Review

Tenderization Mechanism of Meat during Post-mortem Aging: The Calcium Theory of Meat Tenderization

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Five main factors contribute to the overall eating quality of meat; tenderness, taste, odor, color and juiciness. Of these factors tenderness is considered the most important by the average consumer of many countries. Tenderness of meat is the sum total of the mechanical strength of skeletal muscle tissue and its weakening during post-mortem aging of meat. The former depends on species, breed, age, sex and individual skeletal muscle tissue of animal and fowl. Meat tenderness originates in structural and biochemical properties of skeletal muscle fibers, especially myofibrils and desmin intermediate-filaments, and of the intramuscular connective tissue, the endomysium and perimysium, which are composed of collagen fibrils and fibers. The mechanical stability of the endomysium and perimysium increases markedly with chronological aging, and the endomysium and perimysium end up in extremely regular and thickened patterns. Therefore, meat produced from old animal and fowl is tough and of lower eating quality. To obtain meat of high quality, reasonably fattened animal and fowl should be slaughtered, and post-mortem aging of meat at around 4°C for a certain period is required. Aging periods are usually more than 14 days, 6-7 days and 0.5-1 day for beef, pork and chicken, respectively. In practice high quality beef of Japanese Black cattle is aged for 3-4 weeks. Both tenderness and flavor are improved during this time. Figure 1 shows changes in shear-force value of raw beef, pork and chicken during post-mortem aging. The shear-force value, which represents toughness of meat, reduces to 50-60% of the initial value in all meats. Tenderness of beef is improved significantly by aging for 28 days, but tenderization of pork almost stops within 10 days post-mortem.

Fig. 1. Tenderization of raw meat during post-mortem aging.
Bovine, porcine and chicken semitendinosus muscles were aged at 4°C, and shear-force values were measured with a rheometer. ○, beef; △, pork; □, chicken.

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of each meat occurs in two steps, a rapid phase first and a slow phase thereafter. The rapid increases in tenderness are mainly due to the structural weakening of myofibrils, and the slow process is caused chiefly by the structural weakening of the endomysium and perimysium, as described in the following summary of our recent research.

For what reason do structures of myofibrils, intermediate filaments and the endomysium and perimysium weaken during post-mortem aging of meat? Meat during aging is under non-physiological conditions; pH decreases ultimately to near 5.5, ATP disappears completely and temperature is maintained at 3–4°C. Furthermore, the sarcoplasmic calcium ion concentration increases ultimately to 0.2 mM due to the loss of the ability of sarcoplasmic reticulum and mitochondria to accumulate calcium ions. This concentration is about 2,000 times that in resting skeletal muscle. The sarcoplasmic calcium ion concentration increases gradually at 4°C and reaches 0.2 mM within 4 days, 3 days and 20 hr post-mortem in beef, pork and chicken, respectively (Ji et al., in preparation). Although endogenous proteases, i.e., lysosomal cathepsins and sarcoplasmic \( \mu \)- and m-calpains, were long believed to attack myofibrils under these non-physiological conditions, it has been proven that cathepsins and m-calpain do not take part in meat tenderization\(^{5,20,23}\). Now, \( \mu \)-calpain is the only possible candidate protease for the hydrolysis of myofibrillar proteins\(^{21}\). On the other hand, proteolysis is very limited in post-mortem muscle\(^{2,27}\), and most chemically detectable proteolysis occurs after the major post-mortem changes in tenderness are observed\(^{11,37}\). No definitive work has been presented which demonstrate a role of myofibrillar catabolism in the process of post-mortem tenderization\(^{63}\). This summary is still true, unfortunately.

My research derived its origin in findings of two kinds of post-mortem changes in myofibril structure\(^{44}\): fragmentation of myofibrils due to weakening of Z-disks, and restoration of rigor-shortened sarcomeres caused by weakening of rigor linkages formed between actin and myosin. Recently, I have proposed ‘the calcium theory of meat tenderization’ on the basis of the following facts\(^{50,53}\): weakening of Z-disks and rigor linkages are reactions specific to calcium ions at 0.1 mM regardless of proteolysis, and the same changes in connectin (titin) filaments, nebulin filaments and desmin molecules as those observed on aged meat can be induced by the treatment of isolated myofibrils with a solution containing 0.1 mM calcium ions and 70 \( \mu \)M leupeptin, the most effective inhibitor of proteases in myofibril preparation\(^{35}\). I report in this paper the molecular mechanism of these phenomena, and the structural weakening of the endomysium and perimysium during post-mortem aging of meat.

### Structural Weakening of Myofibrils

#### Weakening of Z-disks

The Z-disks of vertebrate skeletal muscle connect neighboring sarcomeres and possess a structure strong enough to transmit the tension developed by the interaction of thin and thick filaments of individual sarcomeres. We found that Z-disks are characteristically weakened during post-mortem aging of meat, and myofibrils tend to break by homogenization into fragments composed of 1–4 sarcomeres\(^{46}\). The structure of Z-disks is non-enzymatically weakened by calcium ions. The calcium-induced weakening of Z-disks occurs without concomitant release of \( \alpha \)-actinin and has a maximum at 0.1 mM calcium ions and a minimum at pH 6.5 \textit{in vitro}\(^{9}\). The ultimate pH of post-mortem muscle can be controlled within the range from 5.65 to 7.13 by subcutaneous injection of various amounts of insulin in living rabbits to exhaust them by convulsion. The relationship between the degree of the Z-disk weakening at 4 days post-mortem and the ultimate pH value indicates that the structure of Z-disks is only slightly weakened in post-mortem muscles of ultimate pH values between 6.3 and 6.7, with a minimum effect at pH 6.5. It is weakened when exposed to acidic pHs below 6.1 and neutral pH, and myofibrils are easily broken into fragments by homogenization\(^{47}\). This pH-dependence of the post-mortem weakening of Z-disks is wholly consistent with that of the calcium-induced weakening of Z-disks in isolated myofibrils.

It is generally accepted that the structure of Z-disks is composed of two phases, Z-filaments and amorphous matrix\(^{19}\). When glycerinated fiber-bundles of
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rabbit psoas muscle are treated with a solution containing 0.1 mM calcium ions to induce the structural weakening of Z-disks non-enzymatically, a clear geometrical configuration of Z-filaments can be observed with removal of amorphous matrix materials under an electron microscope. Even after a prolonged treatment, which results in severe depletion, entangled Z-filaments are left in the original region of Z-disks. Immuno-electron microscopic observations show that antibodies against α-actinin bind to the entangled Z-filaments. Therefore, we have concluded that α-actinin is a component, probably a key one, of Z-filaments. Although the structure of intact Z-disks is most resistant to alkali, Z-disks, from which amorphous matrix materials are removed, split into halves after treatment with 1N NaOH. It seems likely that these materials cement neighboring Z-filaments; once the cementing materials are solubilized by 0.1 mM calcium ions, Z-disks are weakened, and easily split by alkali or homogenization.

We have recently identified the chief components of the amorphous matrix-materials as lipids. The content of lipids in bovine semitendinosus muscle Z-disks is 1.8 g per 100 g of myofibrillar proteins. They are composed of phospholipids, triacylglycerols, cholesterol and free fatty acids; the proportions are 65.8, 23.2, 8.6 and 2.4%, respectively (Takahashi et al., unpublished). Although there are many proteins known to be present in the Z-disk, for example, Z-protein, Eu-actinin, glycogen phosphorylase (amorphin) and β-actinin (capZ), the sum of these minor proteins can be assumed as less than 1% of the total myofibrillar protein. Because the amount of α-actinin is 2.9% of the total myofibrillar protein in bovine semitendinosus muscle, the known proteins occupy about 4% of the total myofibrillar protein at most. The total amount of lipids and proteins coincides well with the estimation from the results obtained by interference microscopic observations that the amount of Z-substance is 6% of the total myofibrillar material.

The weakening of Z-disks is induced by the liberation of phospholipids. The Z-disk weakening agrees well with the liberation of phospholipids during post-mortem aging of beef, pork and chicken at 4°C. The amount of α-actinin remains constant in these processes, which is confirmed by Hwan and Bandman. Further examples are results obtained by in vitro experiments using isolated myofibrils; the Z-disk weakening coincides with the liberation of phospholipids responding to calcium ion concentrations, pH and temperature. Since calcium ions bind to phospholipids, it is very probable that phospholipids would bind electrostatically to α-actinin under physiological conditions, and that this interaction would be broken by the binding of calcium ions at 0.1 mM to phospholipids, resulting in the liberation of phospholipids from Z-disks. The liberation of phospholipids is considered to be the main cause for Z-disk weakening during post-mortem aging of meat. The weakening mechanism of Z-disks will be fully elucidated by an actual proof that binding of calcium ions changes the solubility of phospholipids.

Weakening of rigor linkages

One aspect of the weakening of myofibrillar structures is the restoration of rigor-shortened sarcomeres. The restoration of the sarcomere length indicates that rigor linkages formed between actin and myosin have been weakened. The state of contraction measured by sarcomere length is associated with tenderness of meat.

We have found a novel myofibrillar protein, paratropomyosin, which modifies the actin-myosin interaction. Although paratropomyosin is exclusively located at the A-band and I-band junction region of sarcomeres in freshly prepared myofibrils, it is released from its original position by 0.1 mM calcium ions and translocated onto thin filaments during post-mortem aging of meat. Purified paratropomyosin binds to F-actin and strongly inhibits the Mg-ATPase activity of actomyosin and myofibrils. It relieves the rigor tension generated in glycerinated muscle fibers, and restores rigor-shortened sarcomeres. Thus, paratropomyosin dominates the competition for myosin binding sites on actin due to its greater affinity for them, and speeds dissociation of actin and myosin.

The rate of the post-mortem translocation of paratropomyosin from the A-I junction region of sarcomeres onto the thin filaments in the A-band,
where rigor linkages have been formed, agrees well with the rate of increase in length of rigor-shortened sarcomeres. The sarcomere lengths are found to be maximum at 10, 7 and 1 day post-mortem in beef, pork and chicken, respectively. Thus, translocated paratropomyosin weakens rigor linkages and brings about the recovery in the length of rigor-shortened sarcomeres. Paratropomyosin stimulates the resolution of rigor mortis, and is a key factor in meat tenderization during post-rigor aging. The weakening of rigor linkages begins immediately after development of full rigor in all meats tested. This fact coincides well with Erdös' finding that the stiffness of post-mortem muscle begins to decrease again soon after it reaches the maximum level.

Bendall stated that dissociation of rigor linkages requires a supply of ATP. However, no plausible source of ATP has ever been found in post-rigor muscle and this theory has few adherents today. The available evidence on the resolution of rigor mortis points much more strongly to an ATP-independent mechanism that involves paratropomyosin. The suggestion by Wierbicki et al. that actomyosin (rigor linkages) dissociates into actin and myosin during post-mortem aging of meat is proved by the participation of paratropomyosin.

**Splitting of connectin (titin) filaments**

Connectin, a huge molecule over 3,000 kDa of chain weight, is an elastic protein of vertebrate striated muscle. It was found by Maruyama et al., and later named titin by Wang et al. It exists as a very thin filament connecting the thick (myosin) filaments with the Z-disk in a sarcomere. A single connectin filament of about 1.25 μm in length equals one molecule of α-connectin (T1). Connectin filaments are responsible for the passive tension generation in stretched muscle and play a role in positioning the thick filaments at the center of each sarcomere. The elasticity of skeletal muscle tissue is largely due to the properties of connectin filaments. On the other hand, post-mortem muscle loses its elasticity and becomes plastic with time. The loss of elasticity is considered to be closely related to tenderization of meat.

We have found that α-connectin is split into β-connectin (T2) and a 1,200-kDa subfragment during post-mortem aging of meat at 4°C, and that the same splitting takes place, when isolated myofibrils are treated with a solution containing 0.1 mM calcium ions and 70 μM leupeptin. The splitting of connectin filaments occurs in the highly elastic I band region about 0.34 μm away from the Z-disk, and results in the decrease in elasticity of aged meat. In post-mortem muscle, the amount of α-connectin decreases with aging time and β-connectin is formed together with a 1,200-kDa subfragment. There is no sign of further degradation of either β-connectin or the subfragment, while α-connectin is degraded by proteases first to β-connectin and the subfragment, followed by degradation into various polypeptides of lower chain weights. The splitting of connectin filaments is a common phenomenon in all meats of domestic animal and fowl we have observed (five species). However, the rate of the decrease in the amount of α-connectin varies with the origin of meat; α-connectin disappears after 10 days storage in bovine semitendinosus muscle, and within 1 day in chicken pectoralis superficialis muscle (Tatsumi et al., unpublished). The decrease in elasticity of post-mortem muscle is closely related to the decrease in the amount of α-connectin. The initial rate of these two changes is characteristic of beef, pork and chicken and agrees well in each meat. It is clear that the elasticity of post-mortem muscle is lost by the splitting of connectin filaments into β-connectin and the 1,200-kDa subfragment.

The splitting of connectin filaments in isolated myofibrils depends on the calcium ion concentration; it begins above 0.01 mM and reaches a maximum at 0.1 mM. The same type of splitting of connectin filaments is induced by magnesium ions; the concentration required for the splitting, however, is two order of magnitude higher than the calcium ion requirement. The Ca-specific splitting of connectin filaments is induced by magnesium ions; the concentration required for the splitting, however, is two order of magnitude higher than the calcium ion requirement. The Ca-specific splitting of connectin filaments takes place in all myofibrils prepared from various skeletal muscles of cattle, swine, chicken and rabbit in the presence of 0.1 mM calcium ions and 70 μM leupeptin. The Ca-specific splitting of connectin

filaments is also affected by temperature. It increases almost linearly with increasing temperature and reaches a maximum at 30°C. There is no sign of further degradation of β-connectin and the 1,200-kDa subfragment during the Ca-treatment of myofibrils at temperatures from 5°C to 40°C.

We have proposed that non-enzymatic cleavage of some peptide bonds between β-connectin and 1,200-kDa subfragment is induced specifically by the binding of calcium ions at non-physiological concentrations (above 0.1 mM), because calcium ions bind to the β-connectin portion of connectin filaments. The calcium-binding site on β-connectin is restricted to a 400-kDa fragment, which is produced by tryptic digestion of β-connectin, and 12 moles of calcium ions bind to 1 mole of the fragment (Tatsumi et al., unpublished). There is a possibility that the N-terminal of the 400-kDa fragment is located at the junction between β-connectin and the 1,200-kDa subfragment, and its N-terminal region has a high affinity for calcium ions. The fact that higher concentrations of magnesium ions are required to induce the splitting of connectin filaments suggests that magnesium ions bind to the calcium binding site on the 400-kDa fragment at lower affinity, as well as calcium-binding proteins of EF-hand type. These results support our proposal that connectin filaments are split specifically by 0.1 mM calcium ions.

Fragmentation of nebulin filaments
Nebulin is another giant filamentous protein with a chain weight of about 800 kDa. One molecule of nebulin spans the space between the Z-disk and the free end of thin (actin) filaments as an extremely thin filament. We have found that nebulin disappears during post-mortem aging of all meats of various animals and fowls, though the initial lag time and rate of decrease vary with the species. We have found that nebulin disappears during post-mortem aging of all meats of various animals and fowls, though the initial lag time and rate of decrease vary with the species. It is caused by the fragmentation of nebulin filaments. Nebulin filaments break up into five subfragments having chain weights of 200, 180, 40, 33 and 23 kDa. The same fragmentation takes place when isolated myofibrils are treated with a solution containing 0.1 mM calcium ions and 70 μM leupeptin. The fragmentation of nebulin filaments begins at calcium ion concentrations above 0.01 mM and reaches a maximum at 0.1 mM, and is induced also by magnesium ions of two orders of magnitude higher than the calcium ion requirement, as in the case of connectin filaments. The dependences on pH and temperature are identical to the Ca-specific splitting of connectin filaments. Nebulin is proved to be a calcium-binding protein; the ions bind to the 100, 40 and 23 kDa subfragments.

Based on these findings, we have proposed a non-enzymatic fragmentation of nebulin filaments induced by 0.1 mM calcium ions. There is a homology between the splitting of connectin filaments and the fragmentation of nebulin filaments. The Ca-specific fragmentation of nebulin filaments must destabilize the organization of thin filaments, and thus contribute to the tenderization of meat during post-mortem aging. In addition to the weakening of Z-disks, the weakening of rigor linkages formed between actin and myosin and the splitting of connectin filaments, all of which we have already reported to be important factors in the tenderization of meat during post-mortem aging, the fragmentation of nebulin filaments appear to be a fourth factor. All of these forms of structural weakening of myofibrils occur non-enzymatically at 0.1 mM calcium ions in vitro.

Weakening of Intermediate Filaments
Depolymerization of desmin intermediate-filaments
Desmin was found by Lazarides and Hubbard and its molecular weight is about 50 kDa. It polymerizes to form intermediate filaments of 10 nm in diameter in situ. Desmin intermediate-filaments surround each Z-disk and extend between Z-disks of adjacent myofibrils; they function in muscle fibers primarily as three dimensional matrix which interconnects individual myofibrils to one another and to the cell membrane at the level of their Z-disk. In this manner desmin intermediate-filaments provide a framework that mechanically integrates myofibrils during the contraction and relaxation of skeletal muscle. Desmin is classified as a cytoskeletal protein because of its function.

Recently, we have found that desmin intermediate-filaments disappear during post-mortem aging of meat, and that intermediate filaments, which have
been polymerized from purified desmin, are depolymerized by 0.1 mM CaCl₂ (unpublished data). After addition of 0.1 mM calcium ions, the viscosity of desmin intermediate-filaments falls rapidly, and no intermediate filament can be observed under an electron microscope. Desmin is a calcium-binding protein and properties of desmin molecules are changed by the binding of 0.8 moles of calcium ions to 1 mole of desmin, resulting in depolymerization of intermediate filaments. These findings clearly demonstrate that the depolymerization of desmin intermediate-filaments during post-mortem aging of meat is caused by a direct action of calcium ions, and powerfully support the calcium theory of meat tenderization. The dependence of the structural weakening of myofibrils described above and the depolymerization of desmin intermediate-filaments on calcium ion concentrations is summarized in Fig. 2. All structural weakenings, which contribute to tenderization of meat, take place at calcium ion concentrations above 0.01 mM, and reach a maximum at 0.1 mM. My theory is founded on these experimental facts.

**Fragmentation of desmin molecules**

Desmin molecules disappear during post-mortem aging of beef at 15°C⁴⁴), and Hwan and Bandman⁶) have showed using anti-desmin monoclonal antibodies that fragments of desmin are present in aged beef. We have studied the fragmentation mechanism of desmin using anti-desmin antiserum to detect all fragments produced (Kanawa and Takahashi, unpublished). When bovine, porcine and chicken semitendinosus muscles are aged at 4°C, the amounts of desmin decrease to 70–80% of the initial values within 14, 8 and 2 days post-mortem in beef, pork and chicken respectively, and the disintegrated desmin molecules appear as 43–46, 40 and 34–36 kDa polypeptides. A similar fragmentation is observed in isolated myofibrils treated with a solution containing 0.1 mM calcium ions and 70 μM leupeptin. The fragmentation depends on calcium ion concentrations: it begins above 0.01 mM in the same manner as the structural weakening of myofibrils, and reaches a maximum at 10 mM. Surprisingly, it does not depend on temperature in the range from 5°C to 40°C. This fact denies any participation of proteases. Moreover, desmin purified from chicken pectoralis superficialis muscle is fragmented into 43–46, 42 and 34–36 kDa polypeptides by the treatment with a solution containing 0.1 mM calcium ions and 70 μM leupeptin, as in the cases of aged meat and isolated myofibrils.

These facts suggest that properties of desmin molecules are markedly changed by the binding of calcium ions. The depolymerization of desmin intermediate filaments takes place prior to the fragmentation of molecules. These disintegrations of the three dimensional matrix of desmin intermediate-filaments must result in tenderization of meat.

**Weakening of Endomysium and Perimysium**

The structural integrity of skeletal muscle fiber-bundles is maintained by the intramuscular connective tissue. The structure of this tissue is functionally divided into three hierarchical domains: the endomysium ensheathes individual skeletal muscle fibers; the perimysium surrounds a group of muscle fibers, and the epimysium ensheathes the whole muscle. As
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the epimysium is too tough to eat and is promptly removed from meat, the toughness of meat results mainly from properties of the endomysium and perimysium. The major component of the endomysium and perimysium is collagen. Collagen molecules assemble into fibrils, and fibrils into fibers in situ. In early studies it was shown that the solubility of collagen is affected by neither temperature nor time of post-mortem aging of meat\textsuperscript{38,39}, suggesting that this component remains unchanged at the molecular level. However, perimysial collagen is later shown to be damaged and partially solubilized during post-mortem aging of beef\textsuperscript{41,42}. The thermal shrinkage-temperature of bovine intramuscular collagen decreases by 7-8°C within 7 days post-mortem\textsuperscript{48}, and the isometric tension of intramuscular collagen decreases at 21 days post-mortem in beef\textsuperscript{49}. These results indicate post-mortem alterations in the endomysium and perimysium.

Three-dimensional networks of collagen fibrils in the intramuscular connective tissue have seldom been observed in detail by electron microscopy because of the signal disruption caused by muscle fibers. In the last few years, a method of cell maceration has been developed, which allows detailed examination of the three-dimensional arrangement of collagen fibrils in various connective tissues under a scanning electron microscope\textsuperscript{36}. Cellular elements are eliminated by this method, and collagen fibrils and fibers are exposed. We have applied this method to bovine semitendinosus muscle and showed the three-dimensional organization of collagen fibrils in the endomysium, perimysium and epimysium\textsuperscript{32}.

We have utilized this method to observe the structural weakening of the endomysium and perimysium during post-mortem aging of beef\textsuperscript{33}, pork (Fang et al., in preparation) and chicken\textsuperscript{26}. In bovine semitendinosus muscle immediately post-mortem, the honeycomb structure of the endomysium, which is constructed by the sheaths of 60-100 μm in diameter for housing individual muscle fibers, is clearly visible. The sheaths of the endomysium are membranous and consist of tightly arranged collagen fibrils 30-70 nm in diameter. The perimysium is composed of several layers of 100-200 μm-thick sheets surrounding the endomysia. The wavy sheets of perimysium consist of collagen fibers in which the fibrils are in close contact with each other. The well-ordered anatomical arrangement of collagen fibrils and fibers in the endomysium and perimysium seems to play an important role in the physiological function of skeletal muscle tissue.

These structures of the bovine endomysium and perimysium remain unchanged for up to 10 days post-mortem, but a progression of structural alterations is clearly visible after 14 days post-mortem. In the endomysium and perimysium of semitendinosus muscle aged for 28 days at 4°C, gaps of various sizes open everywhere; the honeycomb structure of endomysium markedly deforms, and the perimysial sheets disintegrate into ribbon-like structures. Endomysial sheaths become lacy. At higher magnifications, the endomysium resolves into individual collagen fibrils which are arranged loosely, though are neither broken nor torn. A closer view of the perimysium shows that the thick sheets of the perimysium separate into collagen fibers of 4-8 μm in diameter, where collagen fibrils lie more or less in parallel. These results indicate that the structural changes in the endomysium and perimysium are induced by the dissociation of collagen fibrils and fibers from endomysial sheaths and perimysial sheets, respectively. The structural changes in, i.e., the weakening of, the endomysium and perimysium are closely related to the tenderization of beef. As shown in Fig. 1, the shear-force value of bovine semitendinosus muscle decreases rapidly up to 10 days post mortem, and then gradually until 28 days post-mortem. The gradual decrease after 10 days post-mortem seems to be mainly due to the structural weakening of the endomysium and perimysium. Thus, the intramuscular connective tissue shows the effect on tenderization of extended aging (3-4 weeks) of beef. Similar structural weakening of the endomysium and perimysium occurs at 4°C after 5 days and 12 hr post-mortem in porcine and chicken semitendinosus muscle, respectively. It is clear that an extended post-mortem aging is necessary to obtain satisfactorily tenderized meat.

The question remains why the endomysium and perimysium dissociate into collagen fibrils and fibers...
during post-mortem aging of meat. Collagen fibrils and fibers of the endomysium and perimysium are embedded in ground substances, proteoglycans and glycoproteins. We have found that proteoglycans in bovine *semitendinosus* muscle are degraded during post-mortem aging. The degradation of proteoglycans is almost certain to be the most important process in the separation of collagen fibrils and fibers, which contributes toward tenderization of meat. When the arrangement of proteoglycans in bovine *semitendinosus* muscle immediately post-mortem is observed under a transmission electron microscope, proteoglycans are associated with collagen fibrils at regular intervals of 65 nm in the perimysium. Collagen fibril-associated proteoglycans in the perimysium remain unchanged up to 14 days post-mortem. In bovine *semitendinosus* muscle aged for 28 days at 4°C, the greater part of the proteoglycans associated with collagen fibrils disappears. If the amount of proteoglycans in the isolated perimysium is determined by the hexuronic acid method, it remains unchanged within 7 days post-mortem, followed by a gradual decrease up to 28 days post-mortem. The results indicate that proteoglycans are closely associated with collagen fibrils in the endomysium and perimysium immediately post-mortem, linking collagen fibrils and stabilizing collagen fibers. Proteoglycans associated with collagen fibrils are degraded with time post-mortem, the linkage between collagen fibrils weakens, and collagen fibrils and fibers separate from the endomysium and the primysium, respectively. The mechanism responsible for the degradation of proteoglycans during post-mortem aging of meat is unknown. There are two possibilities; proteoglycans degrade spontaneously under non-physiological conditions in post-mortem muscle, or they are degraded enzymatically. Further research is needed to clarify the reasons for the disappearance of proteoglycans. It seems reasonable to assume that the post-mortem changes in proteoglycans is induced by 0.1 mM calcium ions under non-physiological conditions, as occurs in the myofibril structures.

**Conclusion**

Since Hoagland *et al.* suggested in 1917 that proteolysis was involved with post-mortem tenderization of meat, numerous investigations have been carried out to demonstrate the role of proteolysis in tenderization of meat. This process turned out to occur to a very limited extent in post-mortem muscle, however. Davey and Gilbert showed that the proteolytic degradation, which occurs in *longissimus thoracis* (dorsi) muscle of beef carcasses during 30 days aging at 2°C, accounts for only 2.3% of the meat protein, and they denied any direct relationship between proteolysis and tenderizing of meat during post-mortem aging. Hamm described in his review paper that proteolysis of muscle proteins can not play major part in the tenderization of meat during normal aging. I think that their opinions were foresighted. The direct action of calcium ions described in this paper clears up many questions, which could not be well understood in the past. Calcium ions have a dual function in post-mortem muscle; the rise of sarcoplasmic calcium ion concentrations to 3–5 μM induces rigor-contraction, and the further rise of the calcium ion concentration to 0.1 mM weakens structures of myofibrils, desmin intermediate-filaments and probably the endomysium and perimysium, thereby bringing about tenderization of meat.

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