

Rheological properties of heat-induced gels of myosin solubilized in a low ionic strength solution containing L-histidine

Yuri Yoshida¹, Toru Hayakawa¹, Junichi Wakamatsu¹, Tomohito Iwasaki², Isamu Kaneda², and Takanori Nishimura¹

¹ Meat Science Laboratory, Graduate School of Agriculture, Hokkaido University, N-9, W-9, Kita-ku, Sapporo, 060-8589, Japan

² Faculty of Dairy Science, Rakuno Gakuen University, 582 Midoricho Bunkyo-dai, Ebetsu, 069-8501, Japan

Abstract— Myosin is generally considered to be insoluble in a low ionic strength solution. However, we have shown that myosin is solubilized in a low ionic strength solution containing L-histidine. To clarify its processing characteristics, we investigated rheological properties and structures of heat-induced gels of myosin solubilized in a low ionic strength solution. Myosin in a low ionic strength solution containing L-histidine formed transparent gels at 40-50°C, while myosin in a high ionic strength solution formed opaque gels at 50°C or more. The former showed a fine network consisting of thin strands, and the latter showed a coarse and porous network by SEM. Dynamic rheological analysis showed that the storage modulus of myosin in a low ionic strength solution increased drastically from 45°C up to 50°C, while that in a high ionic strength solution increased drastically from 45°C up to 75°C. The loss tangent of heat-induced gel of myosin in a low ionic strength solution was 0.23, while that of myosin in a high ionic strength solution was 0.08. The heat-induced gel of myosin in a low ionic strength solution is a “weak gel” and less elastic than that of myosin in a high ionic strength solution.

Keywords— myosin, gelation, rheological properties

I. INTRODUCTION

Water-holding capacity and binding properties are important for meat products. These properties are substantially derived from the heat-induced gelation of myosin, the major protein of myofibrils (Fukazawa et al., 1961). Myosin molecules aggregate to form filamentous polymers under the physiological condition. While myosin exists as monomers in the high ionic condition above 0.3. Myosin forms gels by heating under appropriate conditions regardless of its state, monomeric or filamentous form. However, there is discernible difference between heat-induced gels of monomeric myosin after heating (Ishioroshi et al.,

1979). Furthermore, the rigidity of heat-induced gels of filamentous myosin depends on the length of the filament before heating (Yamamoto et al., 1988). These results suggest that properties of heat-induced gels depend on the state of myosin molecules before heating.

We have investigated the solubilization of myofibrillar proteins in water or a low ionic strength solution (Ito et al., 2003), in order to use myofibrillar proteins in various ways, such as a liquid diet for elderly people. Recently, we demonstrated that myosin molecules disperse as monomers and are solubilized in a low ionic strength solution containing L-histidine (Hayakawa et al., 2009). The rod of myosin in a low ionic strength solution containing L-histidine was longer than that in a high ionic strength solution. Furthermore, we demonstrated the elongation of light meromyosin in a low ionic strength solution containing L-histidine (Hayakawa et al., 2010). These results suggest structural changes in the helical tail portion of myosin in a low ionic strength solution containing L-histidine.

The heat-induced gelation of myosin occurs by two steps; the first one is the aggregation of the globular head portion of myosin and the second one is the formation of three-dimensional network by the helical tail portion (Samejima et al., 1981). Thus, we hypothesized that monomeric myosin in a low ionic strength solution containing L-histidine might have different gelation properties from that in a high ionic strength solution. In this study, we investigated rheological properties and structures of heat-induced gels of myosin solubilized in a low ionic strength solution containing L-histidine in order to clarify its processing characteristics.

II. MATERIALS AND METHODS

A. Preparation of myosin

Myosin was prepared from chicken breast muscle according to the method of Perry (1955). Briefly, minced muscle was extracted with modified Guba-Straub solution (0.3 M KCl, 0.05 M EDTA, 0.1 M KH_2PO_4 , 0.05 M K_2HPO_4) for 15 min and centrifuged at 1,200g for 10 min. The supernatant was diluted with 14 volumes of cold distilled water and centrifuged at 2,500g for 10 min. The precipitate was dissolved in 0.3 M KCl, pH 7.0 and ultracentrifuged at 100,000g for 60 min. The supernatant was diluted with 9 volumes of cold distilled water and centrifuged 1,200g for 30 min. The precipitate was dissolved in 0.6 M KCl, pH 6.5 and dialyzed against the same solution. After dialysis, the solution was ultracentrifuged at 180,000g for 120 min. The obtained supernatant was used as myosin.

B. Solubilization of myosin in a low ionic strength solution

Myosin was solubilized in a low ionic strength solution containing L-histidine by dialysis against a solution of 1 mM KCl and 5 mM L-histidine for 48 hours at 4°C. The dialyzed myosin suspension was ultracentrifuged at 100,000g for 120 min, and the supernatant was defined as 'solubilized myosin' in a low ionic strength solution containing L-histidine.

C. Preparation of heat-induced gel

Myosin solutions (2-6 mg/ml) were placed in test tubes, and the tubes were heated for 10 min at 30, 40, 45, 50, 60 and 70°C in a water bath.

D. Scanning electron microscopy

Heat-induced myosin gels were fixed in 2.5% glutaraldehyde containing 0.1 M phosphate buffer, pH 7.2 for 3 days. The gels were rinsed three times with distilled water for 15 min, cut out into 5-mm cubes and post fixed in 1% OsO_4 overnight. The specimens were dehydrated in a series of graded concentration of ethanol and dried by the t-butyl alcohol freeze-drying method. The dried specimens were mounted on aluminum stubs, coated with gold and observed under

a scanning electron microscope (S-800, Hitachi, Japan) with an accelerating voltage of 10 kV.

E. Measurement of dynamic viscoelasticity

The viscoelastic characteristics of myosin were continuously monitored during heating with a rheometer (Carri-med, TA Instruments, USA). Myosin solutions (2-6 mg/ml) were placed between two plates (40 mm in diameter) and were heated at the rate of 2°C/min from 25°C to 75°C. The measurements were carried out at a fixed frequency of 1 Hz. The dynamic viscoelasticity frequency sweep measurements (1-100 rad/sec) were performed in a controlled-strain rheometer (ARES, TA Instruments, USA). Rheological tests were carried out at 45°C for myosin in a low ionic strength solution and 70°C for myosin in a high ionic strength solution.

III. RESULTS AND DISCUSSION

First, we examined the gel formation ability of myosin solubilized in high (0.6 M KCl) and low (1 mM KCl) ionic strength solutions. The myosin in a high ionic strength solution (0.6 M KCl) formed opaque gels at 50°C or higher in the concentration range above 3 mg/ml (Fig. 1A). On the other hand, myosin in a low ionic strength solution containing L-histidine formed transparent gels at 40-50°C in the concentration range above 4 mg/ml. At 60°C or more, myosin in a low ionic strength solution containing L-histidine did not form a gel (Fig. 1B). These results suggest that the gelation properties of myosin in a low ionic strength solution containing L-histidine are different from those of myosin in a high ionic strength solution. Next, we investigated the ultrastructures of these gels by scanning electron microscopy. When myosin in a high ionic strength solution was heated to 70°C, the gel showed a coarse and porous network (Fig. 2A). On the other hand, when myosin in a low ionic strength solution with L-histidine was heated to 45°C, the gel showed a fine network consisting of thin strands (Fig. 2B).

We investigated the dynamic rheological properties of the myosin by measuring the storage modulus (G') and the loss modulus (G'') during heating. The storage

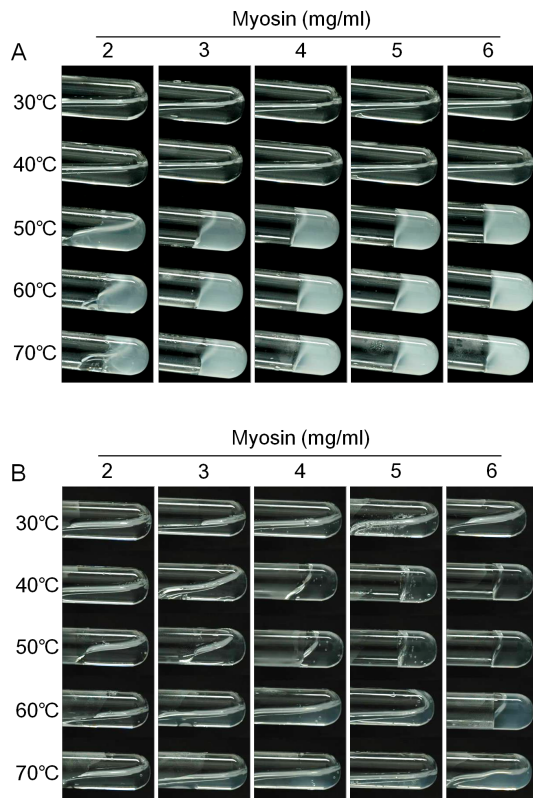


Fig. 1. Effect of heating on gelation of myosin. Myosin solutions (2-6 mg/ml, 1 ml) were placed in test tubes, and the tubes were heated for 10 min at 30, 40, 50, 60 and 70°C in a water bath. (A) Myosin solubilized in a high ionic strength solution (0.6 M KCl). (B) Myosin solubilized in a low ionic strength solution (1 mM KCl) containing L-histidine.

modulus (G') and the loss modulus (G'') indicate the elasticity and the viscosity of materials, respectively. The G' of myosin in a high ionic strength solution increased drastically from 45°C up to 70°C (Fig. 3A). While, the G' of myosin in a low ionic strength solution containing L-histidine increased up to 43°C and decreased until 50°C (Fig. 3B). Furthermore, we examined the frequency-sweep test to determine dynamic viscoelasticities at 45°C and 70°C where the G' values of myosin were the maximum in low and high ionic strength solutions respectively. When myosin in a high ionic strength solution heated at 70°C, the G' and G'' did not depend on the frequency, and the G' was higher than G'' in all the frequency range examined (Fig. 4A). On the other hand, when myosin in a low ionic strength solution

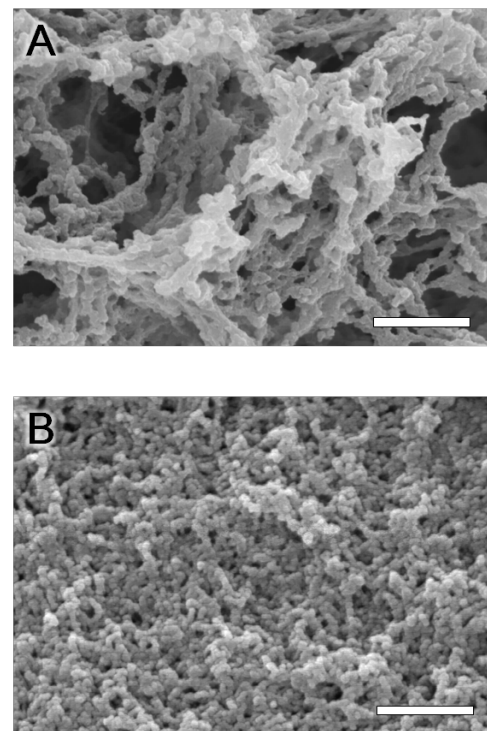


Fig.2. Scanning electron micrographs of heat-induced gel of myosin. Myosin solutions (5 mg/ml) were heated (A: 70°C; B: 45°C) for 10 min, and myosin gels were observed by scanning electron microscopy. (A) Myosin solubilized in a high ionic strength solution (0.6 M KCl). (B) Myosin solubilized in a low ionic strength solution (1 mM KCl) containing L-histidine. Bars are 1.0 µm.

containing L-histidine heated at 45°C, the G' and G'' increased gradually with frequency, and the G' was higher than G'' in all the frequency range examined (Fig. 4B). The loss tangent (G''/G') of heat-induced gels of myosin in a low ionic strength solution was 0.23, indicating a weak gel. While, the G''/G' of heat-induced gels of myosin in a high ionic strength solution was 0.08, indicating an elastic gel.

IV. CONCLUSIONS

Myosin in a low ionic strength solution containing L-histidine forms gels at the lower temperature compared to myosin in a high ionic solution. The heat-induced gel of myosin in a low ionic strength solution containing L-histidine is a “weak gel” and less elastic

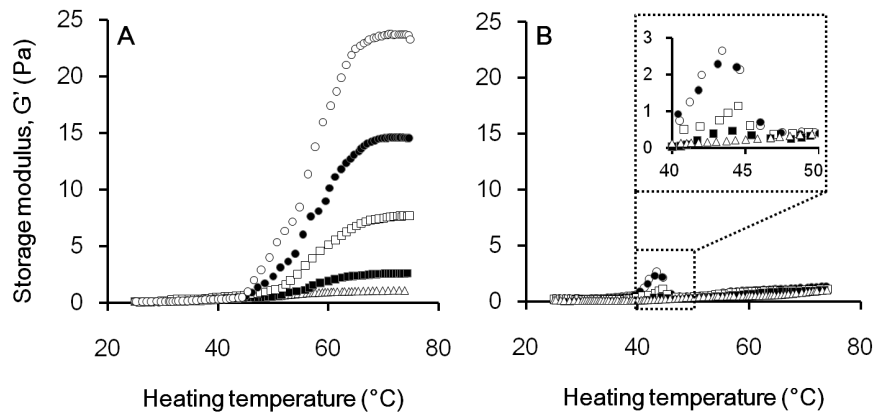


Fig. 3. Changes in storage modulus (G') of myosin during heating. (A) Myosin solubilized in a high ionic strength solution (0.6 M KCl). (B) Myosin solubilized in a low ionic strength solution (1 mM KCl) containing L-histidine. $\circ$: 6 mg/ml; $\bullet$: 5 mg/ml; $\square$: 4 mg/ml; $\blacksquare$: 3 mg/ml; $\triangle$: 2 mg/ml.

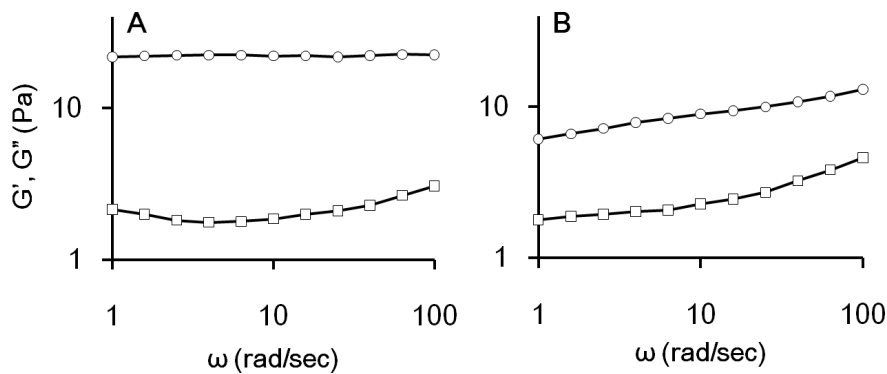


Fig. 4. The dynamic viscoelasticity frequency sweep measurements (1-100 rad/sec) of myosin. (A) Myosin solubilized in a high ionic strength solution (0.6 M KCl). (B) Myosin solubilized in a low ionic strength solution (1 mM KCl) containing L-histidine. $\circ$: Storage modulus (G'); $\square$: loss modulus (G'').

than that of myosin in a high ionic strength solution. In the previous paper, we showed that myosin in a low ionic strength solution containing L-histidine has a longer rod than myosin in a high ionic strength solution (Hayakawa et al., 2009). The structural change of the rod region of myosin in a low ionic strength solution containing L-histidine would affect the interaction between rods during heating, resulting in a weak and transparent gel with a fine network of myosin.

V. REFERENCES

1. Fukazawa T, Hashimoto Y, Yasui T. (1961) Effect of some proteins on the binding quality of an experimental sausage. *Journal of Food Science*, 5: 541-549.
2. Ishiorosi M, Samejima K., Yasui T. (1979) Heat-induced gelation of myosin: factors of pH and salt concentrations. *Journal of Food Science*, 44: 1280-1284.
3. Yamamoto K., Samejima K., Yasui T. (1988) Heat-induced gelation of myosin filaments. *Agricultural and Biological Chemistry*, 52: 1803-1811.
4. Ito Y, Tatsumi R, Wakamatsu J, Nishimura T, Hattori, A. (2003). The solubilization of myofibrillar proteins of vertebrate skeletal muscle in water. *Animal Science Journal*, 74: 417-425.
5. Hayakawa T, Ito T, Wakamatsu J, Nishimura T, Hattori, A. (2009). Myosin is solubilized in a neutral and low ionic strength solution containing L-histidine. *Meat Science*, 82: 151-154.
6. Hayakawa T, Ito T, Wakamatsu J, Nishimura T, Hattori A. (2010). Myosin filament depolymerizes in low ionic strength solution containing L-histidine. *Meat Science*, 84: 742-746
7. Samejima K, Ishioroshi M, Yasui T. (1981) Relative roles of the head and tail portions of the molecule in heat-induced gelation of myosin. *Journal of Food Science*, 46: 1412-1418.