Meat Science 90 (2012) 77–80

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Heat-induced gelation of myosin in a low ionic strength solution containing L-histidine

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ARTICLE INFO

Article history:
Received 15 December 2010
Received in revised form 10 May 2011
Accepted 1 June 2011

Keywords:
Myosin
L-histidine
Heat-induced gelation
Binding properties

ABSTRACT

Binding properties are important for meat products and are substantially derived from the heat-induced gelation of myosin. We have shown that myosin is solubilized in a low ionic strength solution containing L-histidine. To clarify its processing characteristics, we investigated properties and structures of heat-induced gels of myosin solubilized in a low ionic strength solution containing L-histidine. Myosin in a low ionic strength solution formed transparent gels at 40–50 °C, while myosin in a high ionic strength solution formed opaque gels at 60–70 °C. The gel of myosin in a low ionic strength solution with L-histidine showed a fine network consisting of thin strands and its viscosity was lower than that of myosin in a high ionic strength solution at 40–50 °C. The rheological properties of heat-induced gels of myosin at low ionic strength are different from those at high ionic strength. This difference might be caused by structural changes in the rod region of myosin in a low ionic strength solution containing L-histidine.

1. 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, Hashimoto, & Yasui, 1961; Puolanne & Halonen, 2010; Samejima, Hashimoto, Yasui, & Fukazawa, 1969). Myosin molecules aggregate to form filamentous polymers under the physiological condition. However, myosin molecules are dissociated from filamentous polymers with increase of ionic strength and disperse as monomers in the high ionic condition above 0.3. Myosin forms gels by heating under appropriate conditions regardless of its monomeric or filamentous state. However, there is discernible difference between heat-induced gels of monomeric myosin and those of filamentous myosin. The rigidity of heat-induced gels of myosin at pH 6.0 increases with decline of ionic strength below 0.4 M KCl and reached a maximum value at 0.2 M KCl, and decreases below 0.2 M KCl (Ishioroshi, Samejima, & Yasui, 1979). Filamentous myosin forms more rigid gels than monomeric myosin. Furthermore, the rigidity of heat-induced gels of filamentous myosin depends on the length of the filament before heating (Yamamoto, Samejima, & Yasui, 1988). These results suggest that properties of heat-induced myosin 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, Tatsumi, Wakamatsu, Nishimura & Hattori, 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, Ito, Wakamatsu, Nishimura, & Hattori, 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 (LMM) in a low ionic strength solution containing L-histidine (Hayakawa, Ito, Wakamatsu, Nishimura, & Hattori, 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, Ishioroshi, & Yasui, 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 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.

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doi:10.1016/j.meatsci.2011.06.002

2. Materials and methods

2.1. Preparation of myosin

Myosin was prepared from chicken breast muscle as described by Perry (1955). Briefly, minced muscle was extracted with modified Guba-Straub solution (0.3 M KCl, 50 mM EDTA, 100 mM KH_2PO_4, 50 mM K_2HPO_4, pH 6.5) for 15 min and centrifuged at 1200 *g* for 10 min. The supernatant was diluted with 14 volumes of cold distilled water and centrifuged at 2500 *g* for 10 min. The precipitate was dissolved in 0.3 M KCl, pH 7.0 and ultracentrifuged at 100,000 *g* for 60 min. The supernatant was diluted with 9 volumes of cold distilled water and centrifuged 1200 *g* 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 180,000 *g* for 120 min. The obtained supernatant was used as myosin.

2.2. 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. The dialyzed myosin suspension was ultracentrifuged at 100,000 *g* for 120 min, and the supernatant was defined as myosin solubilized in a low ionic strength solution containing L-histidine.

2.3. Preparation of heat-induced gel

Myosin solutions (5.0 mg/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.

2.4. Turbidity measurement

Myosin solutions (1.0 mg/ml) were placed in quartz cuvettes and were heated at 70 °C. The absorbencies at 370 nm were measured when the temperature was at 30, 40, 50, 60 and 70 °C.

2.5. Measurement of dynamic viscoelasticity

Dynamic viscoelasticity of myosin was measured by rheometer (Rheolograph-Sol, Toyo-Seiki, Tokyo, Japan). Myosin solutions (4.0 mg/ml) were heated at the rate of 2 °C/min to 70 °C.

2.6. Transmission electron microscopy

For rotary shadowing, sample proteins were suspended in solutions of 50% glycerol containing 0.4 M ammonium acetate, pH 7.2. Sample suspensions were sprayed on mica sheets and then the platinum–carbon was shadowed, while rotating with an electron beam evaporator (JFD-9010, JOEL, Tokyo, Japan). Following these treatments, the replica was floated off on water and picked up on a copper grid. Samples were observed under a transmission electron microscope (H-800, Hitachi, Tokyo, Japan).

2.7. 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 5 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*-butanol freeze-drying method. The dried specimens were mounted on aluminum stubs, coated with gold and observed under a scanning electron microscope (S-800, Hitachi, Tokyo, Japan) with an accelerating voltage of 10 kV.

3. Results and discussion

Myosin in a high ionic strength solution of 0.6 M KCl formed opaque gels at 50 °C or more regardless of the presence of L-histidine. On the other hand, myosin in a low ionic strength solution containing L-histidine formed transparent gels at 40 and 50 °C (Fig. 1). At 60 °C or more, myosin in a low ionic strength solution containing L-histidine did not form gels. This result suggests that the gelation of myosin in a low ionic strength solution with L-histidine has different properties than myosin in a high ionic strength solution with or without L-histidine. In order to assess the aggregation of myosin during heating, we measured the turbidity of the myosin solution. While the turbidity of myosin in a high ionic strength solution increased rapidly at temperatures over 45 °C, that of myosin in a low ionic strength solution with L-histidine did not increase at temperatures up to 70 °C (Fig. 2). This result suggests that the heat-induced aggregation of myosin in a low ionic strength solution with L-histidine is different from that of myosin in a high ionic strength solution with or without L-histidine.

Next, we investigated the dynamic rheological properties of the myosin by measuring the storage modulus (G′) and the loss modulus (G″) during heating. The storage modulus (G′) and the loss modulus (G″) indicate the elasticity and the viscosity of materials, respectively. The storage modulus of myosin in a low ionic strength solution with L-

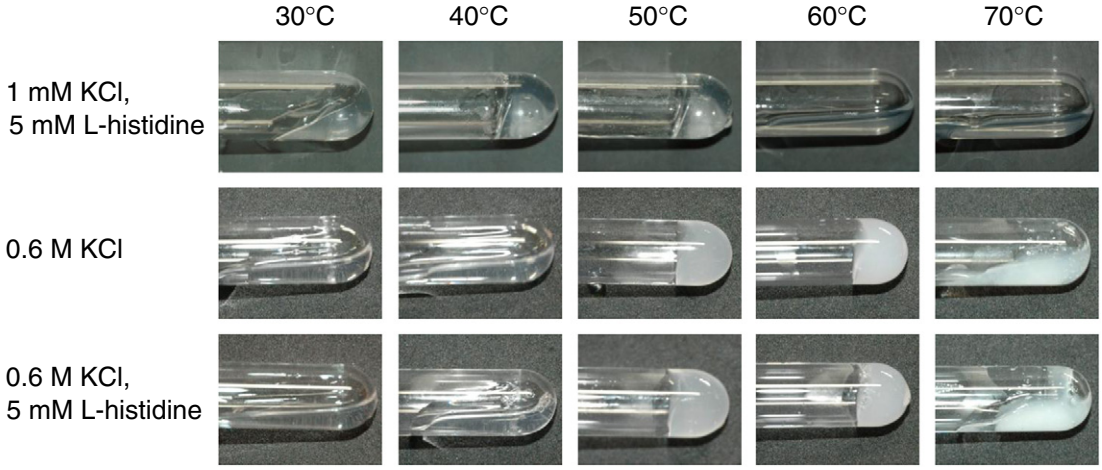

Fig. 1. Effect of heating on gelation of myosin. Myosin solutions (5.0 mg/ml, 1 ml) were placed in test tubes, and the tubes were heated for 10 min at 30, 40, 50, 60 and 70 °C in a water bath.