Some Possible Evidences for an Alteration in the Actin-Myosin Interaction in Stored Muscle

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Received February 26, 1976

Although the exact nature of changes in myofibrillar proteins relating to meat tenderness during postmortem storage is still unknown, there have been some available informations indicating that myofibrillar proteins have undergone two main changes. One of them is the degradation of the Z-disk in myofibril,^{1,2)} which has certainly important effects on meat tenderness. This post-mortem Z-disk degradation is caused by the calcium activated protease discovered and isolated from muscle by Goll and coworkers.^{3,4)} On the other hand, another change is the modification of the actin-myosin interaction that has been supported by structural observation showing elongation of rigor-shortened sarcomeres during postmortem storage.²⁾ The modification of actin-myosin interaction in postmortem muscle has been revealed by changes in the ATPase activity of myofibril⁵⁾ and changes in dissociability of the actin-myosin complex.⁶⁾ The authors⁶⁾ have reported that less ATP is required to dissociate actomyosin prepared from stored rabbit muscle than to dissociate actomyosin prepared from rabbit muscle immediately after death. This has provided some possible evidences that post-mortem aging makes to weaken the actin-myosin interaction.

In this paper we present another possible evidence for changes in dissociability of the actin-myosin complex, which is caused by the breakdown of myosin to heavy mero-

myosin in high temperature stored muscle.

Myofibrils were prepared from fresh and stored rabbit skeletal muscle by the method of Stromer *et al.*²⁾ SDS polyacrylamide gel electrophoresis was done according to the method of Weber and Osborn.⁷⁾

Some comparisons of SDS polyacrylamide gel electrophoretic patterns of myofibrils prepared from fresh and stored muscle (at 4°C and 37°C) were shown in Fig. 1. At the

FIG. 1. SDS Polyacrylamide Gel Electrophoretic Patterns of Myofibrils from Fresh Muscle and Stored Muscle at Low and High Temperature.

All gels were 7.5% polyacrylamide. Gels were loaded with 17 μ g of protein per gel. Electrophoresis was made at current of 8 mA/tube for 3 hr.

- (a) myofibril prepared from fresh rabbit muscle.
- (b) myofibril prepared from stored rabbit muscle for 2 days at 4°C.
- (c) myofibril prepared from stored rabbit muscle for 7 days at 4°C.
- (d) myofibril prepared from stored rabbit muscle for 2 days at 37°C.
- (e) myofibril prepared from stored rabbit muscle for 7 days at 37°C.

low temperature storage there were not seen so much differences among each band of electrophoretic patterns of myofibrils with increase of the stored period. On the other hand, the high temperature-2 days storage led to a visible disappearance of 210,000-dalton band accompanied by a appearance of 150,000-dalton band and a slight appearance of 70,000-dalton band. The high temperature-

TABLE I. SOLUBILITY AND DISSOCIABILITY IN KCl-PYROPHOSPHATE SOLUTION OF MYOFIBRILS FROM FRESH MUSCLE AND STORED MUSCLE

Muscle Stored day-storage temp.	Dissociated myosin myofibril $\times 100$ (%)	P_{40-55} fraction myofibril $\times 100$ (%)
0 day	42.6 $\pm$ 9.1 ^{a)}	0.52 $\pm$ 0.13
2 days-4°C	46.1 $\pm$ 4.9	1.07 $\pm$ 0.70
7 days-4°C	46.7 $\pm$ 7.3	1.15 $\pm$ 0.69
2 days-37°C	31.7 $\pm$ 6.7	1.16 $\pm$ 0.66
7 days-37°C	25.9 $\pm$ 8.0	1.38 $\pm$ 0.59

^{a)} Figures are means or standard errors of 5 samples.

7 days storage resulted in a complete disappearance of 210,000-dalton band and 100,000-dalton band, and led to the appearance of 150,000-dalton band. The alteration of 210,000-dalton band to 150,000-dalton band presumably would result from the breakdown of myosin to heavy meromyosin,⁸⁾ and the disappearance of 100,000-dalton band would be caused by the removal of α -actinin from myofibril preparation or its enzymatic breakdown.

The solubility and dissociability of isolated myofibril in KCl-pyrophosphate solution (0.5 M KCl, 5 mM pyrophosphate, 5 mM MgCl₂, 25 mM Tris-HCl buffer, pH 7.60) were shown on Table I. After 10 min extraction myofibrils were centrifuged at 25,000 $\times g$ for 30 min. The supernatant called provisionally "dissociated myosin," which contained myosin, heavy meromyosin, subfragment-1 and light meromyosin *etc.* At low temperature storage there was seen a slight increase in the amount of dissociated myosin during storage, but at high temperature storage its amount decreased during storage. Subsequently, dissociated myosin was salted out between 40 and 55% ammonium sulfate saturation according to the method of Szent-Györgyi.⁹⁾

This salting-out protein fraction (the P_{40-55} fraction) contained mainly heavy meromyosin and subfragment-1, myosin and light meromyosin being salted out below 30% ammonium sulfate saturation.⁹⁾ Both at low and high temperature storage the amount of the P_{40-55} fraction did not so much increase during

storage.

Considering that there was only seen a very slight increase in amount of the P_{40-55} fraction, instead of a drastic alteration in SDS polyacrylamide gel electrophoretic pattern of myosin to heavy meromyosin at high temperature storage, this result may suggest that at high temperature storage, most likely an unfolding and subsequent aggregation of myofibrillar protein,¹⁰⁾ especially heavy meromyosin was occurring to decrease solubility of the P_{40-55} fraction, although the breakdown of myosin to heavy meromyosin could occur in the stored muscle.

From comparison of SDS polyacrylamide gel electrophoretic patterns of myofibrils prepared from stored muscle at low temperature with those at high temperature, we have found myosin has been progressively cleaved into heavy meromyosin. Increased extent of its cleavage paralleled with increase of stored temperature and stored period suggests the contribution of some protease active in ultimate pH range. In this respect we have recently isolated a new protease from muscle tissue which is maximally active in ultimate pH range and cleaves myosin into heavy and light meromyosin. It has certainly important effects on changes in myofibrillar proteins during postmortem storage. The details of this protease will be reported in the other paper.

A disappearance of myosin accompanied by an appearance of heavy meromyosin in myofibril from stored muscle shows an alteration of thin filament-thick filament cross-linkage,

namely, partially cleavage or weakening of actin-myosin interaction, or increased dissociability of actin-myosin (heavy meromyosin) complex. The latter is explained by the results of Eisenberg *et al.*¹¹⁾ (if not denatured).

Even if actin-myosin cross-linkages in post-mortem muscle would remain to be bound in the absence of ATP, the alteration of actin-myosin binding to actin-heavy meromyosin binding or actin-subfragment-1 binding would lead to weakening of thin filament-thick filament interaction by the breakdown in myosin side.

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