A:28

Heat-Induced Gelation of Myosin: Roles of Head and Tail Regions of Myosin Molecule

K. Samejima, M. Ishioroshi* and T. Yasui*

Dept. of Dairy Science, The College of Dairying, Ebetsu, Hokkaido, Japan and *Dept. of animal Science, Faculty of Agriculture, Hokkaido University, Sapporo, Hokkaido, Japan

Introduction

Fukazawa et al. (1961) showed myosin to be a key constituent with respect to the desirable binding quality and water-holding capacity in the experimental sausages. In 1969, Samejima et al. reported that of the myofibrillar proteins, only myosin has the ability to influence the heat-induced gelation of model systems. They also suggested that the entire myosin molecule is required to develop desirable gel strengths.

The use of isolated myosin fractions as meat binders has recently studied by Macfarlane et al. (1977), Ford et al. (1978) and Siegel and Schmidt (1979 a,b), and the latter authors suggested the importance of the myosin heavy chain in binding ability since the higher proportion of myosin resulted in a higher binding ability.

From the studies cited above it is apparent that myosin which forms more than 50 % of the myofibrillar proteins play an important role in the quality of processed meat with respect to the binding properties. Thus, the phenomenon of the gelation of myosin has been studied by our laboratory for several years (Yasui et al., 1979 and 1981, Ishioroshi et al., 1979 and 1980 and Samejima et al., 1980 and 1981).

To determine the mechanism of the heat-induced irreversible gelation of skeletal muscle myosin, we have attempted to separate the subfragment 1 (S-1) and the myosin rod which represent the major characteristic properties of myosin. The roles of these subfragments on the heat-induced gelation of myosin molecules was studied by the measurements of rigidity, turbidity by the scanning electron microscopic observation of the ultrastructure of the thermally induced gels.

Materials and Methods

Rabbit skeletal muscle myosin was prepared according to the method described earlier (Yasui et al., 1979) from M. longssimus thorasis and hind leg muscles. Chymotryptic S-1 was made from insoluble myosin (0.12 M KCl) filaments by the method of Weeds and Pope (1977) in the presence of 1 mM EDTA and 1 mM DTT. From the insoluble residue of the S-1 preparation, myosin rod was prepared using the ethanol fractionation procedure previously described by Samejima et al. (1976).

Measurements of rigidity of heat-induced gelation were carried out with a band type viscometer reported previously (Yasui et al., 1979).

Turbidity was measured at 370 nm in 1 cm cuvettes. The optical density changes were followed using the spectrophotometer (Hitachi type 323). Solutions contained 0.6 mg/ml of myosin, S-1 or rod, 0.6 M KCl and 20 mM phosphate buffer (pH 6.0).

Determination of free sulfhydryl groups in protein samples was performed using the procedure of Elman as reported previously (Ishioroshi et al., 1979). For determining the total number of thiols, the proteins were denatured in saturated urea. The molecular weights used were 120,000 for S-1, 220,000 for the rod (Weeds and Pope, 1977), and 480,000 for myosin (Tonomura, 1972).

The measurements of ORD and CD were carried out with a JASCO ORD/UV-5 recording spectropolarimeter equipped with a CD attachment as described by Samejima et al. (1976).

Scanning electron microscopic observations were made on the heat-induced gel of protein solutions using a JEOL JSM-T 200 scanning electron microscope at 15 KV described previously (Yasui et al., 1979).

Results

Fig. 1 shows the heat-treated S-1 (a) and rod (b) solutions under the conditions of 0.6 M KCl, 40 mM phosphate buffer (pH 6.0 or 7.0). Under similar conditions, myosin and myosin B solutions formed heat-gels which remained in the test tube when inverted (Trautman, 1966 and Samejima et al., 1969). S-1 forms an infirm gel at fast stage of heating, but the gel releases water with the time as shown in Fig. 1 (a). Myosin rod forms a stable heat-gel and remains in the inverted tube (Fig. 1 (b)).

7-10 mg/ml of myosin rod or S-1 in 0.5 M KCl pipetted into 5 mm diameter glass test tubes were incubated in a water bath at 60 °C for 30 min. The results of the tensile strength of these gels are indicated in Table 1. Myosin heat-gel shows the highest tensile strength value (0.5 g/cm^2) in comparison with its proteolytic fragments upon heating in the presence of salt. The tensile strength of S-1 sample-gel, however, can not be detected by such apparatus under similar conditions, because it did not make heat-gel though it underwent a thermally induced coagulation. The tensile strength for heat-gel of myosin rod is 0.2 g/cm^2, and that for the mixture of S-1 and rod (at identical protein concentration with that of myosin) is 0.25 g/cm^2.

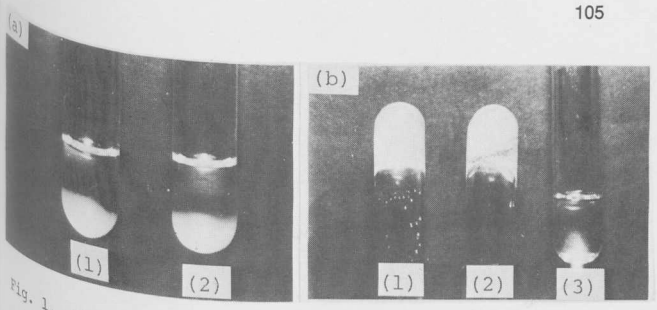

Fig. 1. Heat-Treated S-1 and Rod Solutions.
Protein solutions were heated at 60 °C for 30 min.
(a) S-1 (10 mg/ml) (1):Control (pH 6.0) , (2): with 1 mM DTT
(b) Rod (10 mg/ml) (1): Control, (2): with 1 mM DTT (pH 6.0)
(3): pH 7.0 without DTT.

Table 1. Tensile Strength of Myosin and Its Subfragments.

The measurements were carried out with the RHEO METER type PUD-J (Fuji Rika Kogyo CO.,Japan) under the same conditions of Fig. 1.

Myosin (5 mg/ml)	0.5 (g/cm^2)
S-1 (")	N.D.
Rod (")	0.2
Rod + S-1 (")	0.25

The effect of temperature on the gel formability as expressed by the rigidity indicates that myosin shows the highest value, while the S-1 show the lowest at 65 °C (Fig. 2 (a)). The rod exhibited an intermediate value. It is notable that the heat-induced gelation of both myosin and the S-1, is inhibited by the presence of 1 mM DTT, whereas that of the rod is not affected by 1 mM DTT (Fig. 2 (a)).

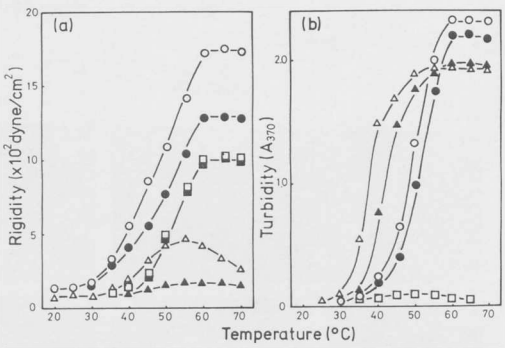

Fig. 2. Changes in Rigidity (a) and Turbidity (b) of Thermally Treated Myosin, S-1 and Rod in The Presence (closed symbols) and Absence (open symbols) of 1 mM DTT.

○,● : Myosin, △,▲: S-1, □,■ :Rod

Turbidity changes during the course of stepwise heating in the basal media containing 0. 6 M KCl and at pH 6.0 and protein at 0.6 mg/ml were followed by measuring absorbance at 370 nm. The S-1 exhibits a rapid and remarkable increase in turbidity starting at 25 °C. Myosin shows a similar change, although initiation of its turbidity change take place at 30 °C (Fig. 2 (b)). However, the rod does not demonstrate a significant increase in turbidity throughout the heating process. Addition of 1 mM DTT to each system retarded the occurrence of turbidity increases at lower temperatures (25-40 °C) for the myosin and the S-1, but had a lesser effect on the final extent of turbidity reached at higher temperatures (60-70 °C)(Fig. 2 (b)).

Fig. 3 presents typical SH content versus temperature profiles for myosin, S-1 and rod under conditions similar to those in Fig. 2. During stepwise heating to 70 °C, the SH content of myosin and S-1 gradually decreased from 9.3 to 7.9 moles/10^5 g and 11.2 to 7.3 moles/10^5 g, respectively, but the rod exhibited no change throughout the heating process. These results, together with the effects of DTT on the gel formability and turbidity of the proteins tested (Figs. 2 (a) and (b)), may confirm the involvement of oxidation of SH groups in the aggregation phenomena of myosin and S-1 during heating. On the other hand, the heat-induced gelation of the rod seems to occur independently of the oxidation reaction of SH groups.

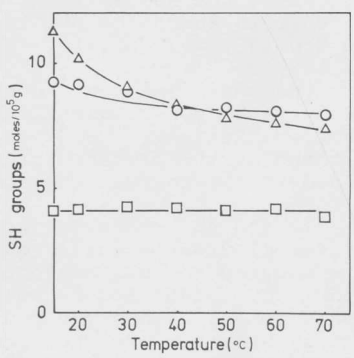

Fig. 3. Changes in The Sulfhydryl Contents of Thermally Treated Myosin (○), S-1 (△) and Rod (□).

Fig. 4 demonstrates a typical helical content versus temperature profile for the rod. The open square symbols in Fig. 4 represent changes in rigidity of the rod shown in Fig. 2 (a). The profiles of the helix-coil transition of the rod as a function of temperature entirely correspond to the changes in the rigidity.

The scanning electron micrographs clearly indicate that an extended network system prevails in the gels of myosin (Fig. 5 (a) and the rod (Fig. 5 (b)), whereas bead-like protein aggregates were observed with the precipitates of the S-1 (Fig. 5 (c)). Mixtures (Fig. 5 (d)) of the S-1 and the rod containing each protein at the same molar ratio as in whole myosin revealed morphological characteristics similar to those of the rod alone (Fig. 5 (b)).

Disccusion

Skeletal myosin molecules are comprised of six polypeptide chains: two heavy chains which run throughout the length of the molecule and form the characteristic coiled coil tail, two identical phosphorylatable light chains probably located near the "hinge" region between the tail and the two heads, and two different alkali light chains which reside in the head regions (Bagshaw, 1977 and Weeds and Pope, 1977). Isolated head regions (S-1) each containing a portion of heavy chain and a single alkali light chain and the tail region (rod) can be prepared by chymotryptic digestion of insoluble myosin filaments (Weeds and Pope, 1977). S-1 preparations retain the ATPase and actin binding properties of myosin and the rod retains the solubility and filament forming properties.

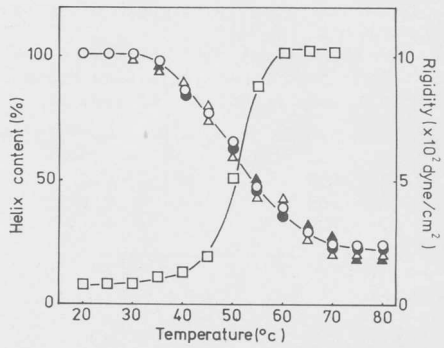

Fig. 4. Temperature Profile of The Rod Obtained from ORD and CD measurement.

The temperature of the protein solutions, which contained 0.5 mg/ml of the rod in 0.6 M KCl with 20 mM phosphate (pH 6.0) in the presence (closed symbols) or absence (open symbols) of 1 mM DTT, was raised stepwisely.

□ : rigidity of rod shown in Fig. 2 (a)
○ : ORD
△ : CD

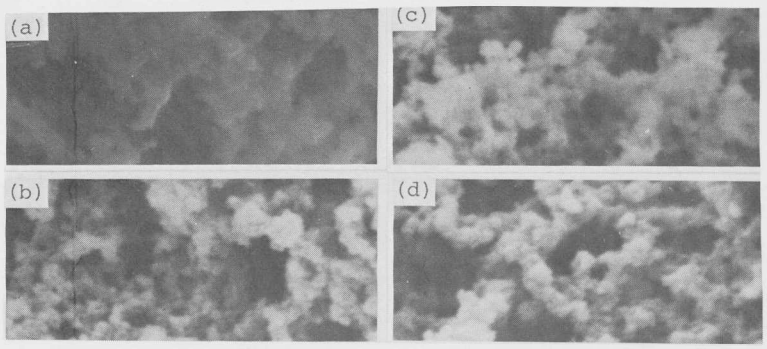

Fig. 5. Scanning Electron Micrographs of Myosin, S-1 and Rod.

(a): Myosin, (b): Rod
(c): S-1, (d): Mixtures of the S-1 and the rod.

The effects of temperature on rigidity indicate that the heat-induced gelation of S-1 differs from the heat-induced gelation of myosin. However, the heat-induced gelation of myosin rod resembles that of intact myosin (Fig. 2 (a)). Electron micrographs of heat-induced gel samples indicate that the rod forms a three dimensional network system like myosin, whereas the S-1 simply aggregates upon heating (Fig. 5). Simply mixing the rod with the S-1 failed to restore the heat-induced gel formability to that of myosin (Table 1 and Fig. 5 (d)), suggesting the necessity of a whole molecule (Samejima et al., 1969) or heavy chain of the molecule in which the S-1 is connected through "hinge region" covalently with the rod (Siegel and Schmidt, 1977 b), for the full development of the heat-induced gelation.

The bonds between the binding sites created by the helix-coil transition (Fig. 4) are likely to be non-covalent in nature, because there seems to be no participation of the SH-SS reaction (Fig. 3). However, the heat-induced gelation of myosin and the S-1 are appreciably affected by the presence of DTT (Fig. 2 (a)), indicating that oxidation of SH groups is involved in the thermal aggregation of the head portion of myosin molecules.

In the sol state the rod chains have 100 % helical conformation, but when a number of helix-coil transitions (Fig. 4) sufficient to provide cross-links for a continuous network have occured the sol converts to a gel. The unfolding tends to keep the protein in the random coil state and hence to prevent the gel from becoming progressively more tightly cross-linked. Thus the rod forms gels, which have optical clarity, are elastic and do not show syneresis. On the other hand, in the case of intact myosin chains with two heads the gel becomes progressively more tightly cross-linked and hence more rigid (Fig. 2 (a)), as the S-1 portions associate to form aggregates which act as "super-junctions". This binding can provide extra cross-linkages for the framework. When aggregates are large and numerous enough, the gel may lose optical transparency.

Summary

Myosin and the myosin rod formed gels which are firm enough to remain in the test tube when inverted, but the S-1 showed very poor gelation upon heating. The scanning electron micrographs indicated that an extended network system prevails in the gels of myosin and the rod, whereas bead-like protein aggregates were observed with the precipitates of the S-1. The rigidity profiles of rod showed essentially the same as those of myosin, but those of S-1 were different from myosin. The S-1 exhibited a rapid and remarkable increase in turbidity starting at 25 °C. Myosin showed a similar change, although initiation of its turbidity change took place at 30 °C. However, the rod did not demonstrate a significant increase in turbidity throughout the heating process. The changes in SH content of myosin and S-1 during heating indicated that the oxidation of SH groups was involved in their aggregation phenomena. On the other hand, the heat-induced gelation of the rod seemed to occur independently of the oxidation reaction of SH groups.

From the results obtained in the present study, we concluded that two features of the heat-induced gelation of myosin, aggregation and three dimensional network formation were

formed to be imparted by the S-1 and the rod, respectively. The former involves SH-SS reaction and the latter relates to conformational changes arising from a partially irreversible helix-coil transition during heating.

References

Bagshow, C. R. , Biochemistry 16:59-67 (1977).
Ford, A. L., Jones, P. N., Macfarlane, J. J., Schmidt, G. R. and Turner, R. H., J. Food Sci. 43:815-818 (1978).
Fukazawa, T., Hashimoto, Y. and Yasui, T., J. Food Sci. 26: 541-549 (1961).
Ishioroshi, M., Samejiam, K. and Yasui, T., J. Food Sci. 44: 1280-1284 (1979).
Ishioroshi, M., Samejima, K., Arie, Y. and Yasui, T., Agric. Biol. Chem. 44:2185-2194 (1980).
Macfarlane, J. J., Schmidt, G. R. and Turner, R. H., J. Food Sci. 42:1603-1605 (1977).
Samejima, K., Hashimoto, Y., Yasui, T. and Fukazawa, T., J. Food Sci. 34:242-245 (1969).
Samejima, K., Takahashi, K. and Yasui, T., Agric. Biol. Chem. 40:2455-2464 (1976).
Samejima, K., Ishioroshi, M. and Yasui, T., 26th European Meeting of Meat Research Workers. at Colorado Springs. 45-48. (1980).
Samejima, K., Ishioroshi, M. and Yasui, T., J. Food Sci. in press (1981).
Siegel, D. G. and Schmidt, G. R. , J. Food Sci. 44:1129-1131 (1979 a).
Siegel, D. G. and Schmidt, G. R. , J. Food Sci. 44:1686-1689 (1979 b).
Tonomura, Y., Muscle Proteins, Muscle Contraction and Cation Transport, University of Tokyo Press, Tokyo. (1972).
Trautman, J. C., J. Food Sci. 31: 409-418 (1966).
Weeds, A. G. and Pope, B., J. Mol. Biol. 111: 129-157 (1977).
Yasui, T., Ishioroshi, M., Nakano, H. and Samejima, K., J. Food Sci. 44: 1201-1204 (1979).
Yasui, T., Ishioroshi, M. and Samejima, K., J. Food Biochemistry in press (1981).