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Mechanism of Heat-induced Gelation of Bovine and Porcine Myosins

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Background:

In general, pork sausages are more palatable than beef sausages. This is thought to be mainly caused by the differences in texture of sausages. Gelation properties of myosin in muscle proteins are shown to be involved in the development of texture of sausages (1). Heat-induced gelation of myosins is thought to be brought about by the successive heat-denaturation of their heads (S-1) and rods. The denaturation of myosin heads leads to their aggregation, and then the denaturation of myosin rods results in the formation of the network by their interactions. Recently, the research on heat-induced denaturation of heads and rods in rabbit (2), chicken (3) and carp (4) myosins by differential scanning calorimetry (DSC) revealed that the temperature in endothermic transition of myosin heads or rods were different among their species. These results indicate possibility that there are also differences in properties between bovine and porcine myosins. And their differences may cause the difference in mechanism of heat-induced gelation of bovine and porcine myosins.

Objectives:

This work was accomplished to examine properties in heat-induced denaturation of bovine an porcine myosins, and to clarify the mechanisms of heat-induced gelation of both myosins.

Materials and methods:

Isolation of bovine and porcine myosins-Bovine and porcine muscles (*M. longissimus. dorsi*) stored at 4 ℃ for 7 days after slaughter were minced and homogenized with Hasselbach-Schneider buffer (pH 6.4), whose volume was 3 times the weight of minced meat. The homogenate was diluted two times with distilled water, and centrifuged at 3,000 x g for 10 min. The supernatant was also diluted two times with distilled water, and centrifuged at 10,000 x g for 15min. The precipitate was washed twice with 0.15 M KCl/5 mM NaN_3 and dissolved in 0.6 M KCl/50 mM K-phosphate buffer (pH 6.5).

SDS-PAGE-SDS-PAGE was done according to Laemmli; gels containing 7.5 % acrylamide were used in all experiments. Gels were stained for 30 min with 0.25 % Coomassie Brilliant Blue R250 in a 50 % (v/v) methanol, 10 % (v/v) glacial acetic acid solution, were destained for 30 min with rapid destain (50 % (v/v) methanol, 10 % (v/v) glacial acetic acid), and were then placed 5 % (v/v) methanol, 7.5 % (v/v) glacial acetic acid solution before photography.

Turbidity measurement-Effects of heating on turbidity of myosin solution were examined using a spectrophotometer. 0.1 % (w/v) of bovine or porcine myosin solution (0.6 M KCl/50 mM K-phosphate buffer (pH 6.5)) was heated at 30-80 ℃ for 5 min and cooled on ice. Then, its turbidity at 340 and 660 nm was measured.

DSC measurement-Endothermic transitions of bovine and porcine myosins were measured using a DSC (MicroCal, MC-2). 0.1 % (w/v) of bovine or porcine myosin solution (0.6 M KCl/50 mM K-phosphate buffer (pH 6.5)) was heated from 15 to 75 ℃ at 1K/min.

Measurement of CD spectrum-Effects of heating on CD spectra of bovine and porcine myosins were analyzed employing a CD spectrometer (Jasco J-720W). 0.005 % (w/v) of bovine or porcine myosin solution (0.6 M KCl/50 mM K-phosphate buffer (pH 6.5)) was heated from 15 to 75 ℃.

Scanning Electron Microscopy (SEM) of heat-induced myosin gels-Myosin gels were formed from 0.5 % myosin solution (0.5M KCl/20 mM PIPES buffer (pH 6.0)) at 65 ℃ for 20 min. The sample of the myosin gel was prepared and observed with SEM (JEOL, JSMT 200) according to the procedures described by Yasui *et al.* (5).

Results and discussion:

Effects of heating on turbidity of myosins-Aggregation of myosin by heating was analyzed by changes in turbidity of bovine and porcine myosin solutions at 340 and 660 nm. Both myosins started aggregating at 40-45 ℃. However, the velocity of aggregation in bovine myosin was larger than that in porcine one, indicating that the property of bovine myosin is different from that of porcine myosin.

Rabbit and chicken myosins was shown to start aggregating about 35 and 45-50 ℃, respectively (6, 7). These reports show that the properties of rabbit and chicken myosins are also different from those of bovine and porcine ones.

DSC thermograms-Two endothermic transitions were observed at 47 and 55 ℃ in bovine myosin and 45 and 50 ℃ in porcine myosin. From previous reports, the transitions at 47 ℃ in bovine myosin and at 45 ℃ in porcine one corresponded to denaturation of their heads, while the transitions at 55 ℃ in bovine myosin and at 50 ℃ in porcine one coincided with denaturation of their rods.

Two endothermic transitions of rabbit (2), chicken (3) and carp (4) myosins were observed at 51 and 56 ℃, 48 and 54 ℃, and 34 and 47 ℃, respectively. The temperatures of transition in bovine myosin were similar to that in chicken one, while the temperatures in porcine myosin were intermediate between those in chicken and carp ones.

Effects of heating on CD spectra of myosins - Changes in α-helix contents of bovine and porcine myosins by heating was examined by the analyses of CD spectra. Before heating (15 ℃), the α-helix content of bovine myosin is higher than that of porcine one, indicating that the secondary structure of bovine myosin was different from that of porcine one, and that the former was more stable than the latter. The α-helix contents of both myosins decreased by heating. The observation of molar ellipticity at 222 nm showed that the denaturation of bovine myosin occurred stepwise, while that of porcine myosin happened continuously. These differences in heat-induced denaturation between both myosins corresponded to the differences in temperatures of endothermic transition of heads and rods between both myosins.

Micro-structure of heat-induced gels made from myosins-Matrixes of gels were made from bovine and porcine myosins by heating (Fig. 1). The meshes of matrixes from porcine myosins were smaller than those from bovine ones, indicating that matrixes of porcine myosin gels were formed more densely than those of bovine myosin gels. It was also observed that the frames of matrixes were a little thicker in bovine myosin gels than in porcine myosin ones.

Conclusion:

Matrix of porcine myosin gel was more dense than that of bovine myosin one. Porcine myosin gel was made by the successive denaturation of heads and rods in myosin, because the temperature of denaturation of myosin rods was near to that of myosin heads. On the other hand, bovine myosin gel was formed by the stepwise denaturation, indicating that aggregation of bovine myosin heads made larger condensates, and then denaturation of myosin rods formed coarse matrixes (Fig. 2).

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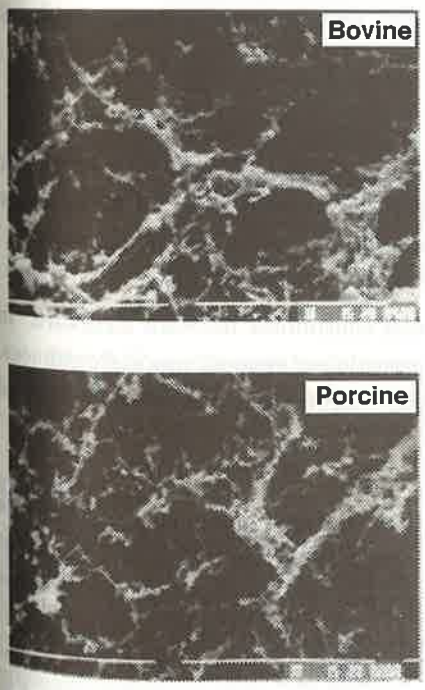

Fig. 1. SEM of heat-induced gels of bovine and porcine myosins
Bar length is 10 μm.

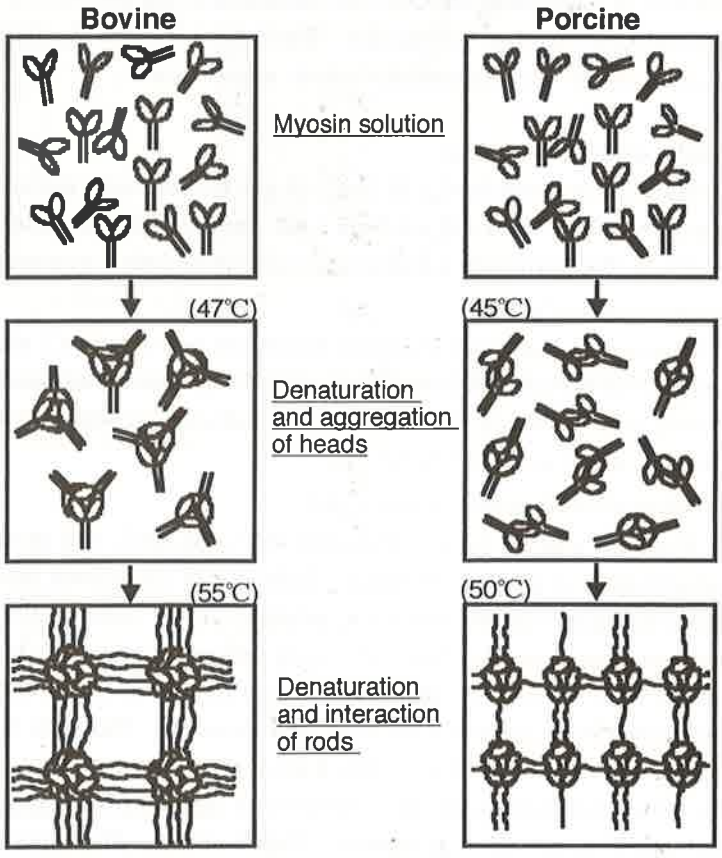

Fig. 2. Mechanism of heat-induced gelation of bovine and porcine myosins