Change of Acto-Heavy Meromyosin ATPase of Rabbit Skeletal Muscle during Postmortem Storage

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Actin-activated heavy meromyosin (HMM) ATPase of at time of death and postmortem muscles has been investigated. The maximum velocity of acto-HMM ATPase was the highest at time of death and decreased with time in postmortem muscles. The affinity of actin for myosin was the greatest in 168-h postmortem muscle and least in at time of death muscle. The study of the cross-reaction between actin and HMM suggests that the properties of both actin and myosin are altered during postmortem storage of muscles. The apparent activation energy of myosin ATPase was decreased with postmortem time, while the polymerization rate of actin was increased.

Muscle undergoes rigor mortis and resolution of rigor during postmortem storage. As ATP decreases in postmortem muscle, rigor mortis occurs, resulting in a loss of extensibility and development of isometric tension. Resolution of rigor follows, with a decline in isometric tension and Z-line degradation (Bendall, 1973; Busch et al., 1972a; Goll et al., 1974). These physical changes are reflected in the textural properties of muscle: both antemortem and aged muscles are tender, while rigor muscles are tough. The physical changes of postmortem muscle may be due to (1) alteration of actin–myosin interaction, (2) Z-line degradation, and/or (3) alteration in connective tissue properties (Busch et al., 1972b; Davey and Gilbert, 1969; Goll et al., 1970, 1974; Fujimaki et al., 1965a; Locker, 1960; Takahashi et al., 1967). Structural changes take place in myosin components during rigor mortis (Huxley and Brown, 1967); however, the nature of the actin–myosin interaction in postmortem muscle is less clear.

In the present study, we have investigated actin-activated heavy meromyosin (HMM ATPase [EC 3.6.1.3] of muscles stored for varying periods after death. It has been found that the affinity of actin for HMM is much higher in postmortem muscles than at time of death, while the maximum velocity of the ATPase activity of postmortem muscles is lower than at death.

MATERIALS AND METHODS

Materials. Myosin was prepared from rabbit longissimus thoracis, semimenbranosus, and biceps femoris muscles at death and after 24- and 168-h postmortem storage at 0 °C according to the method of Tonomura et al. (1961). The muscles had been sprayed with 10 mM NaN_3 solution to protect against bacterial growth and wrapped in a polyethylene bag. Myosin was extracted from postmortem muscles using a modified Guba-Straub solution (0.3 M KCl, 0.15 M potassium 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). HMM was obtained by the method of Lowey and Cohen (1962) except that myosin was digested with trypsin for 4 min at 25 °C. Ammonium sulfate fractionation between 42 and 58% saturation was made. HMM was dialyzed against 50 mM KCl, 10 mM Tris-maleate (pH 7.0), 1 mM dithiothreitol (DTT) prior to use in the assay. Actin was prepared as described by Spudich and Watt (1971); except that polymerization of actin was induced only by the addition of 50 mM KCl (Maruyama, 1975) not by the addition of both 50 mM KCl and 2 mM $MgCl_2$ as described in the original method and the time of polymerization was extended from the original 2 h to overnight.

ATPase Activity. Myosin ATPase activity measurement was carried out in 0.5 M KCl, 4 mM $CaCl_2$, 2 mM ATP, 10 mM Tris–maleate (pH 7.0) at varying temperatures. The inorganic phosphorus liberated was determined by the method of Fiske and Subbarow (1925). The actin-activated HMM ATPase was determined in a medium containing 40 mM KCl, 1 mM $MgCl_2$, 2 mM ATP, and 10 mM Tris–maleate (pH 7.0). The concentration of actin varied between 0.4 and 2.5 mg/mL with constant amount of HMM (0.2 mg/mL).

Viscometry. Polymerization rate of G-actin was determined by relative viscosity of actin (Mannherz et al., 1975) in medium containing 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM β-mercaptoethanol, and 10 mM Tris–HCl (pH 8.0) using an Ubbelohde type viscometer with a buffer outflow time of 35.4 s at 25 °C. The polymerization reaction was started by adding 0.05 volume of 2 M KCl to make a final KCl concentration of 0.1 M.

SDS Polyacrylamide Gel Electrophoresis. SDS polyacrylamide gel electrophoresis was carried out according to the method of Weber and Osborn (1969). The gels were stained with Coomassie Blue.

Protein Concentration. Protein concentration was determined by the biuret reaction (Gornall et al., 1949).

Chemicals. ATP for ATPase assay, trypsin [EC 3.4.21.4], soybean trypsin inhibitor, and DTT were purchased from Sigma Chemical Co. ATP for preparing myosin and actin was purchased from Kyowa Hakko Co. All other reagents were of analytical grade.

RESULTS

Figure 1 shows the double-reciprocal plots of the actin-activated HMM ATPase (Eisenberg and Moos, 1968) of muscles stored for varying periods after death. The maximum velocity and the apparent dissociation constant decreased with postmortem time, i.e., the maximum velocities of acto-HMM ATPase of 24- and 168-h postmortem muscles were approximately 63 and 46% of that of at death muscle, respectively, while the affinity of actin for myosin was approximately 1.9 and 3.5 times greater.

The maximum velocity (V_{max}) and apparent dissociation constant (K_a) of the cross-reacted acto-HMM ATPase are presented in Table I. The apparent dissociation constant was calculated from the intercept on the abscissa, assuming a binding ratio of 4.2×10^4 g of actin/mol of HMM (Rizzino et al., 1970; Elzinga et al., 1973). Results (Table I) indicate that the enzymatic properties of both actin and myosin are somewhat altered during postmortem storage of muscle.

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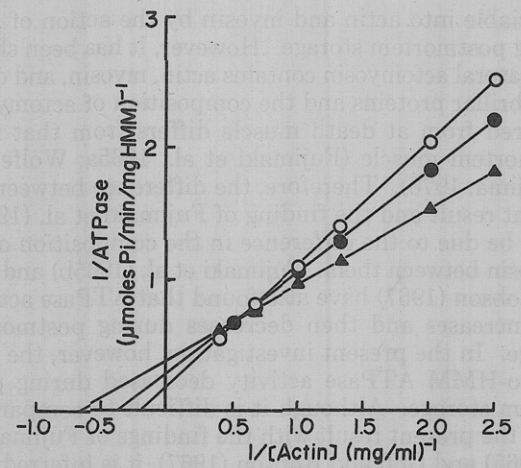

Figure 1. Double-reciprocal plots of actin-activated HMM ATPase vs. actin concentration. The reaction mixture contained 40 mM KCl, 1 mM $MgCl_2$, 2 mM ATP, 10 mM Tris–maleate (pH 7.0), 0.2 mg/mL of HMM, and varying concentrations of actin to a total volume of 2 mL. Acto-HMM prepared at time of death (actin-0 and HMM-0) (○); acto-HMM from 24-h postmortem muscle (actin-24 and HMM-24) (●); acto-HMM from 168-h postmortem muscle (actin-168 and HMM-168) (▲). Each plot in this figure represents an average of three determinations on three different preparations.

Table I. Actin-Activated HMM ATPase of at Death and Postmortem Muscles[a] and Cross-Reacted and Reversely Cross-Reacted Actin-Activated HMM ATPase[b]

	V_{max},[c] μmol of Pi/min·mg of HMM	K_a,[c] M
Actin-0 and HMM-0 (at death muscle)	5.88	1.32×10^{-4}
Actin-24 and HMM-24 (24-h postmortem muscle)	3.70	6.80×10^{-5}
Actin-168 and HMM-168 (168-h postmortem muscle)	2.70	3.72×10^{-5}
Actin-0 and HMM-168	4.76	9.52×10^{-5}
Actin-168 and HMM-0	3.13	5.29×10^{-5}
Actin-0 and HMM-24	4.76	9.52×10^{-5}
Actin-24 and HMM-0	4.00	7.94×10^{-5}
Actin-24 and HMM-168	2.63	6.44×10^{-5}
Actin-168 and HMM-24	2.63	5.95×10^{-5}

[a] The values were calculated from the plots of Figure 1. [b] The values represent an average of two determinations. [c] The maximum velocities (V_{max}) and the apparent dissociation constants (K_a) of the cross-reacted acto-HMM ATPase (actin-0 and HMM-168, actin-24 and HMM-0 and actin-168 and HMM-24) using actin prepared from at death muscle (Actin-0) and from 24- and 168-h postmortem muscles (actin-24 and actin-168) and HMM from at death muscle (HMM-0) and 24- and 168-h postmortem muscles (HMM-24 and HMM-168) and the reversely cross-reacted ones (actin-168 and HMM-0, actin-0 and HMM-24, and actin-24 and HMM-168) were calculated from double-reciprocal plots of acto-HMM ATPase vs. actin concentrations.

Figure 2 shows the Arrhenius plots of myosin ATPase prepared from muscles stored for varying periods after death. The apparent activation energies, E_a, of myosin prepared from at death and 24- and 168-h postmortem muscles, calculated from the slopes of this figure, were 9.6, 7.5, and 7.1 kcal/mol, respectively. Thus, the activation energy of myosin ATPase decreases with postmortem storage time.

White skeletal muscle myosin contains two heavy chains and three kinds of light chains (Weeds and Lowey, 1971). Myosin prepared from postmortem muscles also has this

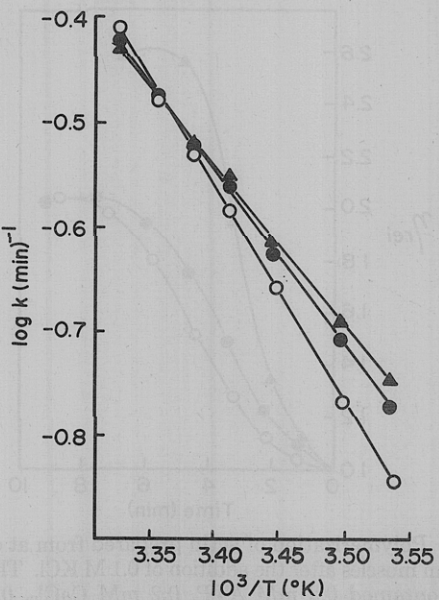

Figure 2. Arrhenius plots of myosin ATPase. Myosin was prepared at death and after 24 and 168 h. ATPase reaction was conducted in a medium containing 0.5 M KCl, 4 mM $CaCl_2$, 2 mM ATP, and 10 mM Tris–maleate (pH 7.0). Myosin prepared from at death muscle (○); myosin from 24-h postmortem muscle (●); myosin from 168-h postmortem muscle (▲).

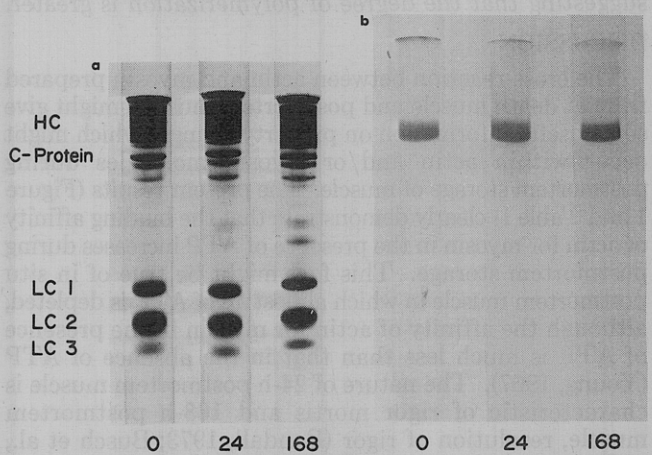

Figure 3. (a) SDS polyacrylamide gel electrophoretograms of myosin prepared from at death and postmortem muscle. Electrophoresis (7.5% gel) was done as described in the Materials and Methods section. A 100 μg sample of protein was loaded on each gel. The number under each electrophoretogram indicates postmortem storage time. HC, heavy chain; LC, light chain. SDS polyacrylamide gel electrophoretograms of actin prepared from at death and postmortem muscle. Twenty micrograms of protein was loaded on each gel (10% gel). The number under each electrophoretogram was the same as in Figure 3a.

substructure (Figure 3a). No light chains were released from the myosin molecule during postmortem storage. In addition, several minor bands having intermediate molecular weights between the heavy chain and the light chain (LC 1) appeared in the electrophoretograms of myosin prepared from postmortem muscles. This was seen in myosin prepared from 168-h postmortem muscles. Figure 3b shows the electrophoretograms of actin. No significant change was found during postmortem storage.

Figure 4 shows the polymerization rate of G-actin prepared from the muscles stored for varying periods after death. The polymerization rate of G-actin after the addition of 0.1 M KCl increased with postmortem storage time of muscle used to prepare the actin sample. In addition, the relative viscosity of actin prepared from 168-h

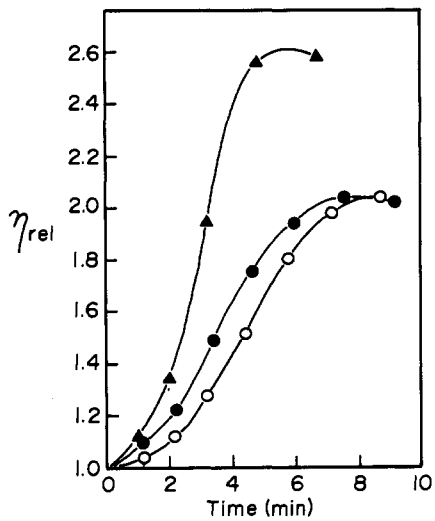

Figure 4. Polymerization of actin prepared from at death and postmortem muscles after the addition of 0.1 M KCl. The reaction mixture contained 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM β-mercaptoethanol, 10 mM Tris–HCl (pH 8.0), and 2 mg/mL of actin. Actin prepared from at death muscle (○); actin prepared from 24-h postmortem muscle (●); actin prepared from 168-h postmortem muscle (▲).

postmortem muscle was much larger than at death, suggesting that the degree of polymerization is greater.

DISCUSSION

The cross-reaction between actin and myosin prepared from at death muscle and postmortem muscles might give some useful information on property changes which might occur within actin and/or myosin molecules during postmortem storage of muscle. The present results (Figure 1 and Table I) clearly demonstrate that the binding affinity of actin for myosin in the presence of ATP increases during postmortem storage. This fact might be true of in situ postmortem muscle in which almost all of ATP is depleted, although the affinity of actin for myosin in the presence of ATP is much less than that in the absence of ATP (Young, 1967). The nature of 24-h postmortem muscle is characteristic of rigor mortis and 168-h postmortem muscle, resolution of rigor (Bendall, 1973; Busch et al., 1972a; Goll et al., 1974). The present results indicate that gross changes in muscle which occur during postmortem storage and, in particular, the resolution of rigor which accompanies tenderization of meat cannot satisfactorily be explained by an alteration in the actin–myosin interaction. Data presented in Table I also indicate that postmortem change in acto-HMM ATPase is due to changes in the properties of both actin and myosin. This consideration is in part substantiated by ATPase of myosin and polymerization rate of actin (Figures 2, 3, and 4). SDS electrophoresis of myosin indicates that a slight degradation of myosin might occur during postmortem storage of muscle. Arakawa et al. (1976) have also suggested the possibility of cleavage of myosin during postmortem storage of muscle. On the other hand, no significant structural change of actin was found during postmortem storage (Figure 3b). Ishiwata (1976) has indicated that the oxidation of a specific SH group of actin alters the polymerization rate of actin. Thus, it appears that the change in the polymerization rate of actin might be due to the oxidation of the SH group.

In addition, the present result that strength of the actin–myosin interaction increases during postmortem storage of muscle is contrary to the finding of Fujimaki et al. (1965a) which showed that actomyosin became more dissociable into actin and myosin by the action of ATP during postmortem storage. However, it has been shown that natural actomyosin contains actin, myosin, and other myofibrillar proteins and the composition of actomyosin prepared from at death muscle differs from that from postmortem muscle (Fujimaki et al., 1965a; Wolfe and Samejima, 1976). Therefore, the difference between the present result and the finding of Fujimaki et al. (1965a) might be due to the difference in the composition of actomyosin between them. Fujimaki et al. (1965b) and Goll and Robson (1967) have also found that ATPase activity first increases and then decreases during postmortem storage. In the present investigation, however, the V_{max} of acto-HMM ATPase activity decreased during postmortem storage. Although it is difficult to compare directly the present result with the findings of Fujimaki et al. (1965) and Goll and Robson (1967), it is inferred that this contradiction is due to the difference in the ratio of actin and myosin in their preparations.

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