Myofibrillar Structural Weakening and Tenderization of Uncooked Cold Shortened Bovine Muscle during Postmortem Storage

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Abstract It is known that bovine muscles stored before the rigor mortis in low temperature cause the cold shortening. This study was carried out in order to clarify the relation of weakening of myofibrillar structure during storage on meat tenderization of cold shortened bovine semimembranosus muscle. The muscle was excised immediately after slaughter from the dressed carcass and divided into muscle blocks, and then stored antiseptically at 4°C. The muscle restrained for two days on the carcass was excised on day 3 postmortem and similarly treated. The value of breaking strength and elasticity of both muscles gradually decreased with the postmortem time. However, the values of both breaking strength and elasticity in the excised muscle decreased but less than the restrained muscle, showing that the cold shortening developed in the immediately excised muscle. Measurement on the weakening of the myofibrillar structures of the cold shortened muscle showed that the weakening of the Z-disks progressed linearly after 3 days postmortem, agreeing with decline of the stiffness of the muscle. The weakening of the interaction between actin and myosin that affects sarcomere length hardly occurred throughout the storage time. And, the degradation of α-connectin rapidly progressed after 4 days postmortem. These results suggest that the weakening of the Z-disks and the degradation of connectin filaments were related to the tenderization of uncooked cold shortened bovine muscle during postmortem storage.


Key words: Bovine muscle, Myofibrillar weakening, Cold shortening, Postmortem ageing

Tenderness is the predominant factor affecting bovine meat quality, so numerous studies have been made on the structural changes of myofibrils, which were closely related to the tenderization of bovine muscles during postmortem ageing. A relationship between sarcomere length and bovine meat tenderness has been observed. Möller et al. have shown a high correlation (0.78) between the fragmentation of myofibrils and the sensory tenderness of cooked bovine muscles, and Olson et al. have demonstrated the relationship between myofibrillar fragmentation and shear force value in three bovine muscles. Moreover, Takahashi and Saito have shown that the decrease in connectin contents agreed well with the decrease in elasticity of muscles. And it has been shown that titin (connectin) of bovine muscle was degraded with postmortem time during ageing. These changes in myofibrillar structures of bovine muscles have been well known as the weakening of myofibrils closely related to meat tenderization during postmortem ageing.

When bovine muscles were excised from the dressed carcass before rigor mortis and stored below 10°C, they shortened excessively and developed shortening-induced toughness, which is what is called ‘a cold shortening’. Such shortened muscles have shown...
little decline in shear force value on cooking. While, Locker and Wild\textsuperscript{8,9} have reported that a marked reduction in yield point occurred when the strips of cold shortened muscles were stretched under the uncooked conditions after prolonged storage, although the extent of the reduction was less than that for aged unshortened muscle. The latter authors indicated the contradiction between the two criteria for ageing : shear force and yield point, as the involvement of different structural elements of muscle\textsuperscript{9}. Thus, the reduction in yield point may indicate that the weakening of myofibrillar structures occurred even in the cold shortened muscles excised immediately after slaughter with ageing time similar to normally excised and stored muscles. However, there have been few reports on the weakening of myofibrils as related to the meat tenderization during postmortem storage of the cold shortened muscles.

This study showed the change in the stiffness of bovine \textit{semimembranosus} muscle that was excised immediately after slaughter from the dressed carcass and stored at \(4^\circ\text{C}\). The relation of the weakening of myofibrils of the muscle to meat tenderization during storage was examined. The relationship between the reduction of the stiffness and the weakening of myofibrils of the uncooked cold shortened muscle during postmortem storage was described.

### Materials and Methods

#### Chemicals

Adenosine 5'-triphosphate disodium salt (ATP) was obtained from Oriental Yeast Co. LTD. (Tokyo, Japan). Ethylene glycol bis (2-aminoethyl ether) tetraacetic acid (EGTA) was purchased from Dojindo Laboratories (Kumamoto, Japan). Other chemicals of analytical reagent grade were obtained from Wako Pure Chemical Industries (Osaka, Japan).

#### Muscle Sample

Japanese Black cattle were slaughtered according to normal procedures at a local slaughter house. \textit{Semimembranosus} muscle was excised approximately four hours post-slaughter and divided into muscle blocks (weighing about 200 g). These pre-rigor muscle blocks were treated antiseptically with 1 mM \(\text{NaN}_3\) and wrapped individually with polyethylene film, and then stored at \(4^\circ\text{C}\). The muscle restrained on the dressed carcass for two days at \(4^\circ\text{C}\) were excised on day 3 postmortem and similarly treated.

#### Tenderness measurement

At a specified postmortem time, muscle pieces 1.3-1.5 cm thick parallel to the fiber axis were cut out, and breaking strength and elasticity were measured by a creep meter (RE-3305, Yamaden Co. LTD., Tokyo, Japan) using an 8 kg load cell with a No. 5 plunger (5 mm in diameter). A muscle piece was placed on the stage of the creep meter and compression rupture test was done at 1 mm/s compression rate. When the plunger penetrated into the muscle piece, the force needed for first breaking point was taken as breaking strength, and Young’s modulus was taken as a measure of elasticity from the recorded stress-strain curve.

#### Preparation of myofibrils

Myofibrils were prepared from the remaining portion of the muscle block used in the tenderness measurement by the method of Etlinger et al.\textsuperscript{2}, except that the final washings with Triton X-100 and with deoxycholate were omitted.

#### Measurement of the weakening of the Z-disks

Degree of the weakening of the Z-disks was determined by measuring the myofibrillar fragmentation index (MFI) by the method of Takahashi et al.\textsuperscript{17}) under a phase contrast microscope (OPTIPHOT-104, Nikon Co. Ltd, Tokyo, Japan).

#### Measurement of the amount of filaments separated from myofibrils

The amount of filaments separated from myofibrils by ATP was measured according to the modified method of Takahashi et al.\textsuperscript{19}). A mixture containing 4 mg/ml myofibrils, 0.15 M KCl, 5 mM MgCl\textsubscript{2}, 1 mM EGTA, 4 mM ATP, and 10 mM K-phosphate buffer (pH 7.0) was gently homogenized at 540 rpm for 100 sec with a teflon homogenizer on ice. Filaments released from myofibrils into the media were separated by centrifugation at 5,000 rpm for 10 min and the protein concentration of the supernatant was determined. The amount of separated filaments from myofibrils was expressed as the percentage of total myofibrillar proteins.

#### Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)
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Fig. 1. Changes in breaking strength of both bovine semimembranosus muscle excised immediately after slaughter and the muscle restrained on carcass during storage at 4°C. The first breaking force was taken as breaking strength when a plunger penetrated into a muscle block. ○, excised muscle; △, restrained muscle. Values represent means and standard deviations for 10–46 measurements.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis of myofibrils was carried out according to the procedures of Laemmli5) using 12% polyacrylamide gel and Tatsumi and Hattori21) using a 2% polyacrylamide gel containing 0.5% agarose. The destained gel was scanned densitometrically with a Densito-Pattern Analyzer (EPA-3000, Maruzen Petrochemical Co. LTD., Tokyo, Japan) at 650 nm and the amount of α-connectin was expressed as the percentage of total amount of connectins from the densitogram20).

Determination of protein concentration

Protein concentration was determined by the biuret method31) and BCA protein assay16) using bovine serum albumin as a standard.

Results and Discussion

The results of tenderness measurement of bovine semimembranosus muscles excised immediately after slaughter from the dressed carcass and stored at 4°C (described as excised muscle) and the changes in the muscles restrained on the carcass for 2 days (described as restrained muscle) are shown as breaking strength and elasticity in Figs. 1 and 2, respectively. The breaking strength of the excised muscle was ca. 160 g at 0 day postmortem and increased to some extent four days after the death, but not significantly (Fig. 1). The value of 160 g in breaking strength (0.82 kg/cm², calculated from the diameter of the plunger) was the same order as that in yield point (0.99 kg/cm²) measured by Locker and Wild9), when the strips of cold shortened sternomandibularis muscle was extended on day 2 postmortem. Thereafter, the breaking strength decreased gradually with postmortem time and reached almost a constant level at 13 days postmortem. While the breaking strength of the restrained muscle was ca. 145 g at 3 days postmortem. The value was lower than that of the excised muscle, although there was no significance between both. However, the breaking strength of the restrained muscle rapidly decreased to almost a constant value of ca. 54 g at 9 days postmortem, and then gradually decreased at the value which was significantly lower than the breaking strength of the excised muscle. The same tendency for elasticity in both the excised muscle and the restrained muscle to that of breaking strength (Fig. 2) was observed. Elasticity of the restrained muscle was significantly lower than that of the excised muscle during prolonged storage.

The breaking strength and elasticity showed that
both the excised and the restrained muscles in uncooked state were tenderized with the progress of postmortem time, and that the extent of the tenderization in the excised muscle was less than that of the restrained one. In the restrained muscle, the weakening of the Z-disks expressed by myofibrillar fragmentation index (MFI), that was an index of the meat tenderization\(^1\), progressed with time until 14 days postmortem (Fig. 3). And the result of SDS-PAGE of myofibrils prepared from the muscle at 17 days postmortem showed no remarkable change during storage in myofibrillar proteins except for the appearance of 30,000-dalton band\(^15\) (data not shown), suggesting that the restrained muscle was obviously tenderized during the storage. The tenderization in the excised muscle is in support of the work of Locker and Wild\(^9\) who have shown the decline in the yield point of the cold shortened muscle during storage. Moreover, when myofibrils prepared from the excised muscle were observed by a phase contrast microscope, the sarcomere had shortened excessively at early postmortem time and its length was not restored during prolonged storage (data not shown). This implies that the excised muscle with such shortened sarcomere could cause toughness on cooking\(^4,6\), although the tenderness of the cooked muscle was not determined at present study. Thus, it is clear that cold shortening occurred in the muscles excised immediately after slaughter from the dressed carcass and stored at 4°C.

It has been suggested that the yield point in extending the cold shortened muscle strips was the point of failure of the myofibrillar structure such as the I-filaments anchorage in the Z-disks\(^9\). So, the weakening of myofibrils concerning the meat tenderization during postmortem storage of the excised and cold shortened bovine muscle was examined. Myofibrils were prepared from the remaining portion of the muscle block used in the tenderness measurement, and the weakening of the Z-disks expressed by MFI was measured (Fig. 3). Myofibrillar fragmentation index (MFI) was 0.04 until 2 days postmortem but increased linearly with postmortem time. At 14 days postmortem it reached a value of 0.61 and gradually increased thereafter. Thus, the weakening of the Z-disks occurred in the excised and cold shotened muscle during postmortem storage and the linear increase in MFI coincided with the decrease in the stiffness shown in Figs. 1 and 2. Olson et al.\(^14\) have demonstrated that MFI of bovine *longissimus* and *semitendinosus* muscles stored at 2°C increased from 1 to 13 days.
Fig. 3. Weakening of the Z-disks in myofibrils prepared from bovine *semimembranous* muscle excised immediately after slaughter and stored 4°C. The weakening of the Z-disks was expressed as myofibrillar fragmentation index \([F]/[Σ]\). \([F]\) represents the number of myofibrillar fragments composed of 4–6 sarcomeres and \([Σ]\) represents the total number over 500 myofibrils. ○, excised muscle; △, restrained muscle. Values represent means and standard deviations for 3–12 measurements.

postmortem with the highest increase from 1 to 3 days postmortem. They have also shown that the increase in MFI corresponded to the decrease in Warner-Bratzler shear force values during ageing of bovine muscles retained on carcass. Therefore, the weakening of Z-disks seems to be greatly related to the tenderization of the excised and cold shortened bovine muscle during storage.

Figure 4 shows the weakening of actin-myosin interaction of the excised and cold shortened muscle estimated by determining the amount of actin and myosin filaments separated from myofibrils which serves as an index to estimate the weakening of actin and myosin interaction during postmortem ageing of muscles\(^9\). At 0 day postmortem ca. 7.0% filaments of all myofibrillar proteins were separated under the condition with ATP. This value increased slightly after 4 days postmortem and ca. 10% filaments of myofibrillar proteins were separated at 20 days postmortem. Filaments were hardly separated from myofibrils throughout the storage time under the condition without ATP. Thus, the interaction between actin and myosin in the excised and cold shortened bovine muscle was only slightly weakened into an easily dissociable state during postmortem storage. However, the extent of the increase was much less than the previously reported results with other animal species\(^{19,22}\). This difference might be attributed to the excessive shortening of myofibrils occurring in the excised and cold shortening bovine muscle. In fact, the sarcomere length did not recover throughout the storage time, indicating that the extent of the overlap of both filaments was fairly large. Therefore, the effect of the weakening of actin-myosin interaction on the decline of the values in tenderness measurements shown in Figs. 1 and 2 seems to be very small in the excised and cold shortened bovine muscle.

A cytoskeletal protein, connectin\(^{12}\), has been shown to undergo degradation from α-connectin to β-connectin and 1,200 kDa polypeptide during postmortem ageing of muscles\(^{20}\). The extent of the connectin degradation is considered being important in meat tenderness\(^{1,10,18}\), because connectin constitutes elastic filament in the sarcomere and provides the structural integrity of the muscle cell\(^{12}\). We can detect the α-connectin degradation of the excised and cold shortened bovine muscle during storage by SDS-PAGE analysis using a 2% polyacrylamide slab gel.
Fig. 4. Changes in the amount of filaments separated from myofibrils prepared from bovine *semimembranous* muscle excised immediately after slaughter and stored at 4°C. The amount of separated filaments was expressed as the percentage of total myofibrillar proteins. ●, with ATP; ○, without ATP. Values represent means and standard deviations for three measurements.

Fig. 5. Changes in the amount of α-connectin in myofibrils prepared from bovine *semimembranous* muscle excised immediately after slaughter and stored at 4°C. SDS-PAGE analysis was carried out using a 2% polyacrylamide slab gel containing 0.5% agarose (inset). The amount of α-connectin was expressed as the percentage of the total amount of connectins. Values represent means for two measurements. Abbreviations: myosin HC, myosin heavy chain.
containing 0.5% agarose and estimate the changes in the relative amount of α-connectin from the densitogram of the gel. As shown in Fig. 5, the relative amount of α-connectin was ca. 70% of total connectins at 0 day postmortem and decreased after 4 days postmortem with the increase of both density of β-connectin and 1,200 kDa polypeptide bands (Fig. 5, inset). The α-connectin band disappeared at 11 days postmortem and there was no sign of further degradation of β-connectin and 1,200 kDa polypeptide over the storage time. The degradation of α-connectin was observed in the excised and cold shortened muscle and the decrease in the relative amount of α-connectin almost coincided with the decrease in the stiffness of the muscle as reported previously. Therefore, the degradation of connectin filaments probably contributes to some extent to the tenderization in the excised and cold shortened bovine muscle during postmortem storage.

Figure 6 shows SDS-PAGE patterns of myofibrils prepared from the remaining portion of the muscle blocks used in the tenderness measurement. No remarkable difference during storage was recognized in such major contractile proteins as actin and myosin. The noticeable changes were that the 30,000-dalton band derived from degraded polypeptides of troponin-T appeared after 17 days postmortem. When bovine muscles excised from carcass in pre-rigor state are stored at low temperature (<10°C), cold-induced shortening occurs and cold shortening develops the related excessive toughness of the meat. While, Locker and Wild have shown that the stiffness of cold shortened muscle in the uncooked state decreased by ageing much greater than shear force measured on the cooked one. They suggested that this decrease arose largely due to the postmortem changes in the myofibrillar components. In this study, we confirmed that the stiffness of the excised and cold shortened bovine muscle decreased under the uncooked condition during postmortem storage, and demonstrated that the extent was less than that of the muscle restrained on the dressed carcass for two days. We have also shown that the weakening of myofibrillar structures occurred even in the cold shortened muscle, although each weakening of myofibrillar structures proceeded in the different pattern during storage. As shown in Figs. 3 and 5, the weakening of the Z-disks and the degradation of α-connectin almost coincided with the decrease of the values in tenderness measurement. While, the weakening of the interaction between actin and myosin estimated by the amount of separated filaments hardly occurred over the storage time (Fig. 4) and major contractile proteins of myofibrils such as actin and myosin were not degraded during the storage (Fig. 6). The only slight increase of the amount of separated filaments seems to be due to the extent of the overlap between actin and myosin filaments which was large when the excessive shortening of myofibrils occurred in the excised and cold shortened bovine muscle. As sarcomere length affects the bovine meat tenderness, the excessive shortening of myofibrils is probably a cause for the less decline of the values in the tenderness measurement during storage of the muscle. Therefore, we conclude that both the weakening of the Z-disks and the degradation of the connectin filaments seemed to affect the decrease in the stiffness of the uncooked cold shortened bovine semimembranous muscle during postmortem storage.
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References


