Myosin ATPase and Acto-Heavy Meromyosin ATPase in Normal and in Pale, Soft and Exudative (PSE) Porcine Muscle

Sam Kyung SUNG,* Tatsumi ITO** and Kenjiro IZUMI***

*Laboratory of Chemistry and Technology of Animal Products, Faculty of Agriculture,
Kyushu University, Fukuoka 812, Japan*

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An investigation was conducted on myosin and actin-activated heavy meromyosin (HMM) ATPase activities in normal porcine muscle stored for varying periods of time after death. Studies were also made on temperature dependent myosin ATPase, initial burst of ATPase and actin-activated HMM ATPase in normal and in pale, soft and exudative (PSE) porcine muscle. The maximum velocity of acto-HMM ATPase of normal muscle decreased considerably with postmortem time, while the apparent dissociation constant decreased slightly. The maximum velocity of acto-HMM ATPase of postmortem normal muscle was approximately two-times larger than that of the corresponding PSE muscle. However, almost no difference was found in the apparent dissociation constant. The size of the initial burst of phosphate-liberation of myosin prepared from normal muscle was approximately 1.2 mol/mol of myosin and from PSE muscle 0. It is assumed that the lack of contractility of PSE muscle was brought about by two basic myosin malfunctions: one, the irreversible binding of myosin to actin filament and the other, the functional damage of myosin ATPase, responsible for the formation of phosphorylated complex, even when dissociable.

Pale, soft and exudative (PSE) porcine muscle known as a low quality pork occurs in some types of pigs after slaughter.¹⁾ The occurrence of PSE muscle is also dependent upon the physiological conditions of muscle and the condition for producing PSE muscle is developed during early rigor process of muscle.^{2,3)} It has been established that high temperature and low pH occurring in an early postmortem muscle cause the denaturation of myofibrillar proteins.^{4~9)} Fuchs¹⁰⁾ showed that with the increase in temperature the inhibitory action of troponin-tropomyosin system in actomyosin was progressively reduced. Park *et al.*¹¹⁾ showed that calcium sensitivity of myosin B prepared from PSE muscle was partially lost. In a previous paper, we have reported that normal myofibril prepared from 24 hr postmortem muscle is able to

contract upon addition of Mg^{2+} -ATP, while PSE myofibril contract in part or cannot contract, even though PSE myofibril retains partially its Mg^{2+} -ATPase activity.¹²⁾ This result suggests that contraction is apparently uncoupled with Mg^{2+} -ATPase in PSE muscle.

In the present study, we have investigated myosin and actin-activated heavy meromyosin (HMM) ATPase activities of normal porcine muscle stored for varying periods after death. We have also investigated temperature dependent myosin ATPase, initial burst of the ATPase and acto-HMM ATPase of normal and PSE muscle, and discussed the inter-relationship between the contractility of muscle and the size of initial burst of phosphate-liberation (Pi-liberation). A part of this study has been published elsewhere in a preliminary form.¹³⁾

* Present address: Yeung Nam University, Daegu, Korea.

** To whom correspondence should be addressed.

*** Present address: Muscle Biology Laboratory, University of Wisconsin, Madison, Wisconsin.

MATERIALS AND METHODS

Materials. Selection of normal and PSE porcine muscle was done by measuring pH and testing contractility of

myofibrils of 24 hr postmortem longissimus thoracis muscle as described in a previous paper¹²⁾: the muscles having high pH values (pH 5.44, 5.48, 5.52 and 5.58) and full contractility were classified as normal, and the muscles having low pH values (pH 5.28, 5.24 and 5.18) and no contractility and those having intermediate pH (pH 5.33 and 5.36) and contractility (60% contractility for both muscles) were also classified as PSE and slightly PSE muscle, respectively.

Myosin was prepared from porcine muscle immediately after death and the muscles stored for 24 and 168 hr at 0~5°C by the method of Quass and Briskey¹⁴⁾ with slight modifications: ultracentrifugation was done at 100,000 × g for 2 hr instead of centrifugation at 30,000 × g for 30 min in the original method. A modified Guba-Straub solution (0.3 M KCl, 0.15 M K-phosphate, 1 mM ethyleneglycol-bis-(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), 5 mM ATP, 5 mM MgCl₂, pH 6.5) was used for the extraction of myosin from aged muscles. Myosin was stored in 50% glycerol (v/v) at -20°C until use.

HMM was obtained by the method of Lowey and Cohen¹⁵⁾ with slight modifications: trypsin digestion was done for 4 min at 25°C, and purification of HMM was made by ammonium sulfate fractionation between 42~58% saturation, and then dialyzed against 40 mM KCl, 10 mM Tris-maleate (pH 7.0), 1 mM dithiothreitol (DTT) prior to use in the assay. Actin was prepared according to the method of Spudich and Watt,¹⁶⁾ except that the time of polymerization was prolonged overnight instead of 2 hr in the original method. This prolongation of the time for polymerization brought about no significant change of the SDS polyacrylamide gel electrophoretogram of actin (data not shown).

All operations were conducted at 5°C or below except where otherwise noted, and all determinations were performed within 3 days for HMM and 5 days for actin after preparation.

ATPase activity. The enzymatic assay of myosin ATPase was performed in water-bath at 25°C for 1~8 min with 1 min intervals. The total volume of reaction mixture was 2.0 ml. The reaction was stopped by adding equal volume of cold 10% trichloroacetic acid (TCA), followed by centrifugal removal of the protein precipitate. The inorganic phosphate liberated was determined by the method of Fiske and Subbarow,¹⁷⁾ and the results were expressed as specific activity in terms of μmol of inorganic phosphate liberated/min/mg protein.

The reaction of actin-activated HMM ATPase activity was carried out in the same way as described above, except that the reaction was initiated by the addition of HMM. The measured ATPase activity was corrected by subtracting the ATPase activity of added HMM in the absence of actin from the activity of acto-HMM ATPase. The apparent dissociation constant was calculated from the abscissa, assuming a binding ratio of 4.2×10^{-4} g of actin.^{18,19)}

The initial burst of myosin ATPase was examined in a medium containing high concentration of salt. The reaction was stopped by adding 10% TCA (final concentration 5%) at appropriate times (usually 20, 40, 60, 80, 100, 120 and 150 sec) after the start of the reaction. The amount of liberated inorganic phosphate was determined by the method of Martin and Doty²⁰⁾ with slight modifications.²¹⁾

Contractility and pH measurement. Contractility of myofibrils and pH of muscles were tested according to the methods described in a previous paper.¹²⁾

Protein concentration. Protein concentration was measured by the biuret reaction.²²⁾

RESULTS

Postmortem change of acto-HMM ATPase

Figure 1 shows the double-reciprocal plots of the actin-activated HMM ATPase of normal porcine muscle stored for varying periods after death. In this figure, the intercept on the ordinate gives the maximum velocity (V_{max}) of

FIG. 1. Double-reciprocal Plots of Acto-HMM ATPase of at Death and Postmortem Muscle.

Acto-HMM ATPase activity was measured at 25°C in a medium containing 40 mM KCl, 1 mM MgCl₂, 2 mM ATP, 10 mM Tris-maleate (pH 7.0) and varying concentrations of actin (0.4~2.5 mg/ml) with a constant concentration of HMM, 0.2 mg/ml.

○, at death muscle; △, 24 hr postmortem muscle; □, 168 hr postmortem muscle. Normal porcine muscle (pH₂₄=5.48, contractility 100%) was used in this experiment.

the acto-HMM ATPase; analogously the intercept on the abscissa yields the apparent dissociation constant (K_{app}) of the complex.²³⁾ As shown in this figure, the V_{max} considerably decreased with postmortem time, while the K_{app} decreased a little: the V_{max} of acto-HMM ATPase of 0, 24 and 168 hr postmortem porcine muscle was 5.71, 4.18 and 2.98 $\mu\text{mol Pi/min/mg}$ of HMM and the K_{app} was 1.91×10^{-4} , 1.23×10^{-4} , and 7.44×10^{-5} M, respectively. These results are consistent with the findings on the acto-HMM ATPase of rabbit postmortem skeletal muscle.^{24,25)}

Acto-HMM ATPase of 24 hr postmortem porcine muscle

The V_{max} and K_{app} of actin-activated HMM ATPase of normal and PSE porcine muscles were also obtained from the double reciprocal plots of the ATPase (Table I). As shown in Table I, the V_{max} decreased with decreasing pH and with decreasing contractility of the muscles. The V_{max} of acto-HMM ATPase of normal muscle was approximately two times larger than that of PSE muscle. This indicates that actin activation of HMM ATPase of PSE muscle at infinite actin concentration was less than that of normal muscle. However, almost no difference was observed in the K_{app} .

Arrhenius plots of myosin ATPase

Figure 2 shows the Arrhenius plots of myosin ATPase activity of normal and PSE porcine muscles. Muscles prepared from 24 hr postmortem porcine muscles were used in this experiment. The apparent activation energies, E_a , of myosins prepared from normal, slightly PSE and PSE muscles were 8.64, 6.11 and 5.86 kcal/mol, respectively. Thus the activation free energy of myosin prepared from normal muscle is slightly higher than that of PSE muscle. This suggests that some alterations of enzymatic function of myosin might occur in PSE muscle in which muscle proteins are inactivated during the development of PSE conditions.

TABLE I. ACTIN-ACTIVATED HMM ATPASE OF NORMAL AND PSE MUSCLE

The V_{max} and K_{app} of normal and slightly PSE muscle were the averages of two determinations on two different normal muscles (pH 5.52, contractility 100%; pH 5.44, contractility 100%) and two different slightly PSE muscles (pH 5.33, contractility 60%; pH 5.36, contractility 60%), respectively. The values of PSE muscle were the averages of two determinations on one PSE muscle (pH 5.28, contractility 0%).

Porcine muscle	V_{max} ($\mu\text{mol Pi/min/mg}$ HMM)	K_{app} (M)
Normal	3.846	11.45×10^{-5}
Slightly PSE	3.704	11.45×10^{-5}
PSE	2.041	11.18×10^{-5}

FIG. 2. Arrhenius Plots of Normal, Slightly PSE and PSE Muscle Myosin ATPase.

Myosin ATPase measurements were carried out at varying temperatures in a reaction mixture containing 0.5M KCl, 4mM CaCl_2 , 1mM ATP and 10mM Tris-maleate, pH 7.0. The concentration of myosin was 0.2mg/ml.

○, myosin prepared from normal muscle (pH 5.44, contractility 10%); △, myosin prepared from slightly PSE muscle (pH 5.33, contractility 60%); ●, myosin prepared from PSE muscle (pH 5.28, contractility 0%).

Initial burst of normal and PSE myosins

The determination of the initial burst of Pi-liberation of myosin was conducted on two normal and two PSE myosins. In each case

Regeneration of NADPH by Frozen-thawed Cells of a Blue-green Alga, <i>Synechococcus</i> sp.	Y. MIURA, Y. NAKANO, K. YAGI and K. MIYAMOTO....	845
D-Glucose Dehydrogenase of <i>Gluconobacter suboxydans</i> : Solubilization, Purification and Characterization.....	M. AMEYAMA, E. SHINAGAWA, K. MATSUSHITA and O. ADACHI....	851
Methane Fermentation of Coffee Grounds and Some Factors to Improve the Fermentation.....	S. OI, T. TANAKA and T. YAMAMOTO....	871
Antifungal Activity of Components of Essential Oils...N. KURITA, M. MIYAJI, R. KURANE and Y. TAKAHARA....		945
L-Arginine Production by Arginine Analog-resistant Mutants of Microorganisms.....	H. YOSHIDA, K. ARAKI and K. NAKAYAMA....	959

Pesticide Chemistry

Synthesis of Some <i>O,O</i> -Dialkyl <i>N</i> -[4-(<i>N</i> -heteroarylsulfamoyl)phenyl]phosphoramidothioates as Potential Fungicides.....	S. GIRI and Y. SINGH....	839
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Notes

Penicillin-Binding Proteins in <i>Selenomonas ruminantium</i>	Y. KAMIO and Y. TERAWAKI....	993
Effects of Trialkyltin Chlorides on <i>Escherichia coli</i> Spheroplasts.....	J. YAMADA....	997
Adsorption Specificity of Immobilized Tannin for Proteins and Other Organic Compounds.....	T. WATANABE, T. MORI, T. TOSA and I. CHIBATA....	1001
Effect of Temperature on Growth of <i>Bacillus</i> sp. T-4, a Thermophilic Acidophilic Bacterium.....	F. UCHINO and T. KATANO....	1005
Variation in Casein from Individual Human Milk Samples...N. AZUMA, H. KOBAYASHI and K. YAMAUCHI....		1007
Stopped-Flow Measurement of Reaction Rate of Dihydroxyfumaric Acid with 2,6-Dichlorophenoldophenol.....	H. OBATA, M. TATSUNO and T. TOKUYAMA....	1011
Purification of Oat Debranching Enzyme and Occurrence of Inactive Debranching Enzyme in Cereals.....	J. YAMADA....	1013
Enrichment of <i>n</i> -Alkane Assimilation-Deficient Mutants of <i>Candida</i> Yeast by Synergistic Effect of Nystatin and Pyrrolnitrin.....	K. YANO, M. KAWAMURA and M. TAKAGI....	1017
<i>In Vitro</i> Stimulation by Parathion of Porcine Pancreatic Carboxylesterase Activity toward Triacetin.....	T. KOMURA and H. NAGAYAMA....	1019
Determination of L-Cysteine with L-Methionine γ -Lyase...H. TANAKA, H. IMAHARA, N. ESAKI and K. SODA....		1021
Isolation and Biological Activity of a Peptidal Antibiotic P168.....	A. ISOGAI, A. SUZUKI, S. HIGASHIKAWA, S. KUYAMA and S. TAMURA....	1023

Rapid Papers

Application of Plasmid to ATP Production by <i>E. coli</i>	M. SHIMOSAKA, Y. FUKUDA and A. KIMURA....	1025
1'-(6-Hydroxyoctanoyl) nornicotine and 1'-(7-Hydroxyoctanoyl)nornicotine, Two New Alkaloids from Japanese Domestic Tobacco.....	M. MIYANO, N. YASUMATSU, H. MATSUSHITA and K. NISHIDA....	1029

Short Communications

Mode of Action of a New <i>Aspergillus oryzae</i> Carboxypeptidase O-1.....	M. TAKEUCHI and E. ICHISHIMA....	1033
Mutagenicity of the Gaseous Phase of Protein Pyrolysis Product in <i>Salmonella</i>	A. KUSHI, H. TSUDA, D. YOSHIDA and F. GOTŌ....	1037
New Aspartate Aminotransferase Inhibitor (Gostatin) Produced by <i>Streptomyces sumanensis</i> Nov. sp., NK-23.....	S. MURAO, T. NISHINO, N. KATAYAMA and H. NAGANO....	1039
Isolation of Tremmerogen <i>a</i> -13, a Peptidal Sex Hormone of <i>Tremella mesenterica</i>	M. YOSHIDA, Y. SAKAGAMI, A. ISOGAI and A. SUZUKI....	1043
Structure of Tremmerogen <i>a</i> -13, a Peptidal Sex Hormone of <i>Tremella mesenterica</i>	Y. SAKAGAMI, M. YOSHIDA, A. ISOGAI and A. SUZUKI....	1045
Synthesis and Structure-Activity Relations of Tremmerogen <i>a</i> -13, a Peptidal Sex Hormone of <i>Tremella mesenterica</i>	C. KITADA, M. FUJINO, Y. SAKAGAMI, M. YOSHIDA, A. ISOGAI and A. SUZUKI....	1049

Abstracts

NIPPON NŌGEIKAGAKU KAISHI, Vol. 55, No. 3.....	A7
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contractility of PSE muscle is brought about by two basic malfunction of myosin, namely, one is the irreversible binding to actin filament³³⁾ and the other is the functional damage of the ATPase active site responsible for the formation of the phosphorylated complex, even if it is dissociable.

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