When the muscle cell is at rest, there is a voltage difference across the SL of about −90 mV and the intracellular Ca^{2+} concentration is very low ($<10^{-7}$ M). In the myofibrils, the tropomyosin is situated on the actin filament in a position so as to sterically prevent myosin–actin crossbridge formation. Upon stimulation of a myofiber by a motor neuron, the muscle cell is depolarized at the neuromuscular junction. This voltage change travels along the SL, entering the interior of the muscle via the T-tubule. The DHPR responds to the electrochemical transient across the membrane by undergoing a conformational change that is transmitted across the T-tubule/SR junction to the RyR located in the SR terminal cisternae. The RyR, in turn, responds by opening its channel pore. Calcium ions flow from the lumen of the SR, where the concentration is $>10^{-3}$ M, to the sarcoplasm, thus raising the sarcoplasmic concentration of calcium ions over 100-fold to $>10^{-5}$ M. Ca^{2+} binds to TnC, resulting in a conformational change in the troponin complex that is transmitted to tropomyosin. This results in a shift of the position of tropomyosin along the actin filament. Tropomyosin shifts deeper into the thin filament groove, thereby exposing myosin-binding sites on the actin monomers and enabling actin–myosin crossbridge formation (Figure 16.12) [24].

At this point in the muscle contraction cycle (illustrated in Figure 16.14), the nucleotide-binding pocket of the myosin head contains ADP and inorganic phosphate (P_i), which are products of

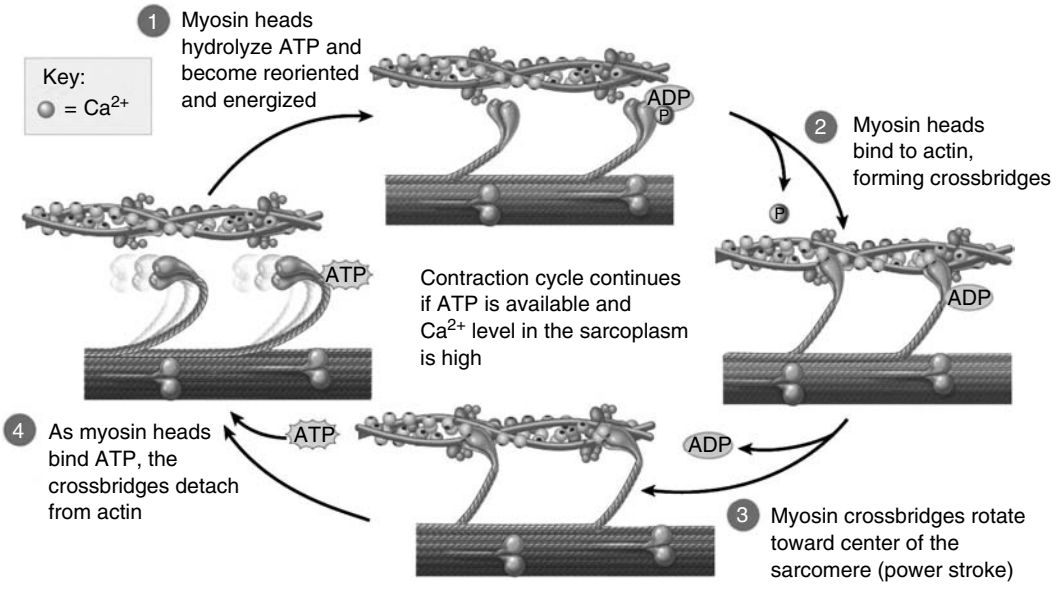


FIGURE 16.14 Crossbridge cycling during muscle contraction. (Reprinted from Tortora, G.J. and B. Derrickson (2006). *Principles of Anatomy and Physiology*. John Wiley and Sons, Inc., Hoboken, NJ.)

hydrolysis of ATP. Exposure of the myosin-binding sites on the actin filament enables weak binding of the myosin head to the thin filament, with the myosin heads binding in an approximately perpendicular orientation relative to the thick filament axis. Upon binding to actin, the myosin head releases P_i, causing a small conformational change in myosin, and strengthening the binding of myosin to actin. This is immediately followed by a large conformational change in the myosin head called the power stroke in which the myosin heads pull the actin filament along the thick filament in a rowing-like motion toward the M-line. ADP is then released from the myosin head, and ATP then binds to the empty nucleotide-binding site that triggers release of the myosin head from actin. Hydrolysis of ATP within the myosin head results in a conformational change that "cocks" the myosin head so that it is again approximately perpendicular to the thick filament, and the reaction series undergoes another cycle of contraction [65]. Because the actin and myosin filaments within a sarcomere are oriented toward the M-line, each contraction cycle results in shortening the distance between the Z-discs, thereby affecting the contraction of muscle (Figure 16.4 and 16.14) [66].

The myosin crossbridge cycling of attachment, power stroke, and detachment continues to maintain tension or contraction as long as neuronal stimulation of the myofiber continues. Upon cessation of the stimulus, sarcoplasmic Ca^{2+} is reduced to resting muscle concentrations by the action of the calcium pump protein. As the sarcoplasmic calcium ion concentration is lowered, TnC is depleted of Ca^{2+} from the low-affinity Ca^{2+}-binding sites, resulting in a conformational change in TnC reverting to the structure associated with the relaxed state of muscle. This conformational change causes tropomyosin to move back to its resting-state position on the actin filament, thereby blocking myosin–actin crossbridge formation, resulting in muscle relaxation.

It is important to recognize the multiple roles of ATP in muscle contraction and to recognize its critical role in conversion of muscle to meat. As the dominant energy source for the cell, hydrolysis of the ATP phosphodiester bond is converted to mechanical energy by myosin resulting in muscle contraction. In addition to the other myriad chemical reactions requiring ATP, the role of ATP in support of the function of the calcium pump is pivotal to the conversion of muscle to meat. As ATP levels decline, the ability of the calcium pump to sequester sarcoplasmic Ca^{2+} is compromised. Finally, it should be evident that after death of the animal, ATP levels will eventually drop to the point at which there is no longer sufficient ATP to bind to myosin heads, which would keep myosin dissociated from actin. The position at the end of the power stroke in which the myosin head is bound to actin is sometimes referred to as the "rigor complex."

16.3.6 Muscle Fiber Types

Skeletal muscle comprises several different types of muscle fibers that display differences in function, including speed of contraction (fast vs. slow) and supporting metabolism (oxidative vs. anaerobic). The muscle fiber types have great adaptive potential and their phenotypic profiles are affected by the type of neuron innervating the muscle fiber, neuromuscular activity, exercise/training, mechanical loading/unloading, hormones, and aging [67]. These fiber types can be defined using histochemical, biochemical, morphological, or physiological characteristics; however, classification of muscle fibers by different techniques does not always agree. Initially, muscles were classified as being fast-twitch (type I) or slow-twitch (type II), on the basis of the speed of shortening of individual fibers [68]. This classification also corresponded to a morphological difference, with the fast-twitch muscles appearing white in some species, and the slow-twitch muscles appearing red. The redness of the fiber correlates with high amounts of myoglobin, which provides a ready source of oxygen in support of oxidative metabolism. The slow-twitch, red fibers generally contain greater amounts of mitochondria and lipids as fuel for oxidative metabolism, whereas fast-twitch fibers are more equipped for anaerobic metabolism fueled by carbohydrates. Later, the type I and type II muscle fibers were reclassified with a bifunctional nomenclature based on the contractile properties and oxidative capacity of the fibers. More specifically, "fast-twitch oxidative" (FOG) muscle contracts with

a faster contractile speed compared with "slow-twitch oxidative" (SO), but has a higher oxidative capacity than "fast-twitch glycolytic" (FG) muscle.

With the advent of immunohistochemical staining methods capable of differentiating myosin heavy chain isoforms, fiber types could be further categorized into type I "slow- red" and type IIa "fast-red," while type IIb "fast-white" was categorized into types IIx and IIb in rodents, and IIx, but not IIb, is expressed in humans [69]. A good correlation exists between type I and SO fibers. However, the correlations between type IIa and FOG and type IIb and FG fibers are more varied. The type IIb fibers do not always rely primarily on anaerobic/glycolytic metabolism, nor do the type IIa fibers always rely primarily on aerobic/oxidative metabolism [70]. With the development of new molecular methods, it is evident that more specific classifications of muscle fiber types will be developed.

16.4 CONVERSION OF MUSCLE TO MEAT

Live muscle tissue has an extensively developed metabolic system designed to support the specific function of muscle, which is to convert chemical energy into mechanical energy. The immediate source of chemical energy is ATP, and the supporting metabolic reactions are geared in large part toward sustaining levels of ATP needed for contraction as well as for maintaining cellular homeostasis. Circulation of blood to the muscle tissue to deliver oxygen and energy substrates and to remove carbon dioxide and metabolic end products is clearly critical to supporting this metabolic machinery.

Upon slaughter of an animal, the initial step in the conversion of muscle to meat is the cessation of blood flow to muscle that occurs at slaughter. Although physiological death of the animal generally occurs within moments after slaughter, the various organs of the body including muscle draw on reserve mechanisms in a futile effort to maintain cellular homeostasis. Without continuous oxygen delivery to the muscle, the myofiber utilizes remaining oxygen bound to myoglobin to support aerobic metabolism. Quickly thereafter, anaerobic glycolysis becomes the dominant metabolic pathway for the generation of ATP. As metabolic end products accumulate and substrates become exhausted, the synthesis of ATP can no longer match the rate at which it is being hydrolyzed. Sarcoplasmic Ca^{2+} concentrations can no longer be sustained at resting state levels, while other ion pumps requiring ATP, such as the Na^{+}–K^{+}–ATPase, become inoperative. The decline of ATP also results in the stiffening of muscle known as rigor mortis; this results from the lack of sufficient ATP to dissociate myosin from actin during contraction. Eventually, endogenous enzymatic activity results in partial degradation of the structure of the myofilament framework. The time course of this series of reactions varies widely from species to species, and as will be seen later, even within a species.

There are few primary reserves of energy that fuel the immediate needs of muscle in the early postmortem state. CrP serves as a rapidly accessible reservoir of high-energy phosphate; as ATP is hydrolyzed to meet the demands of muscle contraction, creatine kinase transfers the phosphate moiety from CrP to ADP, thereby regenerating ATP. The enzyme adenylate kinase (described in Section 16.3.4.2) probably also contributes to the generation of ATP at this point. However, in postmortem muscle, these sources of ATP synthesis are soon exhausted. Glycogen is generally a greater resource for synthesis of ATP. The enzyme phosphorylase cleaves monomeric glucose units from glycogen, yielding glucose-1-phosphate that subsequently enters the glycolytic pathway to generate ATP. In the absence of oxygen, pyruvate is further converted to lactic acid that accumulates in the muscle.

As can be seen in Figure 16.15, the initial generation of ATP from CrP and from glycogen is nearly equal to the rate of ATP utilization, thus sustaining the ATP concentration in the range of 5 mM. As long as ATP levels remain high, the physiological requirements of the muscle are generally supported and the physical characteristics of the tissue during this early stage of conversion of muscle to meat (delay phase) remain similar to those of live muscle. Most notably, the muscle remains

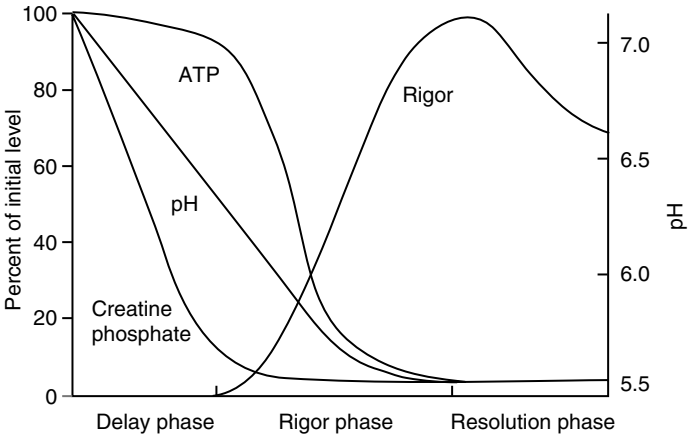


FIGURE 16.15 Schematic representation of postmortem changes in ATP concentration, creatine phosphate concentration, pH, and rigor development as a function of time. (Reprinted from Chiang, W., G.M. Strasburg, and T.M. Byrem (2007). In *Food Chemistry: Principles and Applications*, 2nd edn. Y.H. Hui (Ed.), Science Technology System, West Sacramento, CA.)

pliable and undergoes lengthening when subjected to stretch. However, even as ATP levels remain high, hydrolysis of ATP to support physiological needs results in the generation and accumulation of hydrogen ions that reduces the pH of the muscle. The rate of pH decline closely reflects the accumulation of lactic acid, which serves as a marker of the rate of postmortem glycolysis [9]. As we shall see later, extreme variations in the rate of postmortem glycolysis are often associated with meat quality problems. The length of time associated with the delay phase is quite variable, depending on species, animal genetics, antemortem nutritional status, and management of the animal prior to slaughter, and possibly slaughter method. With red meats, this period may last up to 12 h, whereas with poultry, this period may be in the range of 30 min to 2 h [2].

When CrP is near depletion, the concentration of ATP begins to decline precipitously as utilization exceeds the regenerative capacity. Thus begins the rigor phase (Figure 16.15). As ATP levels fall, there is a corresponding increase in rigor development, measured by the increase in resistance of muscle to stretch. Depletion of ATP reduces the ability of the calcium pump protein to maintain sarcoplasmic Ca^{2+} concentrations in the submicromolar or resting muscle concentration range. Likewise, there is a gradual increase in the number of myosin crossbridges with actin that remain locked because of a lack of ATP to dissociate the two proteins, leading eventually to a maximal stiffening or inextensibility of the muscle. As might be expected, some degree of sarcomere shortening may take place during this period, the magnitude of which is closely related to meat toughness.

The final phase of conversion of muscle to meat is referred to as aging or rigor resolution. This period may last from a few days in poultry, pork, and lamb, to 2 weeks for beef. During the resolution phase, there is a gradual increase in muscle extensibility and tenderness. The basis for these favorable changes is largely the result of proteolytic disruption of the myofiber ultrastructure, most notably at the Z-discs that lose structural integrity rapidly as a function of time. Although development of tension during rigor has important implications in the eventual resolution of rigor, it is widely accepted that proteolysis is largely responsible for the disruption of muscle structure and increase in meat tenderness [71].

16.4.1 Postmortem Degradation of Muscle Proteins

It is recognized that as muscle undergoes conversion to meat, the tenderness of meat tends to increase as a function storage time postmortem. This tenderization may be at least partially the result of degradation of both the myofibrillar and cytoskeletal proteins in muscle [72].

Among the muscle proteases, calpain has been studied most intensively with regard to its role in postmortem muscle protein degradation. Calpain is a calcium-activated, cysteine-protease that is most active in the neutral pH range. Calpain is regulated by a variety of factors, including calcium, phospholipids, and calpastatin, a widely distributed calpain-specific protein inhibitor [73]. Muscle tissue primarily expresses three different calpains: the two ubiquitous calpains and calpain 3 [74]. Ubiquitous calpains include μ-calpain, which requires 5–50 μM Ca^{2+} for its half-activation, and *m*-calpain, which requires 0.25–1 mM Ca^{2+} for its half-activation. These two isoforms are ubiquitously expressed in tissues, suggesting their involvement in basic and essential cellular functions mediated by the Ca^{2+} signaling pathway. Calpain 3 (also called p94, or CAPN3) is a skeletal muscle-specific calpain isoform and it has a lower Ca^{2+} requirement for its activation. Ubiquitous calpains are composed of two subunits: a catalytic subunit of $M_r \sim 80,000$ and a regulatory subunit of $M_r \sim 30,000$. The regulatory subunits are homologous between the two isoforms, but the catalytic subunits are slightly different. Calpain 3 possesses the classical structure of a calpain except that it carries three unique sequences not found in any other calpains. Ubiquitous calpains tend to be concentrated in the Z-discs and treatment of myofibrils with calpains causes rapid and complete loss of the Z-discs. In skeletal muscle cell, calpain 3 binds specifically to certain regions of titin [75]; however, calpain 3 does not cut titin. As the Ca^{2+} concentration increases postmortem, calpains (mainly the ubiquitous calpains) are activated and initiate the degradation of muscle proteins such as troponin-T, titin, nebulin, C-protein, desmin, filamin, vinculin, and synemin [72]. Most of these proteins are either directly attached to (e.g., titin, nebulin), or closely associated with (e.g., filamin, desmin, synemin), or near (vinculin) the myofibrillar Z-discs. When Z-discs are almost completely disrupted, actin and myosin are passively released together with other proteins from the sarcomere and become substrates for other proteolytic enzymes.

Cathepsins are lysosomal proteases that are maximally active at acidic pH, a condition that prevails for the remaining postmortem period, particularly the aging period. At first, this suggests that cathepsin activity is likely to be more important than calpains for achieving the desired tenderization effects during aging. However, when proteinases are incubated with myofibrils, it is the activity of the calpains that closely mimics the proteolytic events in postmortem tenderization. Furthermore, postmortem tenderization is Ca^{2+}-mediated [76], a characteristic only associated with the calpain proteolytic system in skeletal muscle. Still, the importance of cathepsins in aging of meat cannot be overlooked. Their intracellular location and activity on numerous proteins in postmortem skeletal muscle make them logical candidates for postmortem proteolysis and tenderization [77].

The proteosome is a large ubiquitous ATP- and ubiquitin-dependent proteolytic system that may also be involved in myofibrillar protein degradation during aging of meat. The proteosome is able to degrade actin and myosin *in vitro* [78]; however, the proteasome is not able to degrade intact myofibrils. Muscle proteins such as actin and myosin are released from the sarcomere by a Ca^{2+}/calpain-dependent mechanism before they undergo ubiquitination and degradation by the proteasome. In animals overexpressing calpastatin, postmortem degradation by the proteasome is reduced, confirming the involvement of calpains [79]. Therefore, calpain may be the initiator of myofibrillar degradation and the proteasome may be responsible for proteolytic reactions that remove all myofibrillar fragments and hydrolyze them to amino acids.

16.5 NATURAL AND INDUCED POSTMORTEM BIOCHEMICAL CHANGES AFFECTING MEAT QUALITY

Consumer choices in meat purchases are strongly influenced by various product attributes including water-holding capacity, color, fat content, and tenderness of the meat. Meat cuts displaying abnormally light or dark color, or excessive loss of moisture within the package are more likely to be rejected by consumers, thereby downgrading the product value. Likewise, an unsatisfactory

experience by a consumer with an unusually tough cut of meat may result in subsequent product rejection.

The quality attributes of meat are influenced by multiple interacting factors including animal species, breed, genotype, nutritional status, preslaughter handling, and postmortem chilling, processing, and storage. Some specific examples of quality problems and their underlying molecular bases are described in the following sections.

Following aging of meat, the characteristics of the tissue differ substantially from that of live muscle. Postmortem metabolism has led to a decrease in pH from the physiological value of ~7.4 in muscle tissue, to an ultimate pH of ~5.5–5.9 in red meat and poultry. In addition, a degree of contraction has taken place in the tissue prior to the formation of rigor complex.

The consequences of reduced pH are simultaneously beneficial and detrimental to the value of the product. Clearly, the acidic pH of meat will retard microbial growth and thereby extend shelf life compared to the neutral pH of muscle. However, this advantage is offset by the economic loss to processors resulting from loss of water from the tissue as the pH becomes increasingly acidic. The isoelectric point of myosin (the dominant protein in muscle) is approximately 5.0; at this pH, the sum of positive and negative charges is zero, protein–protein interactions are maximal, and protein–water interactions are minimal. As a result, myofibrils shrink and lose much of their water-holding capacity. This loss of water during storage of fresh or cooked product (sometimes referred to as "purge") may be quite substantial, resulting in reduced value because the product bathed in its exudate is unattractive. Moreover, because meat is sold by weight, loss of water equates to loss of product weight and thus, decreased profitability. The product that has lost substantial water content will be perceived by the consumer as having both a reduced juiciness and tenderness. It must also be acknowledged that the watery exudate carries significant quantities of water-soluble vitamins, minerals, amino acids, and other nutrients. These nutrients in the exudate would be lost to the consumer. In addition to reduced water-holding capacity, visual defects may also be brought about by rapid postmortem glycolysis and a low ultimate pH being attained.

16.5.1 Pale, Soft, Exudative Meat

All muscle tissue undergoes a reduction in pH as a result of ATP hydrolysis during the conversion of muscle to meat. However, in aberrant cases, the rate of pH decline is unusually rapid such that most of the pH reduction takes place while the carcass temperature is high. For example, within 45 min postmortem, pig muscle pH is typically in the range of 6.5–6.7 while the temperature is approximately 37°C. In some carcasses, however, the pH may drop to less than 6.0 during the same time period. It is the latter combination of rapidly decreasing pH while the carcass temperature is still high that results in denaturation of some of the contractile proteins, with consequent loss of water-holding capacity, leading to the phenomenon known as "pale, soft, exudative (PSE) meat" [80]. Although these attributes of the fresh cuts are likely to lead to rejection by consumers, the reduced protein functionality of the PSE product in processed meats has also serious economic consequences for the processing industry.

The molecular basis for the PSE meat problem has been the subject of intense investigation over the past half-century. It is clear that antemortem stressors of the animal, such as heat, transportation, physical exercise, mixing of unfamiliar animals, and animal handling, are significant contributors to the problem of meat quality. The exact mechanism by which these stressors result in PSE meat is still unclear, but it is evident from a variety of studies that reduction of antemortem stress results in significant improvement in overall meat quality.

An animal's genotype may further increase its predisposition to an adverse response to stress. In the 1960s it was noted that subsets of pigs within various breeds were particularly susceptible to stressors. The term "porcine stress syndrome (PSS)" was coined to describe the inheritable muscle disorder of the pigs with low tolerance to the effects of stress [81]. These animals typically responded to stressors by developing a condition known as malignant hyperthermia, which is characterized by

severe muscle contracture, respiratory distress, rapid-onset of high fever, and eventually death. Animals with this disorder that did not succumb to the effects of stress were much more likely than normal animals to yield PSE meat.

Studies over the next 30 years eventually led to the identification of a mutation in the RyR that was responsible for PSS. The substitution of thymine for cytosine at nucleotide 1843 in the RyR coding sequence leads to substitution of cysteine for arginine at residue 615 [82]. This mutation leads to excessive calcium release from SR in the stressed animal, which, in turn, triggers severe muscle contracture and eventually malignant hyperthermia in the live pig. Excessive postmortem calcium release in the muscle of the PSS-susceptible pig triggers muscle contraction and associated anaerobic glycolysis with consequent hydrogen ion accumulation and heat production associated with the development of PSE pork.

The problem of PSE meat has classically been associated with pork; however, in the early 1990s, increasing incidence of PSE meat became apparent in the turkey processing industry. The striking similarity in the development of PSE pork and turkey led to the suggestion that a mutation in the RyR is responsible for the problem of PSE turkey [83]. As noted in Section 16.3.4.6, there is a significant difference in the excitation–contraction coupling mechanisms between mammals and birds. Thus, it is possible that if a mutation exists, it may be either in the RyR1 or RyR3 isoforms, or in both. To date, no mutations has been identified in either turkey RyR isoform. However, there are intriguing indications that various alternatively spliced RyR transcript variants can be expressed in turkey skeletal muscle that may alter the tendency to produce PSE meat [84].

Another genetic abnormality that can lead to PSE meat from pigs is the *Napole* (RN) gene. The mutation in this case is a substitution of glutamine for arginine at residue 200 of the γ-subunit of AMP-activated protein kinase [85]. This enzyme plays a variety of roles in muscle including activation of ATP-producing pathways and inhibition of ATP-consuming pathways as well as inactivation of glycogen synthase.

Pigs possessing the dominant RN− allele tend to have much higher glycogen content than pigs with the recessive rn+ allele. The rate of postmortem pH decline in muscle from pigs with the *Napole* gene tends to be normal. However, the high levels of glycogen tend to lead to an extended pH decline and consequently in a very low ultimate pH, resulting in PSE meat with poor protein functionality. In fact, although the water-holding capacity is reduced compared with normal pork, the protein functionality of RN– pork in processed meats is even lower than pork possessing the RyR abnormality. The fact that 65–80% of pork is consumed as processed meats demonstrates the importance of eliminating the *Napole* gene from pigs.

16.5.2 Dark, Firm, and Dry Meat

An occasional consequence of preslaughter stress is antemortem depletion of glycogen stores through stressors, exercise, or excessive fasting. This leads to a product that has the opposite characteristics of PSE meat, and is referred to as "dark, firm, and dry" or DFD meat [86]. The meat color may vary from slightly dark red to extremely dark or nearly black in contrast to the normal cherry red appearance of normal red meat. The problem is most notable in beef, but has also been reported in pork. The lack of adequate glycogen reserves results in early termination of glycolysis, so the ultimate pH remains relatively high (>6.0). The incidence of DFD meat tends to be seasonal, with higher incidence when animals are exposed to sustained periods of cold, damp weather as compared with summer.

Dark, firm, and dry meat has much higher water-holding capacity than normal because the pH is further from the isoelectric point of myosin (~5.0). However, this advantage is strongly offset by the susceptibility of the product to growth of microorganisms and the rejection by consumers because of the abnormal color.

The unusually dark color of this product is a product of the high postmortem pH that keeps the charge on the muscle proteins high, thereby maximizing separation of muscle myofibrils and

reducing light scattering. Active mitochondrial respiration at the higher pH also reduces the fraction of oxymyoglobin in the tissue.

16.5.3 Cold Shortening

In the early 1960s the New Zealand lamb processing industry began receiving complaints from importers in Europe and North America that the meat was excessively tough. This was in contrast to lamb consumed in New Zealand that was not considered to be tough, suggesting that processing and/or storage factors may have been playing a role in the meat toughness [87].

A series of studies from the New Zealand Meat Research Institute elegantly demonstrated the complex relationship between prerigor chilling and meat toughness (Figure 16.16). When prerigor muscles are excised from the bones to which they are attached, they undergo contraction. The extent of contraction depends on the type of muscle (red vs. white), amount of time postmortem, physiological state of the muscle, and temperature. At near-physiological temperatures, the degree of shortening is high. At lower temperatures, the extent of muscle contraction decreases progressively until a nadir is reached in the range of 10–20°C, temperatures at which minimal shortening takes place. If the same muscles are subjected prerigor to still lower temperatures, one notes a dramatic increase in the degree of shortening at temperatures below 10°C. The cold-induced muscle contraction is termed "cold-shortening."

Marsh and Leet [88] subsequently demonstrated the relationship between prerigor cold-shortening and toughness (Figure 16.17). The Warner–Bratzler shear test measures the amount of force required to cut through a fixed size of muscle tissue. Using this test, Marsh and Leet demonstrated that as the degree of shortening increased, the degree of toughness (measured by shearing force) increased, up to the point when muscle had contracted by about 40%. At this point, there is maximal overlap between the thin and thick filaments, and nearly all of the myosin heads are bound

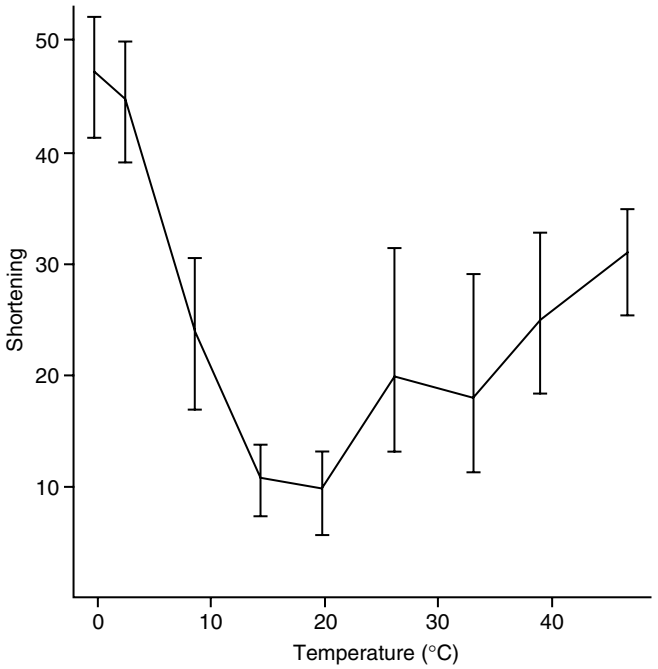


FIGURE 16.16 Shortening of excised prerigor beef muscle as a function of storage temperature. (Reprinted from Locker, R.H. and C.J. Hagyard (1963). *J Sci Food Agric* 14:787–793.)

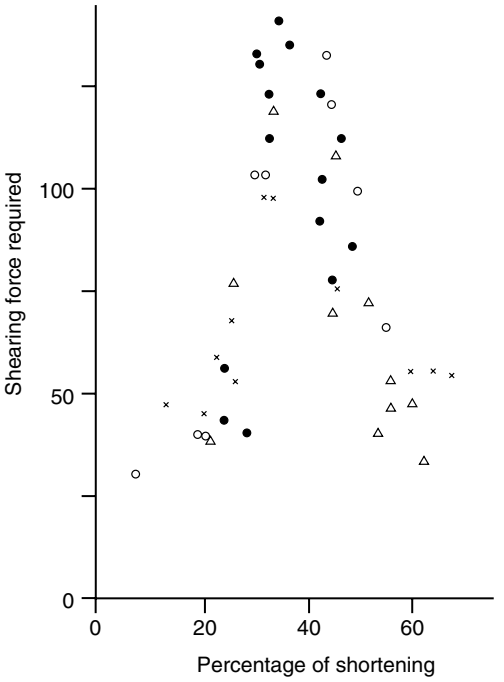


FIGURE 16.17 Meat toughness measured by shear force required to cut through a meat sample as a function of sarcomere shortening. Muscle shortening was induced by storage at various temperatures as well as by thaw rigor. (Reprinted from Marsh, B.B. and N.G. Leet (1966). *Nature* 211:635–636.)

to actin filaments. Beyond this point, additional contraction may occur resulting in a decrease in toughness. The explanation for this observation is that under some circumstances such as lack of tension, muscle contraction may continue to the point that the thick filaments puncture the Z-disc, causing extensive damage to the muscle ultrastructure and thus leading to an increase in tenderness [89]. On the basis of these observations, substantial modifications were made to the postslaughter chilling of lamb carcasses to reduce prerigor chilling rate. Subsequent studies in the United States and Europe have validated the significance of cold-shortening in the red meat industry and led to practices to minimize this problem.

The mechanism underlying the development of cold-shortening is still not completely clear, but the following factors clearly play a role. Muscle must be in the prerigor state for contraction to occur; only during this period are there adequate levels of ATP to provide the energy for contraction as well as for dissociating myosin heads from actin for a subsequent contraction cycle. The temperature must be lower than 10°C, and the closer to 0°C, the greater the degree of shortening. Finally, the muscles most susceptible to cold-shortening are those with the highest percentage of red fibers, for example, beef and lamb or sheep muscles. Cold-shortening occurs to a lesser extent in pork because most pig muscles have higher percentage of white muscle fibers. Relatively little cold-shortening occurs in poultry muscle.

Various lines of evidence suggest that altered postmortem calcium regulation and temperature-dependent changes in enzyme activities are responsible for the cold-shortening phenomenon. As the temperature of the muscle goes below 10°C, anoxic mitochondria and SR lose their ability to retain Ca^{2+} ions. The calcium pump protein would normally keep sarcoplasmic Ca^{2+} concentrations at resting muscle levels. However, at the lower temperatures, the rate of SR reaccumulation of Ca^{2+} is suppressed; thus, sarcoplasmic Ca^{2+} concentrations increase, thereby triggering muscle contraction. Red muscle fibers are more likely to exhibit cold-shortening. They rely primarily on oxidative

metabolism to support energy requirements; thus, they have a higher amount of mitochondria than white fibers. Moreover, red fibers tend to have a less developed SR network, thereby reducing their ability to reaccumulate Ca^{2+} [86].

Strategies to prevent cold-shortening were initially built on the relationship between the rate at which the carcass was chilled and development of toughness. Keeping carcass temperatures in the range of 10–20°C until rigor mortis is established and prevents cold-shortening. However, this approach is in conflict with the desire of lowering carcass temperatures quickly to minimize microbial growth on the surface of the meat. In the 1970s, experiments with electrical stimulation of carcasses demonstrated that the time for development of rigor mortis could be dramatically shortened [90]. This procedure is described in Section 16.5.5, and has now become standard industry practice for most beef and lamb slaughter operations.

16.5.4 Thaw Rigor

A phenomenon closely related to cold-shortening is the severe muscle contracture that takes place upon thawing muscle that had been frozen while still in the prerigor condition. Thaw rigor was first described by Sharp and Marsh [91] who noted that thawing of prerigor whale muscle resulted in shortening of the muscle by as much as 60%, and was accompanied by substantial loss of water-holding capacity. Thaw rigor likely results from structural damage to the SL and SR membranes from ice crystal formation. The loss of membrane integrity results in Ca^{2+} influx into the sarcoplasm triggering muscle contraction because ATP levels are still sufficiently high for shortening to occur. Likewise, membrane damage results in excessive drip loss from the muscle fibers upon thawing. The same approaches to preventing cold-shortening, electrical stimulation or holding muscle on the carcass at temperatures above freezing until the onset of rigor mortis, will serve to prevent thaw rigor.

16.5.5 Electrical Stimulation

Electrical stimulation refers to the application of an alternating electrical current to carcasses of meat animals following slaughter. When applied to carcasses during the early postmortem period, electrical stimulation induces extensive muscle contraction and relaxation, which consequently accelerates the rate of muscle metabolism, ATP turnover, and rigor development. Depending on the voltage, frequency, and duration of the stimulus, this procedure reportedly improves the tenderness, flavor, color, quality grade, retail case life, and processing characteristics of meat and meat products [92].

Improvements in color and quality grade have been primarily responsible for the adoption of electrical stimulation by the processing industry in the United States. In other countries, the primary reason has been improvement in tenderness. Use of electrical stimulation was initially adopted in New Zealand, where the practice of rapid chilling and freezing of lamb carcasses for export led to cold-shortening, thaw-shortening, and increased meat toughness [90]. However, electrical stimulation is also effective in improving meat tenderness in the absence of cold-shortening. Pearson and Dutson [93] discussed mechanisms for tenderization. Lysosomal disruption with consequent release of endogenous proteases has been suggested as a mechanism, based on an increase in the free activity of lysosomal enzymes after electrical stimulation. Thus, increased proteolytic activity prior to and during the aging period could account for a portion of the increased tenderness. Another mechanism appears to be the physical disruption of myofiber integrity caused by the extensive contractions induced with electrical stimulation [94]. Zones of supercontraction (contracture bands) and excessive stretching of the myofilaments adjacent to contracture bands are evident in photomicrographs of meat from electrically stimulated carcasses. On the basis of the physical dimensions of thick and thin filaments, contractions beyond 40% of resting sarcomere length would result in the thick filaments penetrating Z-discs and interacting with thin filaments of adjacent sarcomeres [89]. Other areas must stretch and/or tear to accommodate these zones of supercontraction. An increase in tenderness

would ensue. Although conclusive support is still lacking for the case of physical disruption, the proposed mechanism is consistent with other instances of tenderization associated with excessive contraction.

The beneficial effects of electrical stimulation are thought to occur because of the acceleration in the postmortem conversion of muscle to meat. In essence, stimulated carcasses reach their maximum quality grade sooner, which allows the packer to either increase production at similar quality grade levels or increase quality grade levels at similar production [92]. Where quality grade influences the pricing system, either case is financially more favorable for the packer and accounts for the extensive use of electrical stimulation in the beef industry.

16.6 CHEMICAL CHANGES IN MEAT DURING PRESERVATION

The susceptibility of muscle tissue to microbial spoilage requires that meat be preserved by physical or chemical methods. Refrigeration and freezing provide the most effective means to retard microbial growth as well as to minimize deleterious chemical and biochemical processes in meat and meat products. Traditionally, fresh meat is preserved by salting and partial dehydration that raise the osmotic pressure and lower the water activity thereby suppressing the growth of microorganisms. Irradiation and high-pressure treatments are relatively new preservation interventions that are becoming acceptable in the meat industry. On the other hand, nontraditional packaging systems, such as modified atmosphere packaging, are gaining popularity to extend meat shelf life. All these preservation techniques affect not only the ecology of microorganisms on meat but also the chemical properties of meat and meat products.

16.6.1 Chilling and Refrigeration

In a typical packing plant, animal carcasses are rapidly chilled in a 2–5°C chill cooler to minimize microbial growth; for chicken broilers and fish, the carcasses are usually chilled by immersion in ice slurries. The time duration for a warm carcass to reach its final chilled temperature varies, depending on its size, thickness of subcutaneous fat, and the chilling methods. The chilling time can be as short as 1 h for an ice-chilled broiler carcass and up to 24 h for a 300-kg beef carcass. At some commercial packing plants, cold water showering and high-velocity air are used to facilitate the chilling process.

The rate of chilling affects the enzymatic reactions in postmortem muscle tissue, which in turn affects the quality of aged meat. The major biochemical changes occurring during the early stage of muscle to meat conversion—the pH fall (glycolysis), the depletion of ATP, and muscle contraction—are enzymic processes. As the carcasses are rapidly chilled, these biochemical changes are retarded due to the inhibition of enzyme activities. Lipid oxidation also proceeds more slowly at refrigeration temperatures due to the reduced activity of oxidative enzymes. Inhibition of lipid oxidation would preserve the fresh meat flavor and minimize myoglobin oxidation. However, exposing prerigor muscle to cold temperatures could have an adverse effect on meat tenderness due to increased muscle fiber contraction or shortening, notably for red meat. To minimize cold-shortening, it is imperative that muscle remain attached to the skeleton during chilling, especially in the early stage of postmortem storage. Because the activity of major endogenous proteases involved in meat tenderization during postmortem aging, for example, calpain and cathepsins, is greatly reduced at low temperatures, it is necessary that the carcass or meat be adequately aged to achieve an acceptable level of tenderness.

16.6.2 Freezing

Freezing is one of the most effective methods of preserving meat. When meat and meat products are stored at temperatures below −10°C, microbial growth and enzyme reactions are essentially

curtailed, and hence, quality loss is minimized. However, physical and chemical reactions can still occur in meat during freezing, storage, and subsequent thawing. Chemical changes in frozen meat during storage include discoloration and development of oxidative rancidity, which result from oxidation of myoglobin and unsaturated lipids, respectively, and texture hardening due to protein denaturation and aggregation. These adverse changes are influenced by the rate of freezing and thawing, the duration of frozen storage, fluctuations of the freezer temperature during storage, and the atmospheric condition of the frozen meat. In the case of processed meat, the ingredients added to meat (e.g., NaCl) and the specific processing procedures, such as grinding, chopping, emulsification, and restructuring, can influence the quality and shelf life of the frozen products. Antioxidants are often added to inhibit salt-induced oxidation in frozen meat products.

Freeze-induced protein denaturation, a main side effect of frozen meat, is attributed to physical damage resulting from the formation and accretion of ice crystals, and from chemical processes associated with dehydration and concentration of solutes in the muscle tissue. Freeze-induced protein denaturation is especially notable under slow freezing conditions. At a slow freezing rate, the exterior fluid of muscle cells cools more rapidly than the interior fluid, and when the supercooled extracellular fluid reaches a critical temperature, water separates from solutes and forms ice crystals. As crystallization proceeds, extracellular salt becomes more concentrated, creating an osmotic pressure gradient across the cell membrane. These processes can lead to protein denaturation and disruption of the cell membrane [95]. To prevent protein denaturation, cryoprotectants, such as polyphosphate and polyols (sorbitol, sucrose, polydextrin, etc.), can be incorporated into meat prior to freezing.

The rate of freezing is dictated by freezing methods employed, and follows the order of cryogenic freezing > blast-freezing > still-freezing. Cryogenic freezing, which uses condensed gases such as liquid nitrogen (−195°C) and solid carbon dioxide or dry ice (−98°C), rapidly chills meat to below 0°C and transforms liquid water into ice crystals in a matter of minutes. Blast-freezing air (e.g., −50°C) also enables a rapid heat transfer thereby crystallizing intramuscular water in a very efficient manner. On the other hand, still-freezing allows slow heat dissipation from meat, and can cause damage to muscle cell and proteins. In general, fast freezing promotes the formation of small ice crystals that are uniformly distributed inside and outside the muscle cells, while slow freezing favors the formation of large ice crystals that are much less in quantity and are prevalent extracellularly.

A relatively new freezing technology, known as "pressure-shift freezing," has been introduced as a potential meat quality preservation method. During pressure-shift freezing process, meat samples are chilled to subfreezing temperatures (e.g., −20°C) and will not freeze under a certain high pressure. When the pressure is suddenly released, instantaneous and homogeneous microcrystallization occurs throughout the muscle tissue. Meat processed with pressure-shift freezing reportedly has a minimally altered ultrastructure, reduced protein denaturation, and an improved product quality [96,97].

16.6.3 Pressurization

High-pressure treatment is potentially useful for meat preservation as well as for processing to improve product quality. Hydrostatic pressures ranging from 100 to 800 MPa have been used to destroy pathogenic microorganisms and inactivate spoilage enzymes in fresh meat prior to storage. Nonthermal, high-pressure processing is also used to process postpackaging ready-to-eat meats, such as deli slices, to eliminate potential contamination by *Listeria monocytogenes*. Because the compression energy is low (e.g., 19.2 kJ for 1 L of water under a 400 MPa pressure), covalent bonds are usually not affected. However, high-pressure treatment may disrupt electrostatic and hydrophobic interactions in proteins, thereby rendering them less stable. Because pressurization processes do not depend on additives or temperature, treated meat products will retain their flavor and taste.

Physical modifications in muscle tissue under high pressures include the decrease in the volume of the aqueous phase and a drop in pH. These changes are reversible upon pressure release. However, even a brief exposure to these temporary changes could permanently alter the protein structure

and its association with nonprotein compounds. Pressures at above 100 MPa can cause protein quaternary structure to dissociate into its subunits, monomeric structure to unfold, and can induce protein aggregation and gelation [98,99]. High-pressure treatment separates myosin heavy chains into one-headed monomers, which is followed by head-to-head interaction to form aggregates [100]. Pressure-induced changes in the hydration volume appear to play a major role in the unfolding, dissociation, aggregation, and gelation of muscle proteins. Pressure-treated muscle proteins, such as surimi, can spontaneously form a gel at mild temperatures. This results from increased exposures of hydrophobic side chain groups, allowing protein aggregation to readily occur.

High-pressure treatments of prerigor muscle increase the rate of glycolysis and fiber contraction, which is attributed to multiple factors, including the disruption of SR where calcium is normally stored and the loss of Ca-ATPase activity. The high cytosolic concentration of Ca^{2+} activates enzymes involved in glycolysis (e.g., phosphorylase kinase) and muscle contraction (e.g., myosin ATPase). When the applied pressure is sufficiently high (e.g., >400 MPa), discoloration occurs in raw meat as indicated by an increased L^* value and a decreased a^* value [98]. Indeed, exposures of raw meat to high pressures result in an increased metmyoglobin (brown) content at the expense of myoglobin (red) due to oxidation of heme iron and the denaturation of globin.

A pressure at above 150 MPa can induce extensive changes in sarcomere structure as well, for example, the disappearance of the M-line and H-zone and the loss of integrity of I-band filaments. Surprisingly, these structural changes do not seem to lead to meat tenderness improvement. This may be due to thickening of the Z-discs in costameres and the loss of protease activity [101]. On the other hand, collagen structure is not affected by high-pressure processing.

In high-pressure-treated beef, the level of μ-calpain is markedly reduced during aging [102]. Both μ-calpain and m-calpain are partly inactivated at 200 MPa and completely inactivated at 400 MPa. High-pressure-induced denaturation and the enhanced autolysis of calpains due to the high concentration of cytosolic Ca^{2+} released from SR are responsible for the negative effect. On the other hand, high-pressure application increases catheptic activity (B, D, L, H, and peptidases) due to the disruption of lysosomal membrane. The increased release of cathepsins from lysosomes apparently is sufficient to overcome the pressure-induced denaturation. Nevertheless, the enhanced catheptic activity is not able to compensate for the reduced tenderness resulting from the loss of calpain and structural changes in myofibrils. For this reason, softening of muscle tissue in fish during storage due to excessive proteolytic activities can be overcome by high-pressure treatments that inactivate endogenous proteases, especially lysosomal enzymes.

Extremely high pressures generated with pyrotechnic devices have been invented to tenderize meat. A particular example of such technologies is Hydrodyne® [103]. In this method, encapsulated fresh meat is placed in a sealed, water-filled container situated below the ground level. A small amount of explosive, consisting of a liquid and a solid, generates a shock wave that is in acoustic match with water in muscle. The shock wave produces an extraordinarily high pressure measuring about 680 atm or 10,000 psi at the contact surface with the meat. Meat exposed to such a high pressure exhibits remarkable tenderness improvements and requires less aging time to achieve desirable tenderness. The tenderizing effect is attributed to the disruption of the myofibrils, including the Z-discs. Because Hydrodyne also inactivates microorganisms, it has the additional benefits of preserving fresh meat and enhancing meat safety.

16.6.4 Irradiation

Irradiation as a means to inactivate pathogenic microorganisms has gained acceptance in the meat industry. There are two types of radiations: ionizing radiation and nonionizing radiation. In nonionizing radiation, such as microwave and infrared frequencies, the energy of radiation is not high enough to cause atoms to ionize. Instead, it relies on the heat it generates to destroy microorganisms and, hence, is suitable for heat-processed meat products. In ionizing radiation, a radiation generated by high-speed electrons or radioactive isotopes (γ-radiation) strike atoms to produce ions, and

destruction of microorganisms is therefore more effective. The permissible dosages of irradiation are 1.5–3.0 kilogray (kGy) for poultry and 7.0 kGy for beef.

γ-Irradiation is a proven method of radiation for fresh or raw meat. Although it is effective in reducing microbial contamination, adverse chemical changes to muscle tissue due to radiolysis do occur. For example, γ-irradiation of fresh meat can produce superoxide and hydroxyl radicals. These primary radicals are highly reactive and can react with muscle lipids and proteins to generate secondary radicals and lipid and protein degradation products. Differing from small radicals, which are short-lived in the aqueous environment of meat, protein radicals can be relatively long-lived and can cross-link with one another causing the muscle tissue to harden. On the other hand, degradation of unsaturated lipids in muscle following γ-irradiation leads to the production of various hydrocarbons, particularly alkenes and carbonyl compounds, which contribute to off-flavor of treated products. Volatile sulfur compounds are also produced by γ-irradiation due to radiolytic degradation of side chains of methionine and cysteine residues, and they are the main off-odor volatiles produced in irradiated, vacuum-packaged red meat and poultry [104].

Discoloration is another major consequence of γ-irradiation. Irradiated meat can develop unattractive greenish or brownish gray colors, which appear to be caused by the breakdown of the porphyrin structure of the heme or the formation of sulfmyoglobin [105]. For light-colored meat, such as poultry breast, an intense pink color can form that has been attributed to the formation of a carbon monoxide–myoglobin complex [106]. Because chemical changes caused by γ-irradiation are usually radical-driven processes, the use of vacuum-packaging or incorporation of proper water- and lipid-soluble antioxidants can minimize the negative impact on muscle food quality.

16.7 CHEMISTRY OF PROCESSED MEATS

Processing of meat refers to the application of physical, chemical, and thermal treatments of muscle tissue to increase the product variety, to offer convenience, and to extend meat shelf life. It involves extensive modifications of the physicochemical properties of fresh meat. Processed meats may be separated into three main categories: (1) those in which the structural characteristics of muscle are minimally altered, for example, cured ham and bacon, and corned beef; (2) those with moderately altered muscle structure, for example, sectioned and then restructured roasts and steaks; and (3) those that are extensively comminuted and then reformed, for example, sausage, frankfurters, and many luncheon meats. The chemical changes in the muscle tissue depend on the specific modification procedures and ingredients employed. For example, the development of a stable, pinkish red color in cured ham is due to the chemical reaction of nitric oxide with myoglobin; the formation of stable, fat globules in emulsion-type products is largely attributed to protein–lipid interaction at the water–oil interface; and the adhesiveness and smooth texture of boneless turkey ham result from interaction and gelation of myofibrillar proteins extracted by salt and phosphate.

16.7.1 Curing

The term "curing" refers to the treatment of fresh meat with salt and nitrite (or nitrate) for the purpose of preservation and obtaining desirable color and flavor. The origin of the curing technology is lost in antiquity, but is generally believed to be around 3000 BC. Cured meats have a characteristic pinkish color and a distinct aroma. They include traditional products such as ham, bacon, and summer sausage, and a variety of ready-to-eat products available in the deli or refrigeration section at retail outlets, for example, restructured and sliced turkey ham and deli-type bologna.

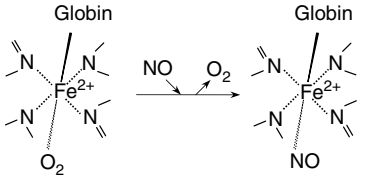
Sodium chloride (NaCl) is the common salt used in cured meats. The main functions of salt, other than imparting flavor, are to extract myofibrillar proteins and to increase osmotic pressure, thus inhibiting bacterial growth and subsequent product spoilage. Although salt is an indispensable ingredient in cured products, the actual curing agent is nitrite (NO_2^-) or nitrate (NO_3^-). Nitrate was

originally approved for color fixation in cured meats, but now it has largely been replaced by nitrite, because the latter is the immediate precursor of nitric oxide (NO), the ultimate curing compound. Nitrate is now restricted to dry-cured products, such as country-cured hams and dry sausages. In making these products, nitrate is slowly converted to nitrite by microorganisms or by reducing compounds, allowing slow curing reactions that presumably produce more desirable flavors and a stable color.

Nitrite is a multifunctional chemical. It induces and stabilizes the pinkish color of lean meat, contributes to the characteristic flavor of cured meat, inhibits the growth of spoilage and pathogenic microorganisms (particularly *Clostridium botulinum*), and retards development of oxidative rancidity. The pinkish red color characteristic of cooked, cured meats results from the reaction of the myoglobin heme with nitric oxide forming the nitrosylmyoglobin pigment. Nitric oxide is derived from nitrite in the presence of reducing compounds such as erythorbic acid. Part of nitrite dissolved in water can form nitrous acid (HNO_2). Under reducing conditions, nitrous acid decomposes to nitric oxide. When nitric oxide binds to the heme iron, it changes the electron distribution in the heme structure, thereby producing a pinkish color. Upon heating, nitrosylmyoglobin is converted to nitrosylhemochromogen, which is more stable due to globin denaturation.

$$NaNO_2 + H_2O \rightarrow HNO_2 + NaOH$$

$$3\,HNO_2 \rightarrow HNO_3 + 2\,NO + H_2O$$



Reducing compounds are added in meat curing mixtures to hasten color development via converting nitrite to nitric oxide, and ferric ion of the heme to ferrous ion. The most commonly used reducing compound is sodium erythorbate (an isomer of ascorbate). Muscle itself also contains endogenous reductants and enzymatic reducing activity, for example, cytochromes, quinines, and NADH, but the reducing power of these factors is relatively small. In addition to reducing metmyoglobin (Fe^{3+}) to myoglobin (Fe^{2+}), and nitrite to nitric oxide, erythorbate also serves as an antioxidant to stabilize both color and flavor, and to decrease the formation of nitrosamines. Phosphates, such as sodium pyrophosphate, tripolyphosphate, and hexametaphosphate, are other curing adjuncts. Phosphates do not directly enter the curing reactions but they function to increase water-holding capacity of muscle and contribute to oxidative stability by chelating pro-oxidative metal ions.

16.7.2 Hydration and Water Retention

As discussed in previous sections, water accounts for 70–80% of the weight in fresh meat. In injected or pumped meat, the water content can exceed 85%. The amount of moisture present in cooked meat determines the product juiciness and influences its tenderness. Water in meat is either bound or in a free form. Bound water is tightly associated with proteins through hydrogen bonds, which is influenced by the surface charge and polarity of protein. Free water is held via capillary forces in different compartments of the muscle tissue, for example, in the spaces between myofilaments, between myofibrils, and outside the fibers. This form of water makes up the bulk of the water in meat (70–90%). In comminuted meats, a large portion of water is also retained via entrapment in the matrix of myofibrillar protein gels. Denaturing conditions, such as frozen storage, oxidation, and rapid acid accumulation postmortem while the muscle temperature remains high, leads to reduced water binding in meat. An example of meat with poor water-binding ability is PSE pork and turkey, which has been discussed in previous sections.

The ability to bind, immobilize, and retain indigenous as well as exogenous water in processed meat is largely attributed to myofibrillar proteins, which are influenced by meat ingredients. High concentrations of monovalent salt (NaCl or KCl) solutions, that is, brine, are commonly incorporated into meat through marination or injection. Hydration and retention of added water are made possible through the NaCl-induced myofibril expansion owing to increased electrostatic repulsion, and thus, transverse swelling [107]. A variety of phosphate compounds, including sodium pyrophosphate, sodium tripolyphosphate, and sodium hexametaphosphate, are used in conjunction with salt to further improve the moisture-retention capability of meat. Injected fresh meat usually contains both salt (0.5–2.0%) and phosphate (0.25–0.40%). When an alkaline phosphate is used, it confers an additional benefit by raising the meat pH from around 5.5–5.6 (which is close to the isoelectric point of actomyosin) to 5.8–6.0 where myosin and most other muscle proteins will bind water more strongly due to increased net charges. A pH elevation would also allow interfilamental spaces to further expand via electrostatic repulsions for additional water to be immobilized.

The mechanism of NaCl- and phosphate-induced meat hydration extends beyond their simple electrostatic repulsion effect. In addition to increasing charge repulsions between adjacent myofilaments, high concentrations of NaCl (e.g., >2.5%) are able to dissociate myosin filaments, creating a bulky polypeptide matrix for moisture retention [108]. Furthermore, at elevated NaCl concentrations, the isoelectric point of myosin shifts to a lower pH due to screening of positive charges ($-NH_3^+$) in proteins by Cl^-. As a result, myosin (or actomyosin) within the normal pH range of meat will carry more surface charges (Figure 16.18). The increased interpeptide electrostatic repulsion enables a stronger protein–water interaction and a greater water-retaining capacity of meat. On the other hand, low concentrations of pyrophosphate and tripolyphosphate (<0.5% or 5–15 mM) are capable of dissociating the actomyosin complex. In the presence of magnesium, the dissociation effect of pyrophosphate is very similar to that exerted by ATP. The detachment of actin from myosin filaments allows water to more readily diffuse into the interfilamental spaces. The dissociation also improves meat tenderness. Significant muscle fiber swelling occurs as the NaCl concentration is raised from 0.1 to about 0.6 M in the absence of phosphate, or to 0.4 M in the presence of phosphate [109]. The extent of swelling and hydration continues until 1.0 M NaCl (~4.0% of muscle weight) is incorporated where the swollen fiber will start to shrink due a salt-out effect.

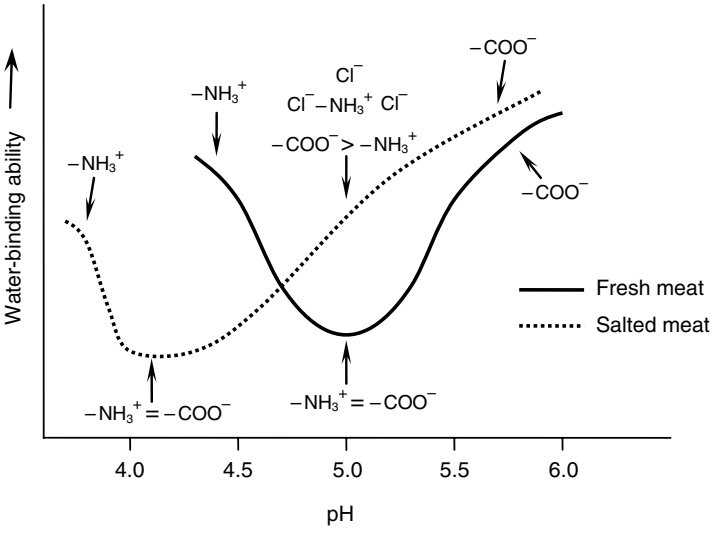


FIGURE 16.18 Schematic representation of the relationship between pH and water binding by proteins in fresh and salted meat.

Hydration of salt- and phosphate-treated meat is accompanied by partial extraction of myofibrillar proteins. Selective removal of proteins from the myofibril backbone may be necessary for the transverse expansion of the protein filaments. Phase contrast microscopy shows that myofibril "swelling" and removal of proteins from the thick filaments (myosin) in the 0.6–1.0 M NaCl solution occur concurrently [107]. The addition of 10 mM pyrophosphate or tripolyphosphate greatly facilitates the hydration process, and causes myosin to be extracted from the ends of the A-band where myosin cross-links with actin. Moreover, the extraction of transverse structural polypeptides, for example, M-protein, X-protein, and C-protein, by the presence of salt and phosphate, seems to promote loosening of myofibril lattices, thereby allowing water pickup by muscle fibers [110,111].

16.7.3 Formation of Protein Gel Matrix

Gelation of proteins is a physicochemical process involved in restructured and comminuted meat products. The gel formation is not only responsible for adhesion of meat pieces and particles, but also plays an important role in water, flavor, and fat binding in cooked products. Gelation in heated muscle foods takes place as a three-step sequential process. The initial unfolding (denaturation) of individual protein molecules is followed by their aggregation, largely through hydrophobic interactions; and at the final step, small protein aggregates or oligomers are cross-linked to form fine strands that eventually lead to a continuous viscoelastic network [112]:

$$\chi P_{\mathrm{N}} \xrightarrow[\text{Denaturation}]{\text{Heating}} \chi P_{\mathrm{D}} \xrightarrow[\text{(aggregation)}]{\text{Heating}} (P_{\mathrm{D}})_{\phi}, (P_{\mathrm{D}})_{\psi}, \cdots \xrightarrow[\text{(cross-linking)}]{\text{Heating/cooling}} (P_{\mathrm{D}})_{\phi} - (P_{\mathrm{D}})_{\psi} - \cdots,$$

where χ is the total number of protein molecules, ϕ and ψ ($\phi + \psi + \cdots = \chi$) are the number of molecules that are aggregated at certain point of the gelation process, P_{N} is native protein, and P_{D} is denatured protein. Examples of gel-type products are bolognas, frankfurters, and various luncheon meats made from comminuted muscle. Because of its adhesion ability, the gel formed at the junction of meat chunks in restructured products (e.g., boneless ham and turkey rolls) is largely responsible for the product integrity and sliceability.

Sarcoplasmic and connective tissue (stromal) proteins play only a minor role in the overall gelation phenomenon in processed meat. Most sarcoplasmic proteins are readily coagulated when salted meat is cooked to 40–60°C and they do not form an ordered, functional gel structure. Partially hydrolyzed collagen (gelatin) is the best-known gelling protein and its gelation is relatively insensitive to ionic strength. Gelatin forms reversible, cold-set gels, which are stabilized by hydrogen bonds. However, dissociation and degradation of collagen into soluble gelatin (the gelling component) requires moist, prolonged heating, a condition that is not commonly employed in producing muscle foods. On the other hand, myofibrillar proteins as a whole are superior gelling proteins, playing a vital role in producing desirable textural characteristics in processed muscle foods. In particular, myosin (prerigor) or actomyosin (postrigor) accounts for most of the gel-forming capacity of the myofibril protein system [113].

In order to form a gel, myofibrillar proteins must be extracted first, and this is usually initiated by mixing meat with salt (NaCl or KCl) and phosphates. The gelling properties of myofibrillar proteins are influenced by the proteins' structure and size, their concentrations, the source or type of meat, and the various processing conditions such as pH, ionic strength, and heating rate. Thus, myosin, which has a large length-to-diameter ratio (approximately 100 nm in length and 1.5–2 nm in diameter), can form a highly viscoelastic gel, whereas actin, which is a globular protein of about one-tenth of the myosin size, is a poor candidate for gelation [114], although it may reinforce myosin gels at a myosin-to-actin ratio (w/w) of about 24 [115]. Myofibrillar proteins from muscle white (FG) fibers form more rigid gels than those from red (SO) fibers, and this is attributed to the different physicochemical characteristics existing between myosin isoforms [116]. This explains why chicken pectoralis major, which consists exclusively of white fibers, and its myosin or mixed myofibrillar

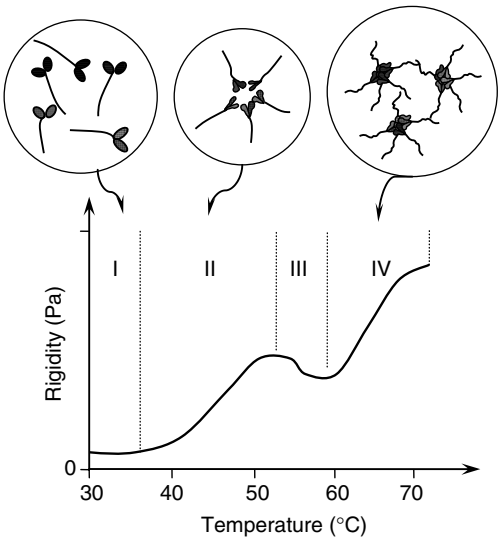


FIGURE 16.19 Schematic representation of heat-induced gelation of myosin in a 0.6 M NaCl, pH 6.0 solution. The four temperature zones show, respectively, (I) no change in myosin, (II) head–head association, (III) structural rearrangement of myosin aggregates owing to unfolding of light meromyosin, and (IV) cross-linking of myosin agglomerates via tail–tail association.

proteins, forms stronger gels than chicken gastrocnemius (preponderantly red fibers) or its proteins under equal meat processing conditions. Another unique property of myofibrillar proteins is that they tend to form the strongest gel at pH around 6.0, although the exact pH optima vary slightly depending on muscle types and animal species.

The mechanism of heat-induced myofibrillar protein gelation is largely accounted for by myosin, the major gelling component in the salt extract of processed meat. Under a typical meat processing condition (pH 6.0, 0.6 M, or 2.5% NaCl), the gelation begins with unfolding of S-1 region of HMM when the protein sol is heated to about 35°C, leading to hydrophobic association through head–head interactions (Figure 16.19). The oligomers then coalesce at about 48°C and form intermolecular disulfide bonds, producing a somewhat elastic characteristic. When the temperature approaches 50–60°C, conformational changes in LMM (rod) occur, creating an open structure that exposes hydrophobic regions and specific side chain groups. The structural change results in a temporary decline in the elastic characteristic of the semigel. For actomyosin, the drop in the gel rigidity in this intermediate temperature region is also related to the detachment of actin. However, the ensuing association of LMM via tail–tail interactions upon further heating leads to the formation of permanent strands and filamentous gel networks with high elasticity and water-binding capacity that are stabilized by disulfide bonds.

Microbial transglutaminase, an enzyme that catalyzes acyl transfer reactions thereby cross-linking proteins through the glutamine–lysine bridge, has a remarkable effect on myofibrillar protein gelation. Incorporation of this enzyme into the gelling solution results in as much as ten-fold increases in myofibril gel strength, and hence, appears to be an excellent food ingredient for use in meat and surimi processing where meat binding is of main importance [117,118].

16.7.4 Fat Immobilization and Stabilization

Fat in processed meat, notably in emulsified products, is immobilized and stabilized by the formation of protein interfacial membrane and protein matrices. During comminution or emulsification, large fat particles or the adipose tissue are broken down to fine granules through shear. As the small fat

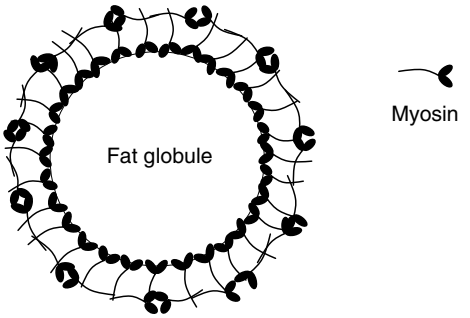


FIGURE 16.20 Schematic representation of a meat emulsion fat globule depicting a myosin monolayer.

globules are formed, they are coated by proteins, which are amphoteric in nature, that is, possessing both hydrophobic and hydrophilic groups. Specifically, the nonpolar groups are imbedded in fat (hydrophobic) while the polar groups extend into the aqueous phase, forming an interfacial film that separates the two immiscible phases (lipid and water). The adsorption of protein on the surface of fat globules decreases the interfacial energy, and denaturation of the adsorbed protein leads to the formation of a protein gel matrix; both these events enhance the emulsion stability. The relative emulsifying activity of muscle proteins follows the order of myosin > actomyosin > sarcoplasmic proteins > actin [119]. The excellent emulsifying capability of myosin is attributed to its unique structure (high length-to-diameter ratio) as well as to its bipolar nature (hydrophobic head and hydrophilic tail). A presumptive monolayer interfacial film, formed predominantly by myosin, is presented in Figure 16.20.

Fat globules in a well-comminuted meat, commonly referred to as "batter," are uniformly distributed in a continuous, yet, complex aqueous phase, which comprises salt-soluble proteins, segments of fibers, myofibrils, connective tissue fibers, collagen fragments, and various ingredients suspended in water. The two emulsion stabilization mechanisms—formation of a protein coating on the surface of fat particles to reduce the interfacial tension and immobilization of fat particles in protein matrices largely through physical entrapment—are applicable to meat batters. The coating, that is, protein membrane that surrounds fat globules, is not homogeneous but is multilayered in nature. Three distinct layers of proteinaceous structure have been observed in thick interfacial protein film of meat batters [120]. They are described as a thin, internal layer coating the surface of the fat globule, probably resulting from depositing proteins onto the myosin or actomyosin monomolecular layer (Figure 16.20). This innermost layer is bound through a diffuse region to another layer of similar density. This second layer is bound to a very thick, diffuse protein coat, forming a stable protein membrane structure.

Similarly, stabilization of fat by protein gel matrix is a complex physicochemical process because the gelling solution, referred to as protein "sol," is not simply proteins suspended in the aqueous solution. Rather, the sol represents a heterogeneous matrix consisting of soluble proteins with some insoluble, hydrated myofibril or fiber fragments suspended in it. Often, nonmeat ingredients (soy proteins, starch, seasonings, etc.) are also present. Hence, the gel formed after cooking may be considered as a composite system where aggregates formed from extracted myofibrillar proteins, fragments of myofibrils, and protein-coated fat globules interact, leading to an interwoven network. Some of the insoluble fibrils may entangle with the gel scaffold, whereas the fat globules may act as fillers in the void spaces of the gel matrix, thereby reinforcing the gel. The protein matrix gel is stabilized by a combination of forces, including hydrophobic and electrostatic interactions, hydrogen bonds, van der Waal's interactions, and covalent bonds, mostly through disulfide linkage. Covalent bonds, such as disulfide linkages, appear to be minor except when oxidation is involved.

The physicochemical and rheological properties of the fat globule membrane and the continuous protein matrices are the determinants of emulsion stability, and they are influenced by many factors,

including pH, viscosity of the aqueous phase, time and temperature of chopping or emulsifying, and the lean meat-to-fat ratio. The composition and properties of both the fat globule membrane and the continuous aqueous phase can be modified by means of ingredients and additives to obtain high-emulsion stability against coalescence at high cooking temperatures. Hydrolyzed soy, gluten, and whey proteins may help stabilize the emulsion system by reinforcing the interfacial membrane and the protein matrix. Treatment of emulsifying proteins with microbial transglutaminase prior to emulsification also enhances the emulsion stability and, additionally, enables the amorphous emulsion to form a stable gel matrix system upon heating [121]. It appears that the enzyme effects by means of promoting the interaction and cross-linking of membranes of fat particles as well as the interaction of the fat globules with segments of the protein network in the gel matrix.

16.7.5 Meat Restructuring

Restructured meats are fabricated and then reshaped products, and they include many formed raw steaks, chops, and roasts that resemble the intact products in texture. They also include a variety of cooked, ready-to-eat meats. Most cooked, ready-to-consume luncheon meats more or less fall into the restructured meat category. Restructured meats can be classified into three main groups, based on the specific method used and meat particle size reduction, that is, the extent of comminution: sectioned (entire muscle or muscle groups) and formed; chunked (coarse meat particles) and formed; and flaked (flakes from frozen meat) and formed.

Regardless of the method of reducing meat particle size, the single most critical factor affecting the product manufacture is the application and the action of salt. Salt (NaCl) is required to extract myofibrillar proteins, particularly myosin and actomyosin. The protein extract is a highly viscous, tacky exudate that provides adhesion between meat particles. For the bind to be effective, however, the protein extract must be converted to a viscoelastic, semisolid matrix, that is, a gel or semigel. This is made possible by heat when the formed meat is cooked. Extraction of salt-soluble myofibrillar proteins is accomplished by mixing meat with at least 0.5 M salt in a mechanical mixer, such as a massager and a tumbler. Tumbling relies on gravitational impact and abrasion from dropping meat pieces (previously tossed) against other meat pieces to extract myofibrillar proteins, while massaging relies mainly on abrasion between meat particles, and between meat and the rotating paddles of the massager to extract proteins. Polyphosphate is also used in conjunction with NaCl to improve protein extraction and moisture retention. Other ingredients, that is, seasonings, nonfat dry milk, whey protein concentrate, soy flour, and nitrite, may also be blended into restructured meat to accentuate the flavor and to enhance binding strength, texture, sliceability, and appearance.

Restructured fresh beef steaks and pork chops by the use of microbial transglutaminase have been successfully manufactured [122]. The enzyme forms covalent cross-links between glutamine and lysine side chains thereby bonding meat particles together. Meat products restructured without heating offer great flexibility and portion control, and can be marketed in specific shapes and sizes.

16.7.6 Chemistry of Surimi

Surimi is a crude myofibrillar protein concentrate prepared by washing minced, mechanically deboned fish muscle to remove sarcoplasmic constituents and fat, followed by mixing with cryoprotectants (usually polyols) to prevent protein denaturation during frozen storage. Surimi is an "intermediate" product because it is further processed to make various kamaboko (fish cakes) and seafood analogs, such as imitation crab meat and lobster, or utilized as a functional ingredient in other products.

A main quality concern with many of the fish species used for surimi is that they contain significant proteolytic activity that is detrimental to the texture of surimi-based products. For example, cathepsins B, L, and an L-like protease are difficult to be completely removed by the washing process. These endogenous proteases exhibit high activities in the 45–55°C temperature range, and thus,

can impair the textural properties of surimi-based cooked seafood analogs by degrading actomyosin [123]. Dry beef plasma protein, egg white, and potato extract are capable of preventing surimi gel softening and, hence, are blended into surimi before cooking is initiated. Small peptides (enzyme inhibitors) present in these additives may compete with fish muscle proteins as substrates for the proteases. Because the majority of the catheptic enzyme superfamily are cysteine proteases, cystatin (a general cathepsin inhibitor) is also used to prevent weakening of the surimi gel during cooking. This inhibitor can be efficiently produced using recombinant technology [124].

An alternative approach to the traditional surimi preparation method is acid or alkaline solubilization. Unlike the traditional surimi process, which employs repeated washing of the minced fish muscle tissue to concentrate myofibrillar protein by removing fat and sarcoplasmic constituents, the solubilization method involves either acid (pH 2.5–3.5) or alkaline (pH 9–10) treatment to solubilize both myofibrillar and sarcoplasmic proteins from homogenized muscle tissue. The soluble proteins are subsequently recovered by isoelectric precipitation (pH 5.0–5.5) [125]. This technique has the main advantage of high product yields (protein recovery >90% compared with 55%–65% from the traditional washing method). The acid solubilization and isoelectric precipitation method is particularly suitable for dark-muscle and fatty fish.

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