Stability of Myofibrillar EDTA-ATPase in Rabbit Psoas Fiber Bundles

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The rate and extent of inactivation of myofibrillar EDTA-ATPase in rabbit psoas fiber bundles at various temperatures between 3 and 42° and at pH values between 5.5 and 7.0 have been studied by measuring the EDTA-ATPase activity. The purification of psoas myofibrils with Triton X-100 permitted the direct measurement of changes in the myofibrillar ATPase activity of psoas fiber bundles stored under various conditions. The myofibrillar ATPase, when the fiber bundles are stored in the medium at 3° and pH 5.5, are quite stable. During storage at 3° for 72 hr, it has been found that myofibrillar ATPase activity remained almost constant between pH 5.5 and 7.0. However, at higher temperatures, the rate of thermal inactivation is increased in the more acidic systems. From the results it has been concluded that the effect of pH on the loss of myofibrillar ATPase is negligible if the fiber bundles are stored near 0° and that the most important factor influencing the denaturation is the temperature at which the fiber bundles are stored.

A naturally occurring phenomenon of some interest is the so-called PSE (pale, soft, and exudative), or watery condition in some types of pig muscle. The condition is characterized by a very rapid pH fall postmortem, and by soft, pale, and watery musculature in the post-rigor state (Briskey and Wismer-Pedersen, 1961; Bendall and Lawrie, 1964; Briskey, 1964). Whale, beef, and rabbit muscles, subjected to high temperature rigor, also develop wateriness, so the condition is of general occurrence under these conditions and is not peculiar to pigs (Bendall and Wismer-Pedersen, 1962; Bendall and Lawrie, 1964). Penny (1967) and Yasui et al. (1973) were able to show that myosin B (natural actomyosin) and myosin prepared from rabbit muscle were considerably denatured under these conditions. These authors demonstrated that both types of protein were sensitive to temperature and pH in the range to be expected in a cooling carcass, i.e., 36–38° and a pH of 6.0 or below within 1 hr of death in those samples exhibiting the highest rate of glycolysis. We can therefore attribute the PSE phenomenon principally to protein denaturation at high temperature and low pH.

In order to investigate what actually occurs in intact muscle during rigor, it is desirable to use a highly organized muscle model whose structural features are similar to that of intact muscle. Yasui et al. (1973) studied the denaturation of myosin in glycerol-treated fiber bundles, which were fixed to application rods at rest length, using extractability and Ca^{2+}-ATPase activity as criteria. Their results indicated that the protein in the fiber bundles was more stable than the isolated protein molecules.

To confirm and extend the conclusion derived from their study, the preferred approach was to investigate directly the myofibrillar ATPase under the conditions of temperature and pH encountered in practice. This has not been done so far for the following two reasons. (1) When the washed myofibrils are exposed to severe conditions, e.g., high temperatures or low pH values, the fibrils then tend to clump together into ill-defined aggregates of different sizes, and (2) although myofibrils have proved useful for the study of the contractile proteins of skeletal muscle, ATPase activity measurements in denaturation studies sometimes have not been very satisfactory because of contamination by mitochondria, sarcolemma, and sarcoplasmic reticulum. We have attempted to overcome those drawbacks by preparing myofibrils from fiber bundles incubated under denaturing conditions (Yasui et al., 1973) and by purifying the myofibrils with Triton X-100 according to the procedure for cardiac myofibrils which was described by Solaro et al. (1971). This paper describes stability changes in thermally treated myofibrillar ATPase as examined by enzymic activity assay.

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MATERIALS AND METHODS

Reagents. The disodium salt of ATP, Triton X-100, ouabain, cytochrome *c*, and quinidine were purchased from Kyowa Hakko Co., Eastman Kodak Co., Sigma Chemical Co., and Nakarai Chemical Co., respectively. All other inorganic and organic salts were commercial products of the best reagent grade available and used without further purification.

Myosin. Myosin was prepared from rabbit Longissimus dorsi muscle according to the method described by Perry (1957).

Myosin B. Myosin B used was extracted from rabbit Longissimus dorsi muscle for 24 hr with Weber-Edsall solution and purified by triple precipitations at 0.2 *M* KCl and dissolution in 0.6 *M* KCl (Szent-Györgyi, 1951). This preparation was found to consist mostly of natural actomyosin and to contain less than 10% of free myosin detectable by ultracentrifugal analysis.

Fiber Bundles. Glycerol-treated fiber bundles were prepared by the method reported previously (Yasui et al., 1973). Washed fiber bundles were prepared by procedures essentially the same as the glycerol treated ones. Freshly prepared fiber bundles which were fixed to bamboo sticks at rest length were immersed in 25 m*M* KCl containing 39 m*M* borate buffer (pH 7.1) and 1 m*M* EDTA at 0–1° for 24 hr with two changes of buffer and then transferred to a solution of 0.1 *M* KCl for another 24 hr with two changes of solution and then used for the experiments.

Myofibrils. Myofibrils were prepared as follows. Fiber bundles were detached from the sticks and cut into small pieces with scissors prior to homogenization in a Virtis homogenizer. This and all subsequent steps were performed at 0–4°. The bundle was homogenized with 8 vol of 0.1 *M* KCl–20 m*M* Tris-maleate (pH 7.0) at 10,000 rpm for 1 min. The homogenate was centrifuged for 15 min at 750*g*. The pellet from this spin was resuspended in the original homogenate volume using the same salt solution described above. This suspension was then centrifuged for 15 min at 750*g*. This sequence of resuspension and centrifugation was repeated 2 more times. The homogenate obtained after this series of steps was termed washed myofibrils.

Further purification of the myofibrils was achieved by resuspending the washed myofibrils in 8 pellet volumes of standard buffer containing 1% Triton X-100. The myofibrils were then centrifuged for 15 min at 750*g*. The myofibrils were then homogenized using a Teflon–glass hand ho-

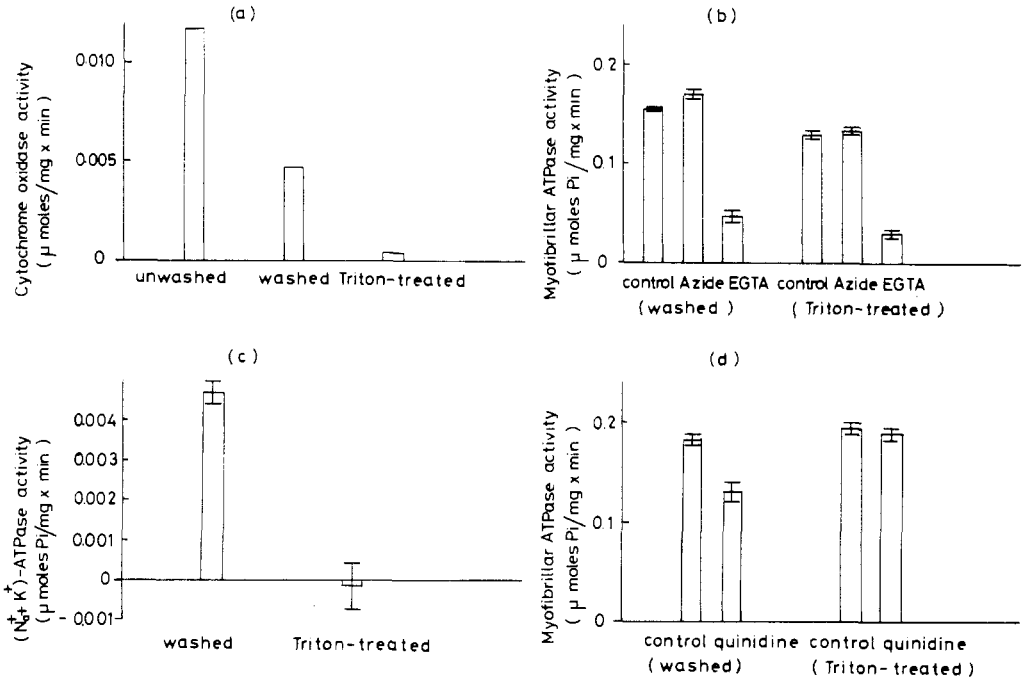

Figure 1. Effect of Triton X-100 treatment on enzymatic activity of psoas myofibrils: (a) cytochrome oxidase activity; (b) effect of azide and EGTA on psoas myofibrillar ATPase activity; the values are expressed as means ± standard error; (c) $(Na^+ + K^+)$-ATPase activity; the values presented are expressed as means ± standard error; (d) effect of quinidine on psoas myofibrillar ATPase activity; the values presented are expressed as means ± standard error.

mogenizer and centrifuged for 15 min at 750*g*. The Triton X-100 treatment was repeated once. The resulting pellets were washed 4 times with 8 pellet volumes of standard buffer to remove the Triton X-100. The purified myofibrils were then resuspended in standard Tris-maleate buffer to a protein concentration of about 10 mg/ml.

Denaturation. The denaturation of isolated myosin and myosin B was studied by incubating a suspension of the enzyme (μ = 0.1) in a buffer of the required pH and temperature and following its loss of ATPase activity with time (Yasui et al., 1973). The denaturation of myofibrillar ATPase in the fiber bundles was carried out by incubating the bundles fixed to application sticks in a buffer of the required pH and temperatures at μ = 0.1. At measured intervals of time, fiber bundles were detached from the sticks and myofibrils were prepared as described previously. ATPase activity was then measured. In the case of incubation at high temperatures such as at 25, 35, and 42°, sodium dehydroacetate (DHA) at a final concentration of 2 m*M* was added to inhibit bacterial growth (Yasui et al., 1973). The incubation medium was refreshed every 24 hr.

Measurement of ATPase Activity. The ATPase measurements for the denaturation study were performed at 25° in the presence of 0.5 *M* KCl, 10 m*M* EDTA (pH 7.0), 20 m*M* Tris-maleate buffer (pH 7.0), and 1 m*M* ATP. The reaction was stopped by the addition of 15% trichloroacetic acid. The phosphate liberated was measured by the method of Martin and Doty (1949). A choice of EDTA as a modifier will be discussed in the text.

ATPase activities, as a criterion of purification of the myofibrillar proteins, were determined from the rate of release of inorganic phosphate. (1) Azide-insensitive and EGTA-insensitive ATPases were measured in the presence of 10 m*M* sodium azide and 2.0 m*M* EGTA, respectively. The protein fractions (0.5–1.0 mg/ml) were incubated in a medium containing 1 m*M* ATP, 2 m*M* $MgCl_2$, 50 m*M* KCl, and 20 m*M* Tris-maleate (pH 7.0) at 25°. (2) $(Na^+ + K^+)$-ATPase was measured as described by Post and Sen (1967), except that Na^+ and K^+ were present in all tubes. The protein fractions (0.5–1.0 mg/ml) were incubated in a medium containing 3 m*M* ATP, 100 m*M* NaCl, 20 m*M* KCl, 5 m*M* $MgCl_2$, 0.5 m*M* H_2Na_2EDTA, 20 m*M* Tris-maleate (pH 7.0), with and without 0.166 m*M* ouabain for 10 min at 37°. (3) In the experiment with quinidine, the alkaloid was added to the standard phosphate solutions. The protein fractions (1–2 mg/ml) were incubated in a medium containing 2 m*M* ATP, 2 m*M* $MgCl_2$, 50 m*M* KCl, and 20 m*M* Tris-maleate (pH 7.0), with and without quinidine at 25°.

Cytochrome Oxidase. Cytochrome oxidase was assayed spectrophotometrically by observing the rate of oxidation of ferrocytochrome *c* at 550 nm (Wharton and Tzagoloff, 1967). The protein fractions (0.1–0.2 mg/ml) were incubated in the medium containing 15 μM ferrocytochrome *c* and 10 m*M* potassium phosphate buffer (pH 7.0) at 37°. The oxidation of the substrate was followed by observing the decrease in absorbance of the incubation medium at 550 nm every 15 sec for 5 min.

Protein Concentration. Protein concentrations of the myofibril suspensions were measured by the biuret procedure (Layne, 1957).

RESULTS

Purification of Myofibrils. The extent of mitochondrial contamination of myofibrils (as indicated by cytochrome oxidase activity) is shown in Figure 1a. Treatment with Triton X-100 reduced the activity to nearly immeasurable levels. Judging from the data, unlike the case with cardiac myofibril (Solaro et al., 1971), the extent of mitochondrial contamination is extremely low; i.e., the initial level of cytochrome oxidase activity of psoas myofibrils (unwashed) was 0.013 μmol/mg per min, whereas that of cardiac myofibrils was 0.49 μmol/mg per min. This low level of contamination is also confirmed by the observation that Triton X-100 treatment exerts little effect on azide-sensitive ATPase activities (Vigers and Zieglar, 1968) of washed myofibrillar preparations (Figure 1b).

A second type of membrane which might be expected to contaminate myofibrils is the sarcolemma. The $(Na^+ + K^+)$-ATPase activities of the treated and nontreated myofibrils are shown in Figure 1c. Triton X-100 reduced this ATPase activity completely.

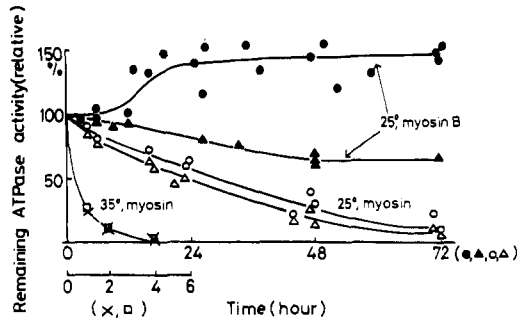

Figure 2. Changes in ATPase activity of myosin and myosin B during storage at pH 7.0: (○,●,□) Ca^{2+}-ATPase activity; (△,▲,×) EDTA-ATPase activity; incubation, myosin (2 mg/ml) or myosin B (2.5 mg/ml), 0.1 *M* KCl, 20 m*M* Tris-maleate (pH 7.0), and 2 m*M* DHA, at 25°; reaction, myosin (0.2 mg/ml) or myosin B (0.25 mg/ml), 0.5 *M* KCl, 50 m*M* Tris-maleate (pH 7.0), 1 m*M* ATP, 5 m*M* $CaCl_2$, or 10 m*M* EDTA.

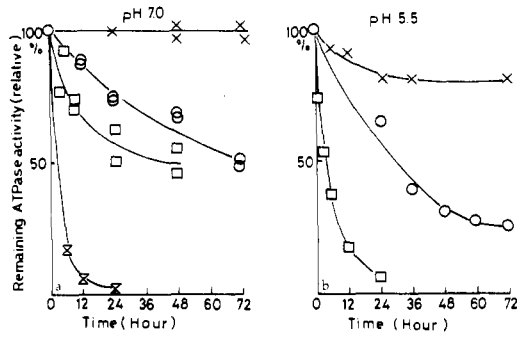

Figure 3. Changes in ATPase activity of Triton-treated myofibrils from glycerol-treated fiber bundles. Fiber bundles fixed to the application rods were incubated in 0.1 *M* KCl, 20 m*M* Tris-maleate (a, pH 7.0; b, pH 5.5), and 2 m*M* DHA at various temperatures: (×) 3°; (○) 25°; (□) 35°; (⧖) 42°. Reaction mixture for ATPase assay: 0.5 *M* KCl, 10 m*M* EDTA, 20 m*M* Tris-maleate (pH 7.0), and 0.5–1.0 mg of protein/ml at 25°.

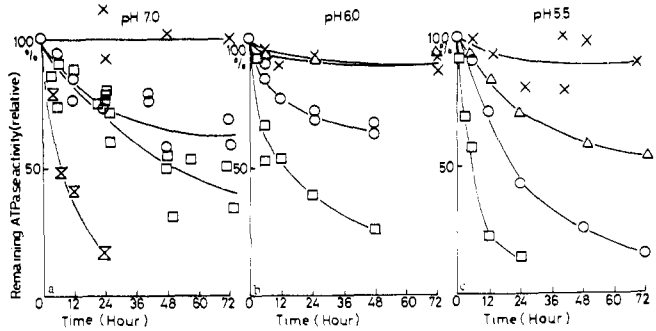

Figure 4. Changes in ATPase activity of Triton-treated myofibrils from freshly prepared fiber bundles at various temperatures and pH values of 7.0 (a), 6.0 (b), and 5.5 (c). Symbols and details are the same as in Figure 3, except that △ stands for 12°.

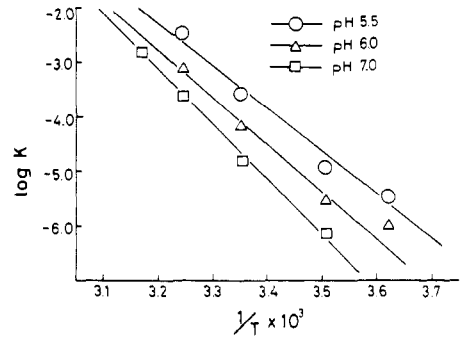

Figure 5. Arrhenius plot of the rate of denaturation of myosin at different pH values: (○) pH 5.5; (△) pH 6.6; (□) pH 7.0; incubation, 0.1 *M* KCl, 20 m*M* Tris-maleate, 2 m*M* DHA, and 2 mg of protein/ml at different temperatures; measurements of ATPase activity were the same as in Figure 2.

We also wished to determine if the sarcoplasmic reticulum impurity was eliminated by Triton X-100. Since the ATPase activity of the sarcoplasmic reticulum is inhibited by quinidine (Fuchs et al., 1968), we tested its effect on the ATPase activities of both washed and Triton-treated myofibrils. Although the washed myofibrils were inhibited 28.2% by quinidine, the ATPase activity of the treated fibers was not influenced by quinidine (Figure 1d). Since quinidine blocks the ATPase of mitochondria as well, one may conclude from this result that the ATPases of both sarcoplasmic reticulum and mitochondria are removed by Triton. Furthermore, it has been shown by Solaro et al. (1971) that the contractile proteins are not inactivated by Triton X-100.

ATPase Activities of Thermally Treated Myosin and Myosin B. To find appropriate conditions for the ATPase assay to reflect correctly the degree of inactivation due to environmental changes, ATPase activities were assayed using myosin and myosin B under the same conditions.

As seen in Figure 2, Ca^{2+}- and EDTA-activated myosin ATPase activities decreased at the same rate at 35° and at a similar rate at 25° as the denaturation proceeded. The results coincide well with those so far obtained in our laboratory (Hashimoto et al., 1959; Yasui et al., 1960). Myosin B ATPase, however, behaves differently under the same conditions (Figure 2). EDTA-activated ATPase decreased gradually with time of incubation, while an increase was observed in the Ca^{2+}-activated ATPase at a middle stage of the incubation, which remained elevated during the 72-hr storage period at 25° and pH 7.0. Our preliminary experiments on myofibrillar ATPase indicate that it behaves in a similar way. In the following experiments, therefore, EDTA-ATPase activity was determined as an indicator of inactivation.

Effect of Temperature and pH on the Myofibrillar ATPase in the Rabbit Psoas Fiber Bundles. Figures 3a and b show the changes in ATPase activity of myofibrils in glycerol-treated fiber bundles incubated at pH 7.0 and 5.5, respectively. It is evident that at 3°, myofibrillar ATPase is stable irrespective of environmental pH, whereas a marked increase in both the rate of and the extent of inactivation was observable as the temperature is increased. The results support our previous conclusion (Yasui et al., 1973) that myosin ATPase in the fiber bundles is stable at low temperatures regardless of the pH value of the incubation medium. More detailed experiments on freshly prepared washed fiber bundles are illustrated in Figures 4a–c. The trend of inactivation of myofibrillar ATPase under various conditions was found to be essentially the same as in Figure 3, indicating the stability of myofibrillar ATPase at low temperatures; even stored at pH 5.5 myofibrillar ATPase decreased only slightly at the early stages of the storage and then kept an almost constant level for 72 hr (Figure 4c).

Stability of Isolated Myosin ATPase and of Myofibrillar ATPase in the Fiber Bundles. When isolated myosin is incubated under the conditions used for fiber bundles it denatures rapidly depending upon the pH and temperature of the medium. As reported repeatedly in previous papers (Yasui et al., 1960, 1973; Takahashi et al., 1962), the reaction proceeds according to a first-order law. Data obtained were plotted semilogarithmically, and inactivation rates of myosin ATPase were calculated. The rate constants were then plotted against temperature (Figure 5).

Table I. Half-Life Time of ATPase Activity and Extractability and First-Order Rate Constants of Inactivation

Conditions of denaturation	Half-life time of ATPase activity, hr		First-order rate constants of denaturation, K_D, sec^{-1}		Half-life time[a] of extractability, hr
	Myosin A	Myofibril	Myosin A	Myofibril	
pH 5.5, 3°	39	>>72	3.350×10^{-6}		>>72
pH 5.5, 25°	0.8	20	2.623×10^{-4}	7.689×10^{-6}	
pH 5.5, 35°	<0.08	6	3.455×10^{-3}	3.059×10^{-5}	
pH 7.0, 3°	>>72	>>72	0	0	
pH 7.0, 25°	13	>72	1.479×10^{-5}		60
pH 7.0, 35°	0.83	48	2.437×10^{-4}	3.434×10^{-6}	48

[a] Values are quoted from Yasui et al. (1973).

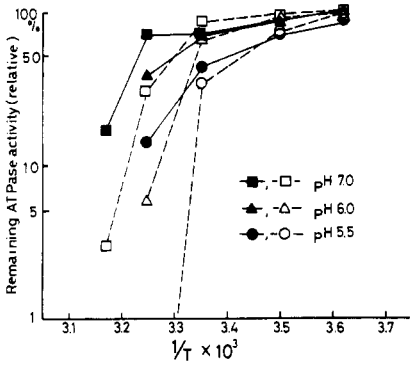

Figure 6. Logarithmic plot of the remaining ATPase activities of 1-hr incubated myosin (open symobols) and 24-hr incubated Triton-treated myofibrils (solid symbols) as a function of temperature at different pH values: (□,■) pH 7.0; (△,▲) pH 6.0; and (○,●) pH 5.5.

The slopes at pH 7.0, 6.0, and 5.5 give the activation energies of inactivation as 45.3, 43.5, and 36.2 kcal/mol, respectively, suggesting that myosin ATPase becomes more unstable as the pH value decreases. However, the inactivation of the myofibrillar ATPase shown in Figures 3 and 4 does not necessarily follow the first-order kinetics except for the reactions at higher temperatures and lower pH (see Table I).

To compare the stability of myofibrillar ATPase in the fiber bundles with that of isolated myosin in suspension, we have selected 1-hr denatured myosin and 24-hr denatured washed myofibrils as respective representatives and shown their temperature dependence at three different pH values in Figure 6. It is quite clear that the latter is far more stable than the former under any conditions tested (Figure 6). Especially, at temperatures below 12°, the myofibrillar ATPase activity remains at more than 60% of the original value even after 24-hr storage. At pH 7.0, about 20% of myofibrillar ATPase survives 24-hr storage at 42°, while myosin ATPase decreases to approximately 3% of its original level within 1 hr under the same conditions.

Table I summarizes the results on the stability of myosin and myofibrillar ATPases at different pH values and temperatures in reference to a solubility study in our previous paper (Yasui et al., 1973) which was done by using glycerol-treated fiber bundles. The solubility data are consistent with the present results. It may thus be concluded that changes in ATPase activity reflect the degree of denaturation of the myosin fraction in the fiber bundles. Moreover, one may notice from the half-life time and rate constants data that the lifetime of myofibrillar ATPase in the fiber bundles is from 10 to more than 100 times longer than that of isolated myosin in suspension under any environmental conditions.

Myosin B Suspension. It is of interest to determine why the myofibrillar ATPase is of greater stability than that of the isolated myosin from the muscle. One possible explanation would be that myosin filaments in the rigor state of muscle are desensitized or stabilized toward denaturation through their interaction with actin filaments. If that is so, we might learn more regarding this problem by studying the behavior of actomyosin under the same conditions as used in the present experiment.

The results shown in Figure 7 indicate that the above supposition may indeed be valid; viz., Figures 7a–c exactly mimic Figures 4a–c, respectively.

DISCUSSION

Changes in the ATPase activity of myofibrils in fiber bundles during storage can be successfully followed by purifying myofibrils with Triton X-100 treatment and by using EDTA as a modifier. The Triton treatment has been shown to satisfactorily eliminate contamination by ATPases originating from organelles other than myosin (Figure 1). Thus, the results of this study add to the previously reported evidence that myosin in the fiber bundles is much more stable than the isolated molecules. Inactivation rates and extent of ATPase activity of myofibrils in fiber bundles agree with those data and also the extractability of myosin from the fiber bundles under similar conditions (Table I). This temperature- and pH-induced loss of activity may therefore be conceived to be the reflection of the state of myosin in the fiber bundles. Assuming that muscle pH in most meat animal carcasses does not fall below 6.0 during the first 2–3 hr postmortem and that muscle temperature will have cooled well below body temperature before the ultimate pH value is attained, it would be expected that myofibrillar ATPase activity would remain almost unchanged (Figures 3, 4, and 6) in meat handled through normal market channels.

The inactivation rate of myofibrillar ATPase is slower than that of the isolated myosin ATPase (Table I), being 10 to over 100 times slower depending upon the condition of denaturation. If we take the largest value of 113, then the half-life time of myofibrillar ATPase would be 39 (half-life time of isolated myosin) × 113 = 4407 hr, 184 days, at pH 5.5 and 3°. However, if the pH value of muscle such as rapidly glycolyzing muscles (PSE pork) does fall rapidly before muscle temperature (which first increases above normal body temperature and then begins to decrease) drops below 35°, the half-life time of myofibrillar ATPase would certainly be curtailed to within 6 hr (Table I). The present study indicates the importance of rapid cooling to ensure quality control of meat.

The nature of the stability given to the myofibrillar ATPase may possibly be due to protection of the active site of myosin ATPase through interaction with F-actin. This idea is supported by the evidence shown in Figure 7 in which the ATPase activity of actomyosin rich myosin B is

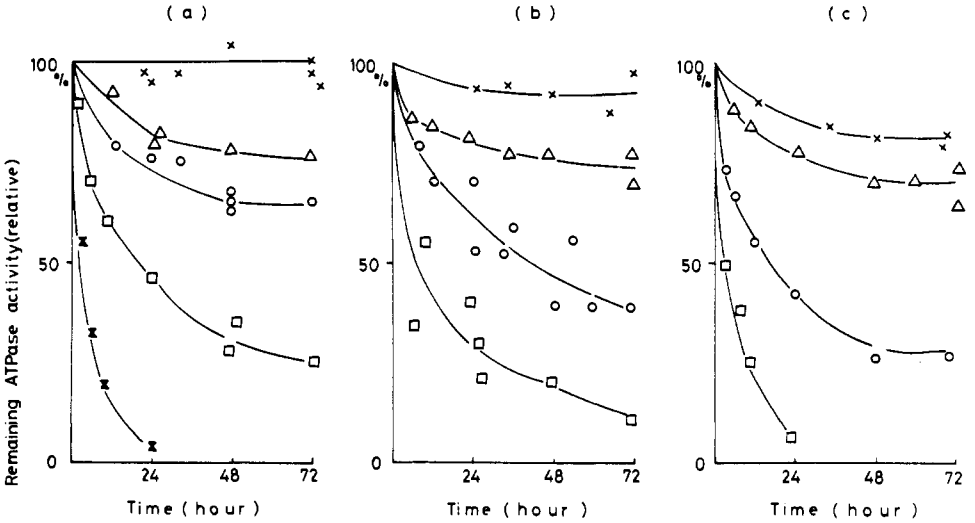

Figure 7. Changes in ATPase activities of myosin B during storage at different pH values and temperatures: (a) pH 7.0; (b) pH 6.0; (c) pH 5.5. Details and symbols were the same as in Figures 3 and 4, respectively.

seen to act like myofibrillar ATPase under the same conditions. Furthermore, Reedy et al. (1965) reported that, judging from the ultrastructure of myofibrils from insect flight muscle in rigor state as seen under the electron microscope, the thin filaments possessed an arrow-headed structure like actomyosin.

Another important finding of this study is that reasonable results can be obtained only when the myofibrillar ATPase is measured in the presence of EDTA (Figure 2). If the modifier is replaced by Ca^{2+}, the decrease in ATPase cannot be detected, but instead an apparent activation-like phenomenon is observable during storage under the moderate denaturing condition (Figure 2, solid symbols). This suggests that the two well-known essential sulfhydryl groups, SH_1 and SH_2, of myosin (Sekine and Kielley, 1964; Sekine et al., 1962; Yamaguchi and Sekine, 1966; Bailin and Bárány, 1972; Reisler et al., 1974) which are located at or near the active site of myosin somehow are involved in this phenomenon. Blocking of both sulfhydryls, SH_1 and SH_2, eliminates both Ca^{2+}-ATPase and EDTA-ATPase activities (Yamaguchi and Sekine, 1966; Reisler et al., 1974). Seidel (1969) has found that myosin preferentially blocked at SH_1 or SH_2 exhibits a high level of Ca^{2+}-ATPase activity but no EDTA-ATPase activity, indicating that both sites are required for the latter activity, whereas either site alone is sufficient for the expression of Ca^{2+}-ATPase activity. Our results in Figure 2 indicate that the Ca^{2+}- and the EDTA-ATPase of myosin do not exhibit any separated behavior under the denaturation conditions. This may suggest that the SH_1 and SH_2 are apparently both being modified with myosin storage.

Several lines of evidence indicate that the binding of actin affects the sulfhydryl sites of myosin. Studies on incorporation of *N*-ethylmaleimide or fluorodinitrobenzene into actomyosin show that actin protects the SH sites from modification (Bárány et al., 1969; Schaub and Watterson, (1972). This protection is afforded mainly to the SH_1 site which, in the absence of actin and under reaction conditions employed by Bárány et al. (1969), is the site of modification. The same conclusion may be derived from the works of Schaub and Watterson (1972) and Seidel (1973). Therefore, in the case of denaturation of myosin B (Figure 2), it is supposed that since the SH_1 site of myosin somehow is protected by actin from modification, the SH_2 site would be preferentially destroyed with subsequent and gradual modification of the SH_1 site. However, it should be recognized at the same time that the decreased affinity of actin for heavy meromyosin or subfragment 1 suggests that the modification of the interaction of the myosin subfragments with actin is due to the proteolytic cleavages introduced into their heavy- and light-chain regions. This may possibly occur when a subtle conformational change in the myosin molecule is brought about through severe environmental changes.

From the lines of reasoning described above, the following two alternative interpretations may be drawn: the rise of Ca^{2+}-ATPase and the fall of EDTA-ATPase activities seen in Figure 2 are due to modification of actin–myosin interaction (1) by proteolytic cleavage of myosin moiety or (2) by subtle conformational changes in myosin or actin produced as the result of denaturation of the molecules. In both cases, myosin molecules would be so arranged as to interact with simple substrate molecules at their SH_1 sites. The former idea is tempting, because of the recent results reported by Yamashita and Hasumi Mimura (1974), where they have shown the activation of Ca^{2+}-ATPase as well as the inactivation of EDTA-ATPase of myosin after treatment with trypsin. Nevertheless, when we consider our previous results (Yasui et al., 1973) that the Ca^{2+}-ATPase of myosin extracted from fiber bundles which were exposed to denaturing conditions did not show activation, the above idea appears unlikely. It is, therefore, fair to presume that the latter possibility may still be attractive and merit further study.

Fujimaki and coworkers (Fujimaki et al., 1965b) reported that both the Mg^{2+}- and Ca^{2+}-ATPase activities of myosin B extracted from rabbit muscle after 2 days of post-mortem storage at 4° were 15–25% higher than the corresponding activities of myosin B at death muscle. This finding was subsequently extended to bovine muscle and to a number of other modifiers including EDTA by Robson (Goll and Robson, 1967; Robson et al., 1967) and to chicken muscle by measuring Mg^{2+}-ATPase by Jones (1972). It is, however, to be mentioned that changes in Mg^{2+}-ATPase of myosin B or myofibrils at low ionic strength during storage may not be only a measure of actin–myosin interaction, but rather a manifestation of the proteolytic degradation occurring in the regulatory protein, troponin complex. As described by Drabikowski et al. (1971), Hartshorne and Dreizen (1972), Head and Perry (1974), and Penny (1974) troponin T and I are susceptible to proteolysis.

Since our experiments were designed to measure the ATPase activities at high ionic strength where no regulatory system, if any, is operative and actomyosin-type ATPase becomes myosin-type, the results may represent the true changes in myosin ATPase activity. Altogether, the evidence so far accumulated strongly implicates the role of local conformational change in the vicinity of the SH_1 site

in myosin in the interaction of myosin with actin, and suggests that the increase in Ca^{2+}-ATPase activity is a universal feature of mammalian skeletal muscle which has undergone some changes during the storage under denaturing conditions. To detect whether or not a local conformational change of myosin in the actin-myosin complex system takes place, measurement of EDTA-ATPase at high ionic strength may be recommended as the best method.

This study provides us with a new approach through which the mechanism of modification of actin-myosin interaction during postmortem storage of skeletal muscle, which has been suggested independently by many workers (Fujimaki et al., 1965a; Gothard et al., 1966; Takahashi et al., 1967; Stromer et al., 1967), will be clarified. Along these lines, investigations on Ca^{2+}- and EDTA-ATPase activities of the myosin-actin/troponin-tropomyosin complex system are now under way in our laboratory.

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Nutritional Evaluation of Protein from Shrimp Cannery Effluent (Shrimp Waste Protein)

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The protein efficiency ratio, PER, was determined for three proteins, casein, ISP (isolated soybean protein), shrimp waste protein, SWP, collected and processed from shrimp cannery effluent, and a mixture of equal proportions of SWP and ISP. With albino rats as the test animals, four isocaloric, isonitrogenous diets (4045 cal/g; 1.6% nitrogen) containing one each of the above protein sources were used to evaluate the effects of SWP on five criteria: food intake, body weight, PER, liver weight, and the ratio of protein to fat in the liver. SWP promoted rat growth 80% as efficiently as casein; moreover, SWP improved protein quality 74% in a soybean diet when SWP replaced half the soybean protein in the diet. A projection of potential practical applications would include incorporation of SWP in canned or processed pet foods and/or use of SWP as an animal feed supplement in poultry or livestock rations, in addition to reduction of water pollution.

Holder (1950) and Novak (1970) stated that protein from fishery products and by-products such as fish meals, condensed fish solubles, fish protein concentrate, and proteins from crude waste meal of crab and shrimp are excellent sources of protein because of their higher values in protein content and quality. Sure and Easterling (1952) found that the amino acid constituents of fish protein are of particular importance when incorporated into a diet of plant protein such as zein, soybean protein, cottonseed meal, and gluten. Also, Combs (1961) and Winchester (1963) indicated that fish proteins can substitute the deficiency in amino acids of plant proteins, which fail to provide the minimal requirements of the essential amino acids for growth and biologi-

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