Change in the Properties of Myofibrillar Proteins during Post-Mortem Storage of Muscle at High Temperature

Yoshihide Ikeuchi, Tatsumi Ito,* and Toshiyuki Fukazawa

Changes in the properties of myofibrillar proteins prepared from muscles stored at 37 °C for varying periods of time were investigated. The extractability of myofibrillar proteins did not change during the first 2 h after death but decreased during 2–6 h, while the pH value of longissimus muscle decreased rapidly to 6.5 after 2 h and to 5.6 after 4 h. The degradation of myofibrillar proteins, especially myosin and troponin, occurred during storage. Myofibrillar ATPase activities in the presence of calcium ions were decreased, while in the absence of calcium ions the activities gradually increased, resulting in a decrease in Ca^{2+} sensitivity of myofibrils during storage. The enzymatic activities of myosin and the affinity of actin for myosin were decreased with increasing storage time. The polymerization rate of actin was increased at first and then decreased with increasing storage time, but the molecular weight of actin and its ability to activate myosin ATPase activity changed little during 12 h of post-mortem storage at 37 °C.

Post-mortem changes in muscle are effected by genetic, physiological, and nutritional conditions of live animals and post-mortem conditioning (Cassens et al., 1975; Cassens, 1977). Bendall (1973) has precisely described physical and chemical changes which take place during the rigor process under various conditions. There are many extensive studies regarding the changes in myofibrillar proteins under the various post-mortem storage conditions, especially in relation to storage temperature, pH, and time, and a significant correlation between storage conditions of muscle after death and the properties of myofibrillar proteins has been established (Wolfe and Samejima, 1976; Samejima and Wolfe, 1976; Yamamoto et al., 1977; Cheng and Parrish, 1978, 1979). Also, it has been well-known that myofibrillar proteins undergo the following specific changes during post-mortem storage: (1) alteration of the actin–myosin interaction (Fujimaki et al., 1965; Goll and Robson, 1967; Ito et al., 1978), (2) loss of Z-line structure (Takahashi et al., 1967; Davey and Gilbert, 1969; Fukazawa et al., 1969), and (3) degradation of myofibrillar proteins, probably due to the action of calcium-activated factor (CAF) and cathepsins (Reddy et al., 1975; Dayton et al., 1976; Olson et al., 1977; Schwartz and Bird, 1977).

Ito et al. (1978) have investigated the changes of acto-heavy meromyosin (HMM) ATPase during post-mortem storage at low temperature (0 °C) and obtained the result that the actin–myosin interaction increases with increasing storage time. Also, we have shown in previous papers (Ikeuchi *et al.*, 1978, 1980) that the Ca^{2+} sensitivities of myofibril and reconstituted acto-heavy meromyosin–tropomyosin–troponin (acto-HMM–TM–TN) complex increase during aging at 0 °C in spite of the appearance of the degradative products of troponin.

High-temperature conditioning brings about the rapid disappearance of ATP and a pH fall (Marsh, 1954) which greatly influence meat quality because myofibrillar proteins are extremely sensitive to low pH and high temperature (Takahashi et al., 1962; Okitani and Fujimaki, 1968; Yasui et al., 1973). Recently, Yamamoto et al. (1979) have shown extensive degradation of myofibrillar proteins during post-mortem storage at high temperature using sodium dodecyl sulfate ($NaDodSO_4$)–polyacrylamide gel electrophoresis.

In the present experiment, changes in the biochemical properties of myofibrillar proteins of muscles stored at high temperature (37 °C) were evaluated and compared with those of muscles stored at low temperature which we previously reported (Ito et al., 1978; Ikeuchi et al., 1978, 1980).

Laboratory of Chemistry & Technology of Animal Products, Faculty of Agriculture, Kyushu University, Fukuoka 812, Japan.

MATERIALS AND METHODS

Materials. Well-fed rabbits were used in the present experiment; they were anesthetized with sodium pentobarbital (90 mg) and *d*-tubocurrarine chloride (15 mg) in order to immobilize the animals at death, since starvation or exhaustion gives a higher value of final pH in muscles. After exsanguination, the carcasses were soaked in 10 mM sodium azide to retard bacterial growth, wrapped in polyethylene bags, and kept in a water bath for designated times (0, 2, 6, 9, and 12 h) at 37 °C. Longissimus thoracic and white hind leg muscles were excised from at-death carcasses (within 15 min after exsanguination). The same portion was sampled from each aged muscle.

Isolation of Myofibrils. The muscles were cut into pieces and then disrupted in about 5 volumes of KCl–borate buffer [100 mM KCl, 39 mM borate, 5 mM ethylenediaminetetraacetic acid (EDTA), and 1 mM β-mercaptoethanol, pH 7.1] with a blade-type homogenizer for 30 s (Briskey and Fukazawa, 1971). After centrifugation at 1500*g* for 15 min, the pellet was rehomogenized in the same solution containing 0.02% Triton X-100 to eliminate the ATPase of the sarcoplasmic reticulum and other organelles. The pellet was washed 5 times with 0.1 M KCl–borate buffer by repeating the centrifugation and suspension. Finally, myofibrils were suspended in the buffer by homogenization with a Teflon hand homogenizer. Myofibrils were stored at −20 °C in 50% glycerol solution containing 2 mM β-mercaptoethanol and 1 mM EDTA. Bacterial growth was not recognized by phase microscopy of myofibrils which has been prepared from muscles incubated at 37 °C for 12 h.

Preparation of Myofibrillar Proteins. Myosin was extracted from high-temperature stored muscles with a modified Guba-Straub solution [0.3 M KCl, 0.15 M phosphate, 2 mM ethylene glycol bis(2-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA), 5 mM $MgCl_2$, and 5 mM ATP, pH 6.5]. Myosin was prepared according to Tonomura et al. (1961) and stored at −20 °C in glycerol solution containing 1 mM EDTA and 2 mM β-mercaptoethanol until use. Heavy meromyosin (HMM) was obtained by the method of Lowey and Cohen (1962) as described previously (Ito et al., 1978). Actin was prepared from acetone-dried powder according to the procedure of Spudich and Watt (1971), except that polymerization of G-actin was induced only by dialyzing against about 100

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volumes of dialyzing solution containing 50 mM KCl and 0.5 mM β-mercaptoethanol overnight at 0 °C.

Measurements of pH Value. A piece of longissimus muscle weighing about 2 g was cut from aged carcasses and homogenized in 10 mL of solution containing 0.15 M KCl and 5 mM sodium iodoacetate (which arrests glycolysis) at pH 7.0 by using a Waring Blendor at a speed of 10000 rpm for 3 min. The pH value measurement was made with a Hitachi-Horiba F-7 pH meter.

Extractability of Myofibrillar Proteins from Myofibrils. Extractability of myofibrillar proteins from myofibrils was essentially carried out according to the method described by Sung et al. (1976). Myofibrillar proteins were extracted from myofibrils for 15 min at 5 °C with the following solutions: (1) Hasselbach–Schneider solution (0.6 M KCl, 10 mM sodium pyrophosphate, 0.1 M potassium phosphate, and 1 mM $MgCl_2$, pH 6.4) and (2) KI solution (0.6 M KI, 6 mM sodium thiosulfate, 2 mM β-mercaptoethanol, 0.5 mM ATP, and 20 mM Tris-HCl, pH 7.5). The extractability of myofibrillar proteins was expressed as a percentage of the total proteins in myofibril suspension.

ATPase Activity. Myofibrillar, synthetic actomysin, myosin, and actin-activated HMM ATPases were measured in a reaction volume of 2 mL. Myofibrils were washed 3 times with 30 volumes of a solution containing 0.1 M KCl, 10 mM Tris–maleate (pH 7.0 or 6.8), and 1 mM β-mercaptoethanol and dialyzed against the same solution overnight to eliminate EDTA and glycerol prior to measuring myofibrillar ATPase activity.

The pCa-dependent myofibrillar ATPase activity was examined under the same conditions as described in a previous paper (Ikeuchi et al., 1978). The concentration of free Ca^{2+} (pCa) was calculated by taking the apparent binding constant of EGTA for Ca^{2+} as 5×10^5 M^{-1} (Ogawa, 1968).

The steady-state enzymatic reactions were done in a water bath for 1–8 min at 25 °C. Conditions of the ATPase assays will be given with the description of the individual experiments. The reactions were initiated by adding ATP and stopped by adding 2 mL of 10% cold trichloroacetic acid (Cl_3CCOOH) to the reaction mixtures. After centrifugation (1500*g*, 10 min), the inorganic phosphate liberated was determined by the method of Fiske and Subbarow (1925).

Measurements of Viscometry. The polymerization rate of G-actin was determined in terms of the relative viscosity of actin at 25 °C (Mannherz et al., 1975) as described in detail by Ito et al. (1978).

NaDodSO$_4$–Polyacrylamide Gel Electrophoresis. NaDodSO$_4$–polyacrylamide gel electrophoresis was carried out according to the method of Weber and Osborn (1969).

Protein Concentration. Protein concentration was determined by the biuret reaction which had been standardized with bovine serum albumin (Gornall et al., 1949).

Reagents. The disodium salt of ATP for the ATPase assay, trypsin (EC 3.4.21.4), soybean trypsin inhibitor, and dithiothreitol (DTT) were purchased from Sigma Chemical Co. ATP for preparing myosin and actin from high-temperature stored muscles was purchased from Kyowa Hakko Co. All reagents were commercial products of the best grade available.

RESULTS

Changes in Myofibril during Post-Mortem Storage at High Temperature. Figure 1 shows the change of the extractability of myofibrillar proteins and pH of muscles during storage at 0 and 37 °C. The pH value of longissimus muscle stored at 0 °C gradually fell during storage

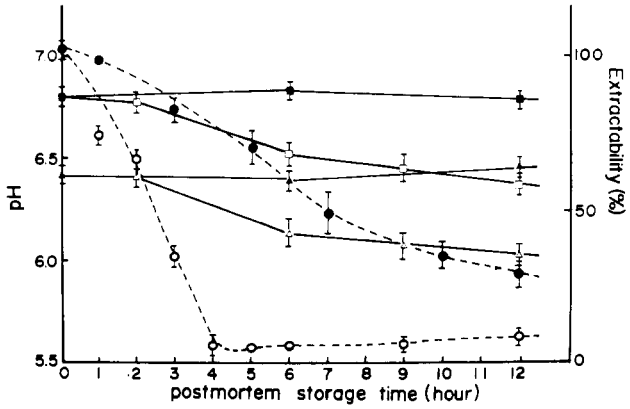

Figure 1. The time-dependent change of pH of muscle and extractability of myofibrillar proteins during post-mortem storage at 0 °C (closed symbols) and 37 °C (open symbols). Measurement of pH in longissimus muscles from three carcasses was carried out as described under Materials and Methods: (●) 0 °C; (○) 37 °C. Extractability of myofibrillar proteins was expressed as the percentage of the total protein in the myofibril suspension: (■) 0 °C, KI solution; (□) 37 °C, KI solution; (▲) 0 °C, Hasselbach–Schneider solution; (△) 37 °C, Hasselbach–Schneider solution.

and attained 5.9 at 12 h after death, while that of the muscle stored at 37 °C fell rapidly from 7.1 to 5.6 and attained the ultimate pH value at 4–9 h after death. This result was consistent with that of Bendall (1973). On the other hand, no apparent change was observed in the extractability of myofibrillar proteins during 12-h of storage at 0 °C and the first 2 h of storage at 37 °C. But after 2 h of storage at 37 °C the extractability decreased rapidly in the case of both Hasselbach–Schneider and KI solutions. The extractability of myofibrillar proteins with Hasselbach–Schneider solution was in good agreement with that of Davey and Gilbert (1968). For an investigation whether or not there is a difference in the extractability of individual myofibrillar proteins between low- and high-temperature storage conditions, a comparative study was made on NaDodSO$_4$–polyacrylamide gel electrophoresis of the extracts obtained from myofibrils with Hasselbach–Schneider solution. Figure 2 shows NaDodSO$_4$–polyacrylamide gel electrophoretograms and densitograms of the gels. Almost all kinds of myofibrillar proteins were detected in the extracts by NaDodSO$_4$ gel electrophoresis. Huxley and Hanson (1957) have also found by interference microscopy that not only the thick filament substances but also the thin filament substances are extracted with Hasselbach–Schneider solution. On comparison of the densitograms of the gels, considerable differences were found in the amounts of each myofibrillar protein in the extracts between at-death and 12-h stored muscles at 37 °C. On the other hand, there was little difference in the ratio of myosin heavy chain to actin in the extracts between both muscles. However, a band of 30000 daltons appeared in the NaDodSO$_4$–polyacrylamide gel electrophoretograms and the densitograms of 12-h stored muscle at 37 °C, although there was a great decrease in the extractability of myofibrillar proteins.

Myofibrillar ATPase activities in the presence or absence of free calcium ions are shown in Table I. In the presence of calcium ions, myofibrillar ATPase activity decreased slightly until 2 h after death and then it decreased more rapidly during 2–6 h after death. In the absence of calcium ions, on the other hand, the ATPase activity increased gradually with increasing storage time. On the basis of the calculation of myofibrillar ATPase activities with and without calcium ions, Table I summarizes the effect of regulatory proteins on the Mg^{2+}-ATPase activity of myo-

Table I. ATPase Activity and Ca^{2+} Sensitivity of Myofibrils Isolated from Muscles Stored at 37 °C[a]

	ATPase act.[b] at 37 °C for post-mortem storage time of				
	0 h	2 h	6 h	9 h	12 h
$+Ca^{2+}$	0.416 ± 0.016	0.391 ± 0.017	0.305 ± 0.013	0.283 ± 0.007	0.261 ± 0.005
$-Ca^{2+}$ (+EGTA)	0.027 ± 0.01	0.029 ± 0.01	0.048 ± 0.007	0.056 ± 0.001	0.058 ± 0.008
Ca^{2+} sensitivity[c]	93.5	92.6	84.8	80.2	77.8

[a] Conditions of assay: 0.5 mg/mL myofibril, 50 mM KCl, 2 mM $MgCl_2$, 2 mM ATP, 20 mM Tris-maleate (pH 7.0), and 0.2 mM $CaCl_2$ or 1 mM EGTA. [b] ATPase activities were expressed as micromoles of P_i per minute per milligram of myofibril as means plus or minus standard error from four determinations. [c] Ca^{2+} sensitivity was defined as [1 − [(activity without Ca^{2+})/(activity with Ca^{2+})]] × 100.

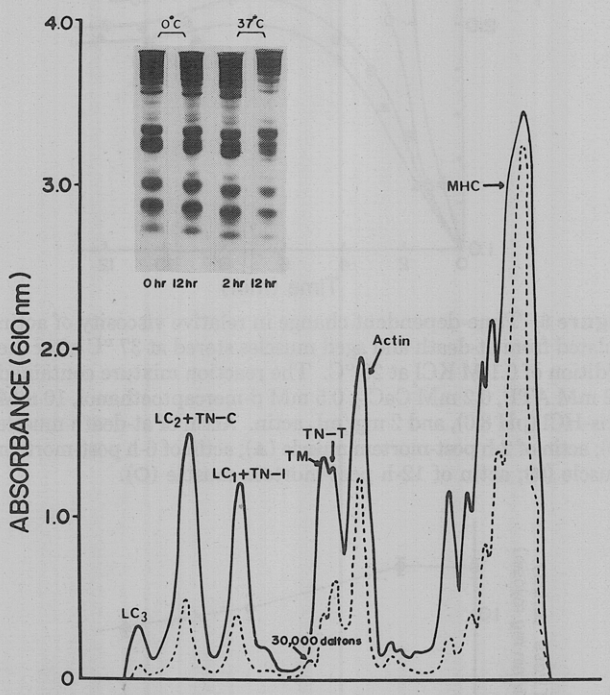

Figure 2. Electrophoretograms and densitograms of $NaDodSO_4$–polyacrylamide gel electrophoresis of the extracts from myofibrils with Hasselbach–Schneider solution. Electrophoresis was made on 10% gels. Densitometry of the gels was performed at a wavelength of 610 nm with a Gilford 2400S spectrophotometer using the absorbance range of 0–4.0. A constant volume (45 μL) of the extract was loaded on each gel. Solid line, at-death muscle; dotted line, 12-h muscle stored at 37 °C. MHC, myosin heavy chain; TN-T, troponin T; TN-I, troponin I; TN-C, troponin C; TM, tropomyosin; LC, myosin light chain.

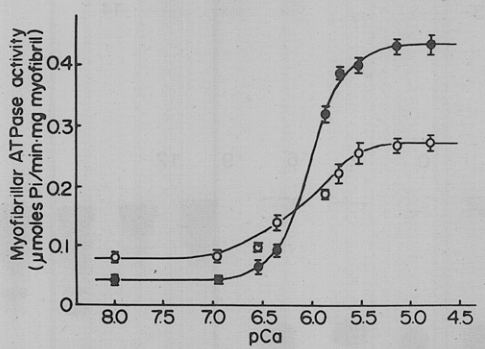

Figure 3. pCa-dependent ATPase activity of myofibrils prepared from at-death muscle (●) and 12-h post-mortem muscle stored at 37 °C (○). ATPase activity was determined in a reaction mixture containing 0.5 mg/mL myofibril, 50 mM KCl, 20 mM Tris–maleate (pH 6.8), 1 mM $MgCl_2$, 2 mM ATP, and varying concentrations of free calcium ion (pCa). Vertical lines represent means ± SEM (n = 3).

fibrils. The Ca^{2+} sensitivity of myofibrils was considerably decreased during storage at high temperature (Table I). For further confirmation of this finding, changes in the regulatory system of myofibrils were investigated by measuring the pCa-dependent ATPase activity. In Figure 3, myofibrillar ATPase activity is plotted as a function of free calcium ions (pCa). As shown in this figure, the ATPase activity of at-death muscle showed a sigmoidal curve, indicating that the regulatory system of myofibril effectively exerts its function (Solaro and Briggs, 1974). However, the pCa-dependent ATPase curve of 12-h post-mortem muscle stored at 37 °C was more gentle than that of at-death muscle, although the midpoint of both pCa curves was almost the same, pCa 6.0. In addition, the specific activity of myofibrillar ATPase at higher calcium ion concentrations (pCa <6.2) was lower than that of at-death muscle, and, conversely, at lower calcium ions concentrations (pCa >6.2) the ATPase specific activity was higher for post-mortem muscle and lower for at-death muscle. This result clearly demonstrates that muscle greatly lost both the enzymatic activity and regulatory function during 12-h post-mortem storage at 37 °C.

$NaDodSO_4$–Polyacrylamide Gel Electrophoretograms of Myofibril, Actin, and Myosin. Figure 4a shows $NaDodSO_4$–polyacrylamide gel electrophoretic patterns of myofibrils prepared from at-death and post-mortem muscles stored at 37 °C. A noticeable change was the appearance of 30 000- and 27 000-dalton components with increasing storage time, which might be the degradative products of troponin T (Dabrowska et al., 1973; Olson et al., 1977; Yamamoto et al., 1979). In addition, several unidentified bands appeared between myosin heavy chain and actin and their density gradually increased with increasing storage time. These bands might be derived from myosin heavy chain or some sarcoplasmic proteins bound to the myofibrils (Yamamoto et al., 1979).

Gel electrophoretograms of actin and myosin prepared from 0-, 2-, 6-, and 12-h post-mortem muscles are also shown in Figure 4b. No apparent change was found in the electrophoretograms of actin during post-mortem storage of muscle at high temperature. However, several polypeptides that might be degradative products of the myosin heavy chain produced by cathepsins (Schwartz and Bird, 1977; Yamamoto et al., 1979), were observed in the samples stored for 2, 6, and 12 h. In addition, the density of these bands increased with increasing storage time.

Change in the Properties of Actin and Myosin during Post-Mortem Storage. The denaturation of F-actin prepared from muscle stored at 37 °C was investigated by measuring synthetic actomyosin ATPase (0-h myosin + 0–12-h F-actin). Actomyosin ATPase activity was slightly decreased with increasing storage time (Figure 5). However, a significant difference was observed in the ATPase activity between at-death and 12-h post-mortem muscle ($p < 0.01$). Figure 6 shows the polymerization rate of G-actin prepared from muscles stored for designated times at 37 °C. The relative viscosity of the F-actin obtained by polymerization did not change during the first 2-h storage of muscle, but it increased during the next 4

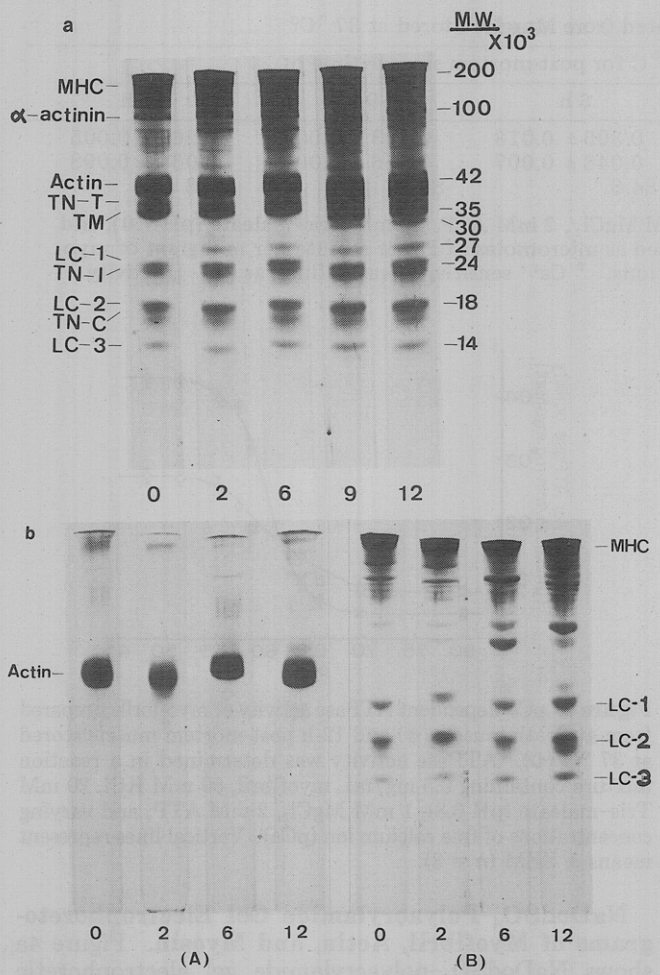

Figure 4. (a) $NaDodSO_4$–polyacrylamide gel (10%) electrophoretograms of myofibrils prepared from at-death and post-mortem muscle stored at 37 °C. The number illustrated under the gels is the post-mortem storage time of the muscle. 100 μg of myofibril was loaded on each gel. MHC, myosin heavy chain; TN, troponin; TM, tropomyosin; LC, myosin light chain. (b) $NaDodSO_4$–polyacrylamide gel electrophoretograms of actin and myosin isolated from at-death and aged muscles stored at 37 °C. The number illustrated under the gels is the post-mortem storage time of the muscle. (A) 20 μg of actin was loaded on each gel (7.5% gel). (B) 100 μg of myosin was loaded on each gel (10% gel). MHC, myosin heavy chain; LC, myosin light chain.

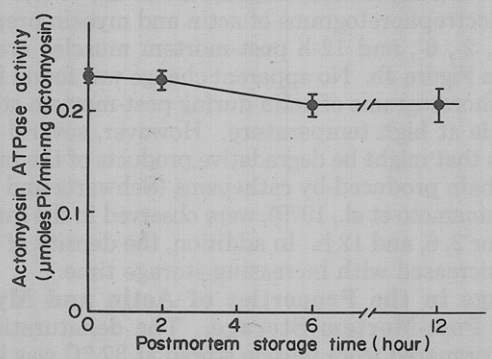

Figure 5. Change in the ATPase activity of synthetic actomyosin prepared by mixing intact myosin with F-actin from stored muscle at 37 °C. ATPase activity was determined under the conditions of 57.5 mM KCl, 11 mM Tris-HCl (pH 7.5), 2 mM $MgCl_2$, 2 mM ATP, 0.6 mg/mL intact myosin, and 0.3 mg/mL F-actin. Vertical lines represent means ± SEM (n = 3).

h and then began to decrease after more prolonged storage time. And after 24 h of storage the relative viscosity of actin decreased to the level of at-death muscle (data not shown in Figure 6). In this experiment, three determi-

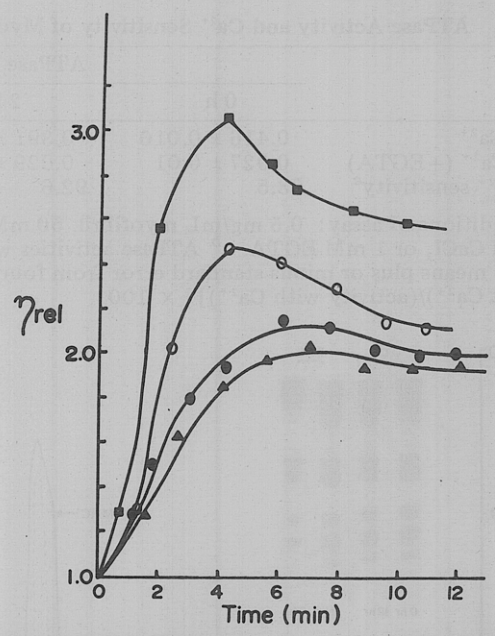

Figure 6. Time-dependent change in relative viscosity of actin isolated from at-death and aged muscles stored at 37 °C after the addition of 0.1 M KCl at 20 °C. The reaction mixture contained 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM β-mercaptoethanol, 10 mM Tris-HCl (pH 8.0), and 2 mg/mL actin. Actin of at-death muscle (●); actin of 2-h post-mortem muscle (▲); actin of 6-h post-mortem muscle (■); actin of 12-h post-mortem muscle (○).

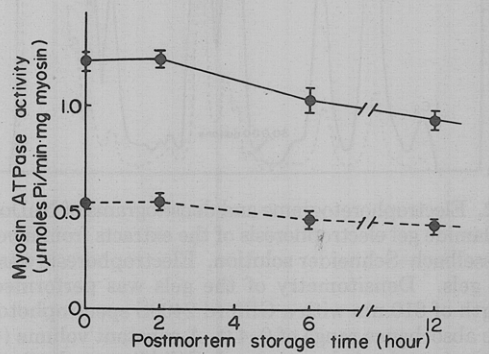

Figure 7. Changes in Ca^{2+}- and EDTA-ATPase activities of myosin during storage of muscle at 37 °C. Ca^{2+}-ATPase assay (●--●): 0.5 mg/mL myosin, 0.25 M KCl, 1 mM ATP, 20 mM Tris-HCl (pH 7.5), and 10 mM $CaCl_2$. EDTA-ATPase assay (●—●): 0.5 mg/mL myosin, 0.5 M KCl, 1 mM ATP, 20 mM Tris-HCl (pH 7.5), and 1 mM EDTA. Vertical lines represent means ± SEM (n = 3).

nations were made on three different samples and similar results were obtained in every determination. In Figure 7, Ca^{2+}- and EDTA-modified ATPase activities of myosin isolated from post-mortem muscles are illustrated. High-temperature storage had no effect on the enzymatic activities of myosin during 2 h after death, but both ATPase activities decreased thereafter. A significant difference was found in both ATPase activities between at-death and 12-h post-mortem muscle ($p < 0.01$). In addition, EDTA-ATPase activity decreased more steeply than Ca^{2+}-ATPase activity during high-temperature treatment. Figure 8 shows the double-reciprocal plots of actin-activated HMM ATPase of muscles stored for designated times. As can be seen in Figure 8, the intercepts on the ordinate and the abscissa give the value of the ATPase of the HMM at infinite actin concentration (V_{max}) and the apparent dissociation constant (K_{app}) for the acto-HMM complex, respectively. From the intercept on the abscissa in Figure 8, it was observed that the binding of actin to

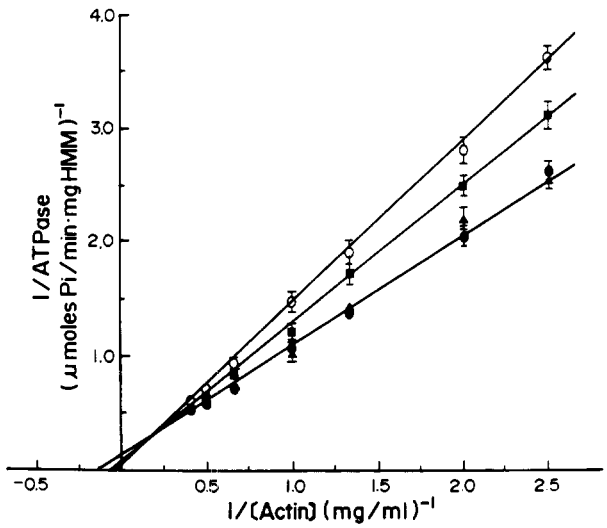

Figure 8. Double-reciprocal plots of actin-activated HMM ATPase vs. actin concentration. Actin and HMM were prepared from muscles stored for the designated time at 37 °C. Actin was mixed with HMM prepared from the same muscle to form acto-HMM complex from the same muscle. All samples contained 40 mM KCl, 1 mM $MgCl_2$, 2 mM ATP, 10 mM Tris–maleate (pH 7.0), 0.2 mg/mL HMM, and varying concentrations of actin (0.5–2.5 mg/mL). Acto-HMM prepared from at-death muscle (●); acto-HMM from 2-h post-mortem muscle (▲); acto-HMM from 6-h post-mortem muscle (■); acto-HMM from 12-h post-mortem muscle (○). Vertical lines represent means ± SEM (n = 3).

HMM (i.e., the affinity of actin for myosin) was gradually weakened during storage at 37 °C, resulting in a decrease of the acto-HMM ATPase activities of muscles with increasing storage time under the assay condition where a lower weight ratio of actin to HMM was employed (actin/HMM = 2:1). The maximum velocities (V_{max}) of at-death and 2-, 6-, and 12-h stored muscle were 6.67, 6.67, 13.33, and 26.67 (μmol of P_i)/(min·mg of HMM), respectively, and the apparent dissociation constants, assuming a binding ratio of 4.2×10^4 mol of actin/mol of HMM (Rizzino et al., 1970; Elzinga et al., 1973), were 1.79×10^{-4}, 1.79×10^{-4}, 3.57×10^{-4}, and 9.48×10^{-4} M, respectively. The V_{max} and K_{app} of at-death muscle were similar to the results of Ito et al. (1978).

DISCUSSION

In order to understand the process of post-mortem change in muscle, it is useful to investigate the changes in the properties of myofibrillar proteins under various post-mortem storage conditions (Arakawa et al., 1970b; Yasui et al., 1973; Wolfe and Samejima, 1976; Olson et al., 1977; Yamamoto et al., 1979). Many factors which influence meat tenderness have been elucidated (Marsh, 1972; Goll et al., 1974). It could be easily estimated that rigorous storage conditions of muscle, which produce low-quality meat like PSE pork (Briskey, 1964), might induce drastic changes in the myofibrillar proteins themselves. Hamm (1977) has recently published general remarks in regard to the changes of muscle proteins during the heating of meat. The results of the present investigation also provide evidence that biochemical properties of myofibrillar proteins were greatly influenced by a prolonged high-temperature conditioning.

As shown in Figure 1, the pH value of muscle stored at 37 °C fell rapidly when compared with muscle stored at low temperature (0 °C). In addition, a considerable loss was observed in the extractability of myofibrillar proteins with a concomitant decrease of the pH value during high-temperature storage of muscle, although the extractability did not decrease until after the pH had declined below 6.5 at 37 °C. In general, a pH fall and high temperature, which give a optimum condition for cathepsins (Schwartz and Bird, 1977), could hand in hand affect the properties of myofibrillar proteins (Takahashi et al., 1962; Yasui et al., 1973; Yamamoto et al., 1979). In fact, ATPase activities of myofibrils, myosin, and actin-activated HMM were decreased with increasing storage time, except that there was no apparent difference in the enzymatic activities between at-death and 2-h stored muscles, which still maintained a high pH value of 6.5 (Figures 1, 7, and 8 and Table I). Penny (1977) has also shown that the denaturation of myofibrillar ATPase prepared from porcine muscle occurs rapidly at 37 °C, but not at all at low temperature (0 °C). However, the loss of the ATPase activities of muscle stored at 37 °C was not as large as we had expected: about 63% for myofibrillar ATPase, 77% for EDTA-ATPase and 81% for Ca^{2+}-ATPase of myosin, and 71% for actin-activated HMM ATPase (when the weight ratio of actin to HMM is 2) of their original activities still remained even after 12-h post-mortem storage at 37 °C, respectively (Table I and Figures 7 and 8). Also, the myosin ATPase activating ability of F-actin was decreased only slightly even after 12 h of storage at 37 °C (Figure 5). The highly remaining activity of myosin ATPase obtained in the present study was consistent with the result of Yasui et al. (1973), who have reported that the ATPase activities of myosin in glycerol-extracted fiber bundles of rabbit psoas muscle, in which myofibrillar proteins are organized in a specific architecture of myofibril, were only slightly decreased by low pH and high-temperature treatment.

A structural change of myofibrillar proteins, especially of myosin and troponin, was revealed by $NaDodSO_4$–polyacrylamide gel electrophoresis (parts a and b of Figure 4). However, it is likely that the degradation of troponin T (the appearance of 30 000- and 27 000-dalton components) is not responsible for the decrease in the Ca^{2+} sensitivity of myofibril, because we have shown that the Ca^{2+} sensitivity of the reconstituted complex of acto-HMM–tropomyosin–troponin does not change during storage of muscle in spite of the degradation of troponin T isolated from muscle stored at 0 °C for 1 week (Ikeuchi et al., 1980). Therefore, the decrease in Ca^{2+} sensitivity may be due to the change in properties of another troponin subunit(s) (Hartshorne et al., 1972). This change may also be due to the modification of the sulfhydryl groups of myosin and actin (Arakawa et al., 1970a). On the other hand, the structural changes of myosin induced by storage at 37 °C seem to be greatly related to the loss of the enzymatic activity (Figures 3, 4, 7, and 8 and Table I).

A change in the polymerization rate of actin also occurred during storage at high temperature (Figure 6), although no structural changes and few biochemical changes of actin were revealed by $NaDodSO_4$–polyacrylamide gel electrophoresis and the synthetic actomyosin ATPase assay, respectively (Figures 4 and 5). On the basis of the report of Ishiwata (1976), Ito et al. (1978) suggested that the change in the polymerization rate of actin might be due to the oxidation of a specific SH group of actin during post-mortem storage at 0 °C. On the other hand, it has been shown that the chemical modification of specific amino acid residues of actin is responsible for the polymerization of actin (Martonosi and Gouvea, 1961; Chantler and Gratzer, 1975). Therefore, we cannot rule out a conformational change of actin during heat treatment since specific amino acid residues of actin may be modified by heat treatment, which brings about the change in the

polymerization rate of actin in addition to the oxidation of the SH group.

As shown in Figure 8, the actin-activated HMM ATPase activity at lower concentration ratios of actin to myosin (actin/HMM = 2:1) and the affinity of actin for HMM decreased with increasing storage time. Moos (1972) has reported that the decrease in acto-HMM ATPase in high-salt solution is due to the decreased affinity of actin for HMM, which, in turn, implies that the increase of actin–myosin interaction causes the increase of acto-myosin-type ATPase activity. Therefore, it may be natural that actin-activated HMM ATPase activity (when the weight ratio of actin to myosin is similar to that in situ; actin/HMM = 1:1.15) shows a similar tendency to that of myofibrillar ATPase activity which decreased with increasing storage time at high temperature (Table I and Figure 8). Inversely, our present results also indicate that the increase in actin–myosin interaction during post-mortem storage at low temperature is responsible for the increase of myofibrillar and myosin B ATPase activities as evidenced by Ito et al. (1978).

In conclusion, it was striking that the muscle stored at high temperature still maintained the enzymatic activities in spite of the structural change of the myosin molecule (Figures 4, 7, and 8 and Table I). The present results, especially in the case of 6- and 12-h post-mortem muscles, indicate that the changes in biochemical properties of individual myofibrillar proteins during storage of muscle at high temperature are associated with the biochemical property changes of myofibrils (Figures 3–8 and Table I), regardless of the decrease of the extractability of myofibrillar proteins during storage (Figures 1 and 2). In addition, these results also indicate the importance of the combination of low pH and high temperature during post-mortem storage for controlling meat quality, because the combination greatly altered the properties of myofibrillar proteins (Figures 1–8 and Table I).

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