Role of Myosin Heavy Chains from Rabbit Skeletal Muscle in the Heat-induced Gelation Mechanism[†]

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Some physico-chemical and heat-induced gelling properties of myosin heavy chains (MHCs) from rabbit skeletal muscle were studied. MHCs were found to be almost devoid of ATPase activity, possibly because of the absence of myosin light chains. MHCs formed precipitates below and ionic strength of 0.3, and above this ionic strength MHCs became soluble. On heating to 65°C at low concentrations of the protein, MHCs lost their solubility and formed aggregates even at high ionic conditions. Partially irreversible change in the conformation of MHC (from helical to random coil) also occurred in heated MHCs.

The heat-induced gel strength of MHCs was found to be almost equal to that of intact myosin molecules with identical protein concentrations. This suggests that the gelling potential of myosin is solely confined in MHC. Although myosin light chains do not seem to contribute to the gelling power of myosin, possibly they provide some stability to the gel if the pH is varied above the optimum value, *i.e.*, 6.0. The addition of actin to a MHC system weakened the gel strength of the latter proportionally to the quantity of added actin.

Since the pioneering findings of Fukazawa *et al.*¹⁾ about the role of myosin in the binding properties of sausage, intensive studies^{2~8)} have been made in our laboratory to unravel the problem of which segment of the myosin molecule carries the significance in the gelling phenomenon. Efforts were directed to this aspect because myosin was found to be a much more complex molecule^{9,10)} than originally thought. Later studies^{11~15)} established that a skeletal myosin molecule consists of six subunits, which include two myosin heavy chains (MHC) and four light chains (LC) [one each of alkali light chains 1 (LC-1) and 2 (LC-2), and two DTNB-light chains].

Based on the enzymic vulnerability at different sites, the myosin molecule has also been

cleaved into different subfragments.^{16~20)} The C-terminal half of MHC which forms the double helical tail or rod is referred to as light meromyosin (LMM). On the N-terminal end, the MHC constitutes two separate heads. This domain is also called subfragment-1 (S-1) of heavy meromyosin (HMM). A flexible region between the head and tail has been designated subfragment-2 (S-2). The two heads may be identical or not. In the latter case, the heavier has been named S-1A and the lighter is called S-1B. Each contains one DTNB-light chain whereas LC-1 is present in S-1A, and LC-2 is located in S-1B.^{15,21,22)} This description holds only for myosin from white (fast) muscle. The light chain composition is different in the case of red (slow) muscle myosin.^{23~25)}

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Some earlier studies from this laboratory have reported the heat-induced gelling behavior of HMM, S-1, total rod (tail) and the LMM segment of myosin molecule.^{2~8)} The present report describes certain physico-chemical and gelling properties of MHC.

MATERIALS AND METHODS

Back and leg muscles from rabbit carcasses were dissected after slaughtering the animal, and immediately cooled in slushed ice. They were minced using a 2mm mesh sieve and myosin was extracted according to Perry's method,²⁶⁾ and then the procedure of Gaetjen *et al.*²⁷⁾ was followed for preparation of MHC from myosin.

Analytical methods. SDS-polyacrylamide gel electrophoresis (PAGE) was performed for myosin and MHC according to the method of Weber and Osborn.²⁸⁾ The electrophoretograms were scanned on a densitometer. The Ca^{2+} -ATPase activity of myosin and MHC was determined according to the method reported previously.²⁾ The changes in turbidity at 370nm were used as an indicator of aggregate formation by MHC under different ionic conditions as reported before by Samejima *et al.*⁴⁾ As described by Samejima *et al.*⁵⁾ the ORD spectra of MHC were taken under different experimental conditions to

determine the conformational changes (helicity) in the molecules. To avoid the formation of visible aggregates, the measurements were performed at pH 8.0.

Gel strength. The gel strength (rigidity) of myosin or MHC was determined according to the method of Yasui *et al.*⁷⁾

Scanning electron microscopy. The three-dimensional structure of gels was examined under a scanning electron microscope as described earlier by Samejima *et al.*⁵⁾

RESULTS

Purity of myosin and MHC

Figure 1 shows the SDS-PAGE densitogram of myosin (A) and MHC (B) indicating that the MHC preparation was essentially free from myosin light chains, and most of the regulatory proteins which are known to be associated with myosin were also not present in the MHC preparation, except a minor quantity of two unknown proteins, which were not eliminated by the method of Gaetjen *et al.*²⁷⁾

Ca^{2+} -ATPase activity

The data on the Ca^{2+} -ATPase activity of myosin and MHC are presented in Fig. 2. It shows that intact myosin molecules exhibited normal ATPase activity under appropriate conditions, but MHCs were almost devoid of any ATPase activity. This substantiates the

FIG. 1. SDS-Polyacrylamide Gel Electrophoretograms of Myosin (A) and of MHC (B) and Their Respective Densitograms.

7.5% gel was used.

FIG. 2. Ca^{2+} -ATPase Activity of Myosin (Δ) and of MHCs ($\circ$) under the Same Conditions.

ATPase activity was measured in 0.5M KCl at 25°C and 20mM Tris-HCl (pH 7.6) in the presence of 10mM CaCl_2 , 1.0mM ATP, and 0.1mg/ml of proteins.

early findings of several researchers^{15,29,30)} that the presence of LC is essential for the ATPase activity of myosin molecules.²⁹⁾

Solubility for ionic strength

Figure 3 illustrates the results on aggregation behavior of MHC at pH 6.0 and different ionic strengths as revealed by changes in turbidity. A sharp transitions in phase occurred at the ionic concentration of 0.3 M KCl. Below this ionic strength aggregates of MHC seem to form, and above 0.3 M KCl concentration MHCs acquire soluble form. This aggregation behavior of MHC as a function of salt concentration was quite similar to that generally reported for myosin monomers.^{31,32)} However, MHC aggregates which formed on heating showed no tendency to be solubilized even when the ionic strength was raised to 1.0 M KCl. This suggests that the MHC aggregates which form under the influence of thermal treatment are irreversible.

Changes in helical content

The ORD spectra in Fig. 4 reveal the changes that occurred in the helicity of MHC which is somewhat smaller than the original myosin during heating at various temperatures and on subsequent cooling. It can be seen that a significant reduction in the helical content of

MHC became apparent at temperatures above 35°C, and MHC almost lost the helical conformation at 65°C. The hysteresis in the cooling curve indicates only a partial recovery in helical content. This may be due to displacement of some side groups of amino acids during heating, which possibly do not return to their original positions on cooling, and hence the MHCs fail to acquire the initial conformation. This relates generally to aromatic amino acids, especially tryptophan which is possibly exposed to a hydrophilic environment.³³⁾ The

FIG. 4. Changes in the ORD Spectra of MHC on Heating to Different Temperatures (○) Followed by Cooling to Different Temperatures (●).

Measurements were carried out in the medium containing 2 mg/ml of MHC in 0.5 M KCl and 20 mM Tris-HCl (pH 8.0) at room temperature.

FIG. 3. Changes in Turbidity and Solubility of MHCs in Different Molarities of KCl Solution at pH 6.0.

MHC (1 mg/ml) in various KCl concentrations were measured for turbidity, and centrifuged for solubility. (○), solubility; (●), solubility after heat treatment (65°C, 20 min); (△), turbidity.

FIG. 5. The Gel Strength of Myosin (△) and MHCs (○) as a Function of Protein Concentration.

Rigidity was tested at 65°C and pH 6.0, and in 0.6 M KCl after 20 min of incubation.

mode of changes in the helicity of MHC as a result of heating was found to be very similar to that reported for myosin rods by Samejima *et al.*⁵⁾

Gelling properties of MHC

Figure 5 shows the rate of increase in heat-induced gel strength of myosin and MHC with increases in protein concentration. Both myosin and MHC formed a gel of almost equal magnitude in strength as a function of protein concentration. The log-log plot reflects only a minor difference in the slope of the lines (1.90 and 1.85, respectively, for MHC and myosin). This suggests that the gel strength potential of myosin lies solely in the MHC, and that the presence or absence of LC makes little difference in the gelling power of myosin molecules in this model system.

Early studies^{2,3,6,8)} showed that the gel strength of myosin increased exponentially by 1.7 to 2.0 power of the protein concentration depending on ionic strength, pH and presence or absence of actin in the system.

Effects of temperature

On heating to 35°C, MHC formed a very weak gel (Fig. 6a), but with further increases in temperature up to 60°C the gel strength in-

creased almost exponentially. Those changes were similar to those reported by Yasui *et al.*⁷⁾ for myosin. Heating beyond 60°C tended to weaken the gel structure of MHC. The inset in Fig. 6a is the differential plot of rigidity *versus* temperatures (dR/dT) which shows two peak temperatures (42 and 55°C), indicating two transition phases that took place in MHC during gel formation.

The changes in the turbidity curve of MHC as a function of temperature (Fig. 6b) substantiate the occurrence of two transitions in MHC during heating. The first transition, which commenced at about 35°C and was completed at 40°C was relatively sharp, whereas the second transition occurred rather gradually in the temperature range between 40 to 60°C. The former possibly represents the loss of α -helix and destruction of electrostatic and hydrophilic interactions³⁴⁾ accompanied by the formation of aggregated MHC, and in latter phase head-to-head interaction might be taking place.⁵⁾ Differential scanning calorimetric studies on myosin have shown one to three transitional temperatures depending on the ionic strength and pH value of the system.³⁵⁾ Different researchers^{36~39)} have given different interpretation of those transitions in the myosin molecules during heating. However, myo-

FIG. 6. a: Influence of Heating Temperature on the Gel Strength of MHCs.

b: Effects of Heating Temperature on the Solubility (○) and Turbidity (Δ) of MHCs.

Protein concentrations were 4.5 mg/ml for (a) and 2.0 mg/ml for (b). Other conditions were the same as in Fig. 5.

(Inset): Derivative Plots as a Function of Temperature. Differential rigidity (dR/dT) of the system at pH 6.0 was plotted against temperature.

sin has been found to exhibit two transitional temperatures^{6,40)} under similar conditions as observed for MHC in the present study. This lends support to the proposition of Locker,⁴¹⁾ that only MHCs coagulate, while light chains dissociate and solubilize during the heating of myosin.

Effects of actin

The data in Fig. 7 show that MHC alone produces the strongest gel (20×10^2 dyn/cm²) at ionic strength 0.6 and pH 6.0. When a part of the MHC content was replaced by actin in the model system, the gel strength of the resulting mixture decreased proportionally with increasing actin content in the system. This behavior of MHCs' gel seems at variance with earlier findings of Fukazawa *et al.*¹⁾ and Yasui *et al.*⁸⁾ that myosin produced the strongest gel at a myosin to actin ratio of 15 (w/w). However, this observation agrees with the conclusion of Ishioroshi *et al.*³²⁾ who had reported that at low ionic conditions the presence of actin weakens the gel strength of myosin. The possible mechanism by which actin increases the gel strength of myosin at high ionic strength has been suggested by yasui *et al.*⁸⁾ In a low ionic strength system, apparently it seems that actin when present possibly interferes with head-to-head interactions, thus the resulting myosin gel is likely to be weak.

Effects of pH

Figure 8 shows the influence of the pH value of the system on the strength of the gel formed by MHC and myosin at 0.6 M KCl concentration on heating to 60°C. It can be seen that the pH optima for maximum rigidity of both gels are almost identical. However, MHCs' gel appears to be more sensitive to variations in pH than that of myosin gel at pH above 6.0. While there was gradual weakening of the structure of myosin gel with increase in pH above 6.0, MHC gel showed an abrupt collapse of structure under those conditions. These differences in gel behavior suggest that perhaps the presence of light chains provides some stability to heat-induced gel on the al-

FIG. 7. Effect of MHC to Actin Ratio (w/w) on Rigidity of the Resulting Gel.

Total protein concentration in the mixture was 5 mg/ml and other conditions were the same as in Fig. 5.

FIG. 8. Effects of Variation in pH Value of the System on the Rigidity of Myosin (Δ) and MHC ($\circ$) Gel.

Conditions were the same as in Fig. 6 except pH.

kaline side of the isoelectric point of myosin, and that syneresis of the gel may occur in the absence of light chains at high pH value.

Scanning electron microscopy

Scanning electron microscopic observations on heat-set gels of myosin and MHC formed at 65°C in 0.6 M KCl (pH 6.0) are shown in Fig. 9. The network structure with globular projections of myosin (A) is also shown similarly in the MHC sample (B). These similar microstructures between myosin and MHC correspond to the results of their rigidity (Fig. 5).

FIG. 9. Scanning Electron Micrographs for Heat-Set Gels of Myosin (A) and MHC (B) Formed at 65°C in 0.6 M KCl.

Bar length is 0.2 μ m.

DISCUSSION

Extensive studies in various laboratories have established the role of myosin in the binding property of restructured meat and meat products.^{1~8,42~46)} However, as already pointed out in the introductory section of this paper, the myosin molecule is a very complex

entity. This complexity aroused the interest of Yasui and his co-workers^{2~8)} in exploring the contribution of different segments of myosin molecule in the heat-induced gelation phenomenon. For this, the myosin molecules were enzymatically cleaved into various subfragments such as total rods, LMM, HMM, S-1, and S-2 under specified conditions, and their

heat-induced gel strength potential was determined in different model systems. These investigations revealed that S-1 fragments formed a very weak gel as compared to that formed by intact myosin molecules. The gels formed by total rods and LMM were intermediate in strength. The fact that disintegrated myosin molecules were unable to form gels of equivalent strength to that produced by intact myosin molecules, suggests that the integrity of the myosin molecule is essential in determining the binding strength on heating.

All these studies indeed revealed several interesting features of gelling behavior of myosin subfragment. However, it was not clear whether or not the myosin light chains, which play an indispensable role in the physiology of muscle,^{29,47,48} have any functionality in the gelation mechanism of thermally processed restructured meat products. On the other hand, Siegel and Schmidt⁴⁶ reported that MHC of myosin molecule played an important role in the binding of meat pieces. The present study not only supports their view but also provides an indirect answer to the above question. It seems that myosin light chains carry no significance in the heat-induced gelation phenomenon of myosin, and that the whole gel strength potential of myosin lies in the myosin heavy chains. However, light chains seem to provide some stability to the myosin gel if pH varies above the optimum value.

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