EFFECT OF TEMPERATURE ON ACTIN-MYOSIN INTERACTION DURING POSTMORTEM STORAGE OF MUSCLE[1]

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INTRODUCTION

It is well known that pH and temperature affect the quality of meat during early postmortem storage of muscle. Izumi et al. (1978) have demonstrated that the pH of muscle when rigor sets in is a critical factor in determining the intensity of the rigor tension of muscle and the intensity of rigor tension decreases with decreasing the pH. The intensity of rigor tension is probably determined by the extent of overlapping of the thin and thick filaments (Gordon et al., 1967; McGrath and Dos Remedios, 1974).

The change in the texture of muscle after slaughter is partly controlled by postmortem conditioning. Electrical stimulation is a useful method for controlling the quality of meat after slaughter (Cross, 1979). High temperature conditioning accelerates the breakdown of ATP and pH fall, resulting in the increase of tenderness (West, 1979, Marsh, 1954).

There are many extensive studies demonstrating the significant correlation between postmortem storage conditions of muscle and the properties of myofibrillar proteins (Wolfe and Samejima, 1976; Samejima and Wolfe, 1976; Yamamoto et al., 1977; Cheng and Parrish, 1978). It has been considered that (1) alteration of actin-myosin interaction, and (2) a loss of Z-line structure, and (3) degradation of myofibrillar proteins, probably due to the action of calcium activated factor (CAF) and cathepsins, and (4) degradation of collagen are the major intrinsic factors influencing the tenderness of meat after slaughter.

Recently, we have shown by investigating the double reciprocal plots of acto-heavy meromyosin (HMM) ATPase that the actin-myosin interaction increases with increasing storage time of muscle at 0°C (Ito et al., 1978). The objective of the present study was to investigate the effect of high temperature conditioning on the chemical nature of the actin-myosin interaction.

MATERIALS AND METHODS

Well-fed rabbits were anesthesized with sodium pentobarbital (90 mg) and d-tubocurrarine chloride (15 mg) in order to avoid the pH change of muscle due to starvation or exhaustion. 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 0, 2, 6, 9 and 12 hr at 37°C. Longissimus thoracis and white hind leg muscles from at-death carcasses (within 15 min after exsanguination) and aged carcasses were used in the present study.

Actin and HMM

Actin was prepared from acetone dried powder by the method of Spudich and Watt (1971), except that polymerization of G-actin was induced by dialyzing against about 100 vol of dialyzing solution containing 50 mM KCl, 0.5 mM β-mercaptoethanol overnight at 0°C. Myosin was extracted from at-death muscle with a Guba-Straub solution and also extracted from high temperature stored muscles with a modified Guba-Straub solution (0.3 M KCl, 0.15 M phosphate, 2 mM ethyleneglycol-bis-(2-aminoethylether)-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 buffer until use. HMM was prepared by the method of Lowey and Cohen (1962) and 42-58% ammonium sulfate saturated fraction was obtained as described previously (Ito et al., 1978).

pH value

About two grams of longissimus muscle was cut from carcasses and homogenized in 10 ml of solution containing 0.15 M KCl and 5 mM sodium iodoacetate, pH 7.0, using a Waring Blender. The pH value was measured with a Hitachi-Horiba F-7 pH meter.

Extractability of myofibrillar proteins

Myofibrils were prepared by the method of Briskey and Fukazawa (1971). Myofibrillar proteins were extracted from myofibrils for 15 min at 5°C with Hasselbach-Schneider solution (0.6 M KCl, 10 mM sodium pyrophosphate, 0.1 M K-phosphate, 1 mM $MgCl_2$, pH 6.4) and KI solution (0.6 M KI, 6 mM sodium thiosulfate, 2 mM β-mercaptoethanol, 0.5 mM ATP, 20 mM Tris-HCl, 7.5).

Myosin ATPase

Ca^{2+}-ATPase activity was measured under the condition of 0.25 M KCl, 20 mM Tris-HCl (pH 7.5), 10 mM $CaCl_2$, 2 mM ATP, 0.5 mg/ml myosin and the condition for determining EDTA-ATPase was 0.5 M KCl, 20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM ATP, 0.5 mg/ml myosin. The reactions were stopped by adding equal volume of 10% trichloroacetic

1. The results cited in this paper are being considered elsewhere for publication in complete form.
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acid and the inorganic phosphate liberated was determined by the method of Fiske and Subbarow (1925).

Actomyosin ATPase activity

Intact myosin from at-death muscle was added to F-actin prepared from high temperature stored muscle. The ATPase activity of the resulting actomyosin was measured in order to estimate the denaturation of actins prepared from the muscles stored at high temperature (37°C) under the following condition; 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 prepared from high temperature stored muscles.

Actin-activated HMM ATPase

The actin activated HMM ATPase activity 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 0.5 and 2.5 mg/ml with constant amount of HMM (0.2 mg/ml).

Protein concentration

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

RESULTS AND DISCUSSION

Figure 1 shows the change of pH value of muscles and the extractability of myofibrillar proteins during postmortem storage at 0 and 37°C. The pH value of longissimus muscle stored at 0°C gradually fell during 12 hr storage, while that of the muscle stored at 37°C fell rapidly and attained the ultimate pH value after 4-9 hr storage. No apparent change was observed in the extractability of myofibrillar porteins during 12 hr storage at 0°C. However, when muscle was stored at 37°C, the extractability of myofibrillar proteins did not change during the first 2 hr storage and it decreased rapidly during prolonged storage in the case of both Hasselbach-Schneider and KI solutions. It is known that a pH fall and high temperature could affect the properties of myofibrillar protein (Yasui et al., 1973; Yamamoto et al., 1979). The present result (Fig. 1) indicate that short time treatment of low pH and high temperature show no appreciable effect on the properties of myofibrillar proteins in muscle, although prolonged treatment of them induced the denaturation of myofibrillar proteins.
Synthetic actomyosin (0 hr myosin + 0-12 hr F-actin) ATPase activity was investigated in order to estimate the denaturation of F-actin. Actomyosin ATPase activity was slightly decreased with increasing storage time (Fig. 2). Figure 3 shows Ca^{2+}- and EDTA-modified ATPase activities of myosin prepared from postmortem muscles.

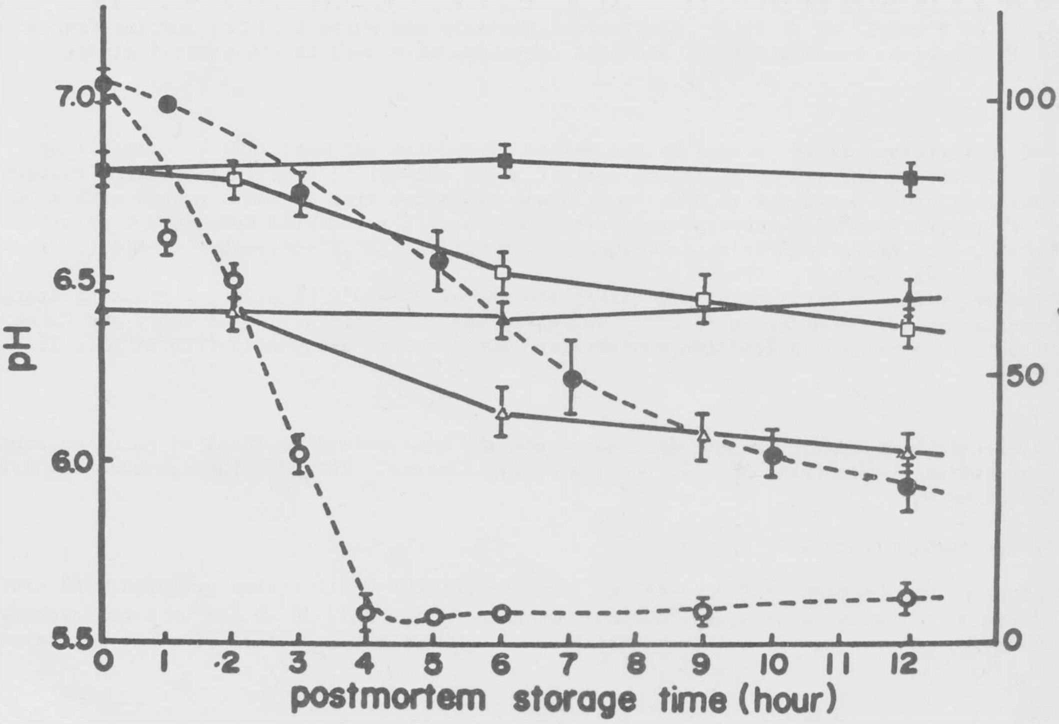

Fig. 1. Time dependent change of pH of muscle and extractability of myofibrillar proteins during postmortem storage at 0°C (closed symbol) and 37°C (Open symbol).
pH of muscle at 0°C, -●- ; pH of muscle at 37°C, -○- . Extractability of myofibrillar proteins was extressed as percentage of the total protein in myofibril suspension. ■ , 0°C-KI solution; □ , 37°C-KI solution; ▲ , 0°C-Hasselbach-Schneider solution; △ , 37°C-Hasselbach-Schneider solution. Vertical lines represent means ± S.E.M. (n=3).

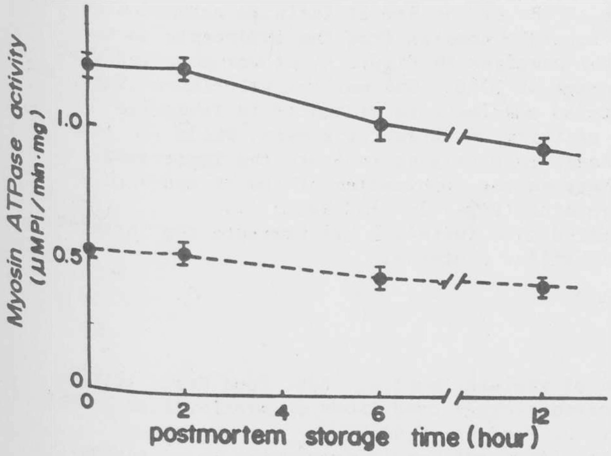

Fig. 3. Changes in Ca^{2+} and EDTA-ATPase activities of myosin during storage of muscle at 37°C.
Dotted line, Ca^{2+}-ATPase; Solid line, EDTA-ATPase. Vertical lines represent means ± S.E.M. (n=3).

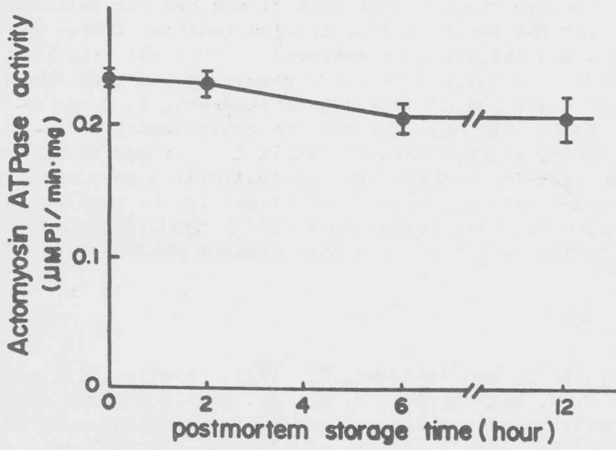

Fig. 2. Change in the ATPase activity of synthetic actomyosin prepared by mixing intact myosin with F-actin from stored muscle at 37°C.
Vertical lines represent means ± S.E.M. (n=3).

Fig. 4. Double-reciprocal plots of actin-activated HMM ATPase versus actin concentration.
Actin and HMM were prepared from at-death and high temperature (37°C) stored muscles. Acto-HMM from at-death muscle, ● ; Acto-HMM from 2 hr postmortem muscle, ▲ ; Acto-HMM from 6 hr postmortem muscle, ■ ; Acto-HMM from 12 hr postmortem muscle, ○.
Vertical lines represent means ± S.E.M. (n=3).

No appreciable change was found in ATPase activities during 2 hr storage at high temperature, but both activities decreased during prolonged storage.
Figure 4 shows the double reciprocal plots of actin activated HMM ATPase of muscle stored at high temperature for designated times. From this figure, we can calculate the ATPase of the HMM at infinite actin concentration (Vmax) and the apparent dissociation constant (Kapp) for the acto-HMM complex from the intercepts on the ordinate and abscissa,respectively. From the intercept on the abscissa in Figure 4, it was observed that the binding of actin to HMM was gradually weakened during storage at 37°C. The maximum velocities (Vmax) and apparent dissociation constant of at-death, 2, 6 and 12 hr stored muscles were presented in Table 1.
It was found that high temperature conditioning weakened the affinity of actin for myosin, while low temperature conditioning strengthened it (Table 1). In addition, the present results also indicate the importance of conditioning temperature for controlling meat quality, because the combination of low pH and high temperature greatly altered the properties of myofibrillar proteins (Figs. 1-4 and Table 1).
In conclusion, high temperature (37°C) conditioning for a limited time (within 2 hr) prevents the increase of the affinity of actin for myosin without denaturation of myofibrillar proteins.

REFERENCES

Briskey, E. J. and Fukazawa, T. 1971. Myofibrillar proteins of skeletal muscle. Adv. Food Res. 19:279.
Cheng, C. S. and Parrish, F. C., Jr. 1978. Effects of postmortem storage conditions on myofibrillar ATPase activity of porcine red and white semitendinosus muscle. J. Food Sci. 43:17.
Cross, H. R. 1979. Effects of electrical stimulation on meat tissue and muscle properties —— A review. J. Food Sci. 44:509.
Fiske, C. H. and Subbarow, Y. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375.
Gordon, A. M., Huxley, A. F. and Julian, F. J. 1966. The variation in isometric tension with sarcomere length in vertebrate muscle fibers. J. Physiol. 184:170.
Gornall, A. G., Bardawill, C. T. and David, M. M. 1949. Determination of serum protein by means of the biuret reaction. J. Biol. Chem. 177:751.
Ito, T., Sung, S. K. and Fukazawa, T. 1978. Change of acto-heavy meromyosin ATPase of rabbit skeletal muscle during postmortem storage. J. Agric. Food Chem. 26:324.
Izumi, K., Ito, T. and Fukazawa, T. 1978. Effects of pH, calcium ions and ATP on rigor contraction in glycerinated rabbit psoas muscle fiber. J. Food Sci. 43:1188.
Lowey, S. and Cohen, C. 1962. Studies on the structure of myosin. J. Mol. Biol. 4:293.
Marsh, B. B. 1954. Rigor mortis in beef. J. Sci. Food Agr. 5:70.
McGrath, P. A. and Dos Remedios, C. G. 1974. The dependence of rigor tension on sarcomere length in vertebrate muscle. Experimentia, 30:1036.
Samejima, K. and Wolfe, F. H. 1976. Degradation of myofibrillar protein components during postmortem aging of chicken muscle. J. Food Sci. 41:250.
Spudich, J. A. and Watt, S. 1971. The regulation of rabbit skeletal muscle contraction. J. Biol. Chem. 246:4866.
Tonomura, Y., Tokura, S., Sekiya, K. and Imamura, K. 1961. Influence of solvent composition on the molecular shape and enzymic activity of myosin A. Arch. Biochem. Biophys. 95:229.
West, R. L. 1979. Effects of high temperature conditioning on muscle tissue. Food Technol. 33(#4):41.
Wolfe, F. H. and Samejima, K. 1976. Further studies of postmortem aging effects on chicken actomyosin. J. Food Sci. 41:244.
Yamamoto, K., Samejima, K. and Yasui, T. 1977. A comparative study of the changes in hen pectoral muscle during storage at 4°C and -20°C. J. Food Sci. 42:1642.
Yamamoto, K., Samejima, K. and Yasui, T. 1979. Changes produced in muscle proteins during incubation of muscle homogenates. J. Food Sci. 44:51.
Yasui, T., Gotoh, T. and Morita, J. 1973. Influence of pH and temperature on properties of myosin A in glycerol-treated fiber bundles. J. Agr. Food Chem. 21:241.

Table 1. Actin-Activated HMM ATPase of at Death and Postmortem Muscles Stored at 37°C [a]

	Vmax (μ moles of Pi/min.mg of HMM)	Kapp (M)
At death muscle (0 hr)	6.67	1.79×10^{-4}
2 hr stored muscle	6.67	1.79×10^{-4}
6 hr stored muscle	13.33	3.57×10^{-4}
12 hr stored muscle	26.67	9.48×10^{-4}
24 hr postmortem muscle (at 0°C) [b]	3.70	6.80×10^{-5}
168 hr postmortem muscle(at 0°C) [b]	2.70	3.72×10^{-5}

a: The values were calculated from the plots of Figure 1. The values represent an average of three determinations.
b: Cited from Ito et al., J. Agric. Food Chem. 26:324 (1978).