EFFECT OF HEATING TEMPERATURE ON THE DEGRADATION OF MYOSIN HEAVY CHAIN

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Background

When meat is simmered in sub-boiling water at 95 ºC for a long time as in the case of stew cooking, such cooking procedure usually improves the tenderness and flavor of meat. It is well known that the increase of tenderness is due to gelatinization of collagen in stroma. In addition to the gelatinization, we have reported that myosin heavy chain (MHC), one of the major myofibrillar proteins, is degraded into some fragments during simmering of beef round meat (Tajima et al. 2001). Furthermore, The degradation of MHC was observed in purified myofibrils heated at 100 ºC. We also demonstrated that the degradation of MHC was promoted by heating under pressure (1.3kg/cm^2, 124 ºC). The molecular weights of the fragments produced by heating were 70 and 58 kDa and that the amounts of them increased with increasing heating time. The temperature and time of simmering of meat are important factors to improve the tenderness.

Objectives

Our objective is to clear the effect of heating temperature on the degradation of MHC during heating. Purified myofibrils suspended in 0.15 M KCl were heated at 70, 80 and 90 ºC, respectively, and Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting were used to identify the derivatives from MHC after heating.

Methods

Heat treatment of myofibrillar proteins. Myofibrils prepared from the beef round meat according to the method of Briskey and Fukazawa (1971) were suspended in 20 volumes (v/w) of 0.15M KCl, and an aliqout (3 mL) of the myofibril suspension in a glass tube was heated in a dry block heater (Model No. DB-1L, MS, Tokyo, Japan) at 70, 80 and 90 ºC for 0.5, 1, 2, and 3 h, respectively. After the glass tubes were centrifuged at 4,000 rpm for 15 min, the supernatant was separated from the residue. 7.5 mL of the sample buffer (5% sodium dodecyl sulfate, 20 mM sodium phosphate buffer, 0.1% 2-mercaptoethanol, pH 7.0) was added to each glass tube, followed by gentle stirring overnight at room temperature to solubilize the residue prior to apply to SDS-PAGE.
Electrophoresis. SDS-PAGE was done according to the method of Laemmli (1970) using a mini-protean II electrophoresis unit (Bio-Rad Lab., Richmond, Calif., USA). The stacking gel and the resolving gel contained 4% and 10% acrylamide, respectively. Samples for the electrophoresis were prepared as follows. An aliquot (1 mL) of each sample (the solubilized proteins from unheated and heat-treated myofibrils and supernatant after the centrifugation of the heat-treated myofibrils) was mixed with 0.2 vol of tracking dye (50 mM 2-[N-morpholine] ethane sulfonic acid buffer, pH 6.5, 5% SDS, 50% sucrose (w/v), bromophenol blue) and 0.1 vol of 2-mercaptoethanol. It was then heated at 60 ºC for 20 min.
Transfer condition and Western blotting. The proteins separated by SDS-PAGE were electophoretically (5 h at 90 V) transferred from the gel to a nitrocellulose membrane (0.45 µm; Bio-Rad) in a Trans-Blot cell unit (Model 250/2.5; Bio-Rad) using 25 mM Tris, 192 mM glycine, and 10% (v/v) methanol buffer (pH 8.39). After blocking, the membrane was incubated in the anti-bovine myosin (whole serum) of rabbit (Polysciences Inc., Warrington, Pa., USA) duluted 1:40 in blocking solution at 4 ºC for 16 h. The succeeding treatment was made according to a common procedure (Nakaya and Watabe 1994).

Results and discussion

In order to clarify the effect of temperature on the degradation of MHC, myofibrils were heated at 70, 80, and 90 ºC for 0.5, 1, 2, and 3 h in 0.15 M KCl, respectively. The electrophoretogram of the unheated myofibrillar proteins (Fig. 1A, 80 ºC, lane 0) shows the bands of MHC, actin, tropomyosin and some of other proteins. When myofibril was heated at 70 ºC for 3-h heating, the change was not observed in the intensity of MHC band during the heat treatment (Fig. 1A, 70 ºC). At 80 ºC, however, the intensity of MHC band reduced slightly and a new faint band having molecular weight of 70 kDa appeared in the 3-h-heated sample. In the sample heated at 90 ºC, 70-kDa band appeared after 2-h heating. In Western blotting pattern of the unheated sample, MHC band and its degraded band of 170 kDa were observed (Fig. 1B lane 0). In blotting pattern of the sample heated at 70 ºC, a new 140-kDa band appeared after 0.5-h heating and a faint 70-kDa band appeared after 1-h heating (Fig. 1B, 70 ºC). In blotting pattern of the sample heated at 80 ºC, 100 kDa band (arrowhead) in addition to the two bands were observed obviously (Fig. 1B, 80 ºC). In case of heating at 90 ºC, the MHC and 170-kDa bands decreased gradually with increasing the heating time and the intense band of 140 kDa and clear bands of 100 and 70 kDa were observed after 0.5-h heating. The 140-kDa band became weaker with increasing heating time and several newly faint bands were observed between 100 and 70 kDa after 3-h heating (Fig. 1B, 90 ºC, lane 3). These results clearly demonstrated that the degradation of MHC was influenced by both of heating temperature and time. We reported in a previous paper that, when myofibril was heated at 100 ºC, 70- and 58-kDa bands were observed after 0.5-h heating. These results suggest that MHC degrades from 170 to 58 kDa gradually during heating.

Fig. 2A shows the electrophoretogram of soluble proteins in the supernatant separated from heated myofibrils by centrifugation. Two clear bands having molecular weight of 39 and 36 kDa were observed in the supernatants of the myofibrils heated at 70, 80, and 90 ºC. These electophoretograms suggest that the heating temperature didn't influence on the degradation of soluble proteins in the supernatant The degraded fragment from MHC were not observed in Western blotting patterns of the supernatant after heating. This result demonstrated that the heat-induced fragments from MHC was insoluble in 0.15 M KCl.

The results in the study showed that MHC was degraded to the fragments having molecular weights between 140 to 58 kDa by heating at >70 ºC and that the degradation was influenced by temperature and time of heating.

Pertinent literature
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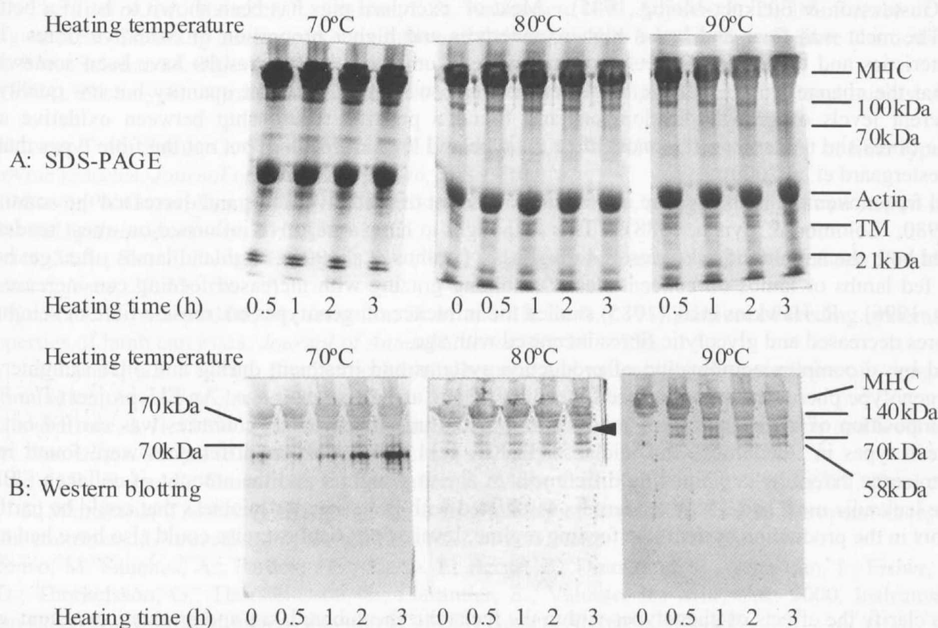

Fig.1. SDS-PAGE and Western blotting patterns of myofibrillar proteins heated at 70, 80 and 90ºC for various times. MHC: myosin heavy chain, TM: tropomyosin, arrowhead indicates 100 kDa

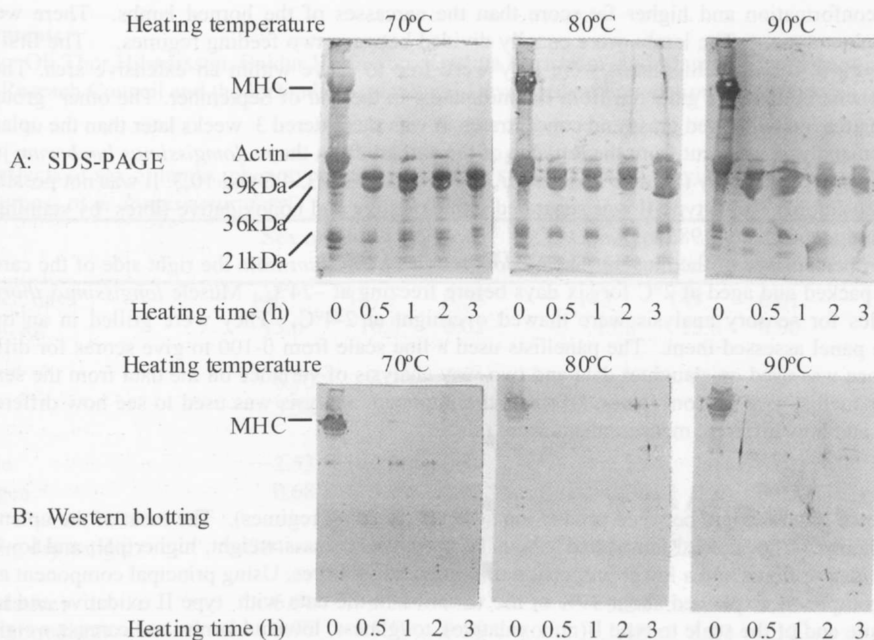

Fig.2. SDS-PAGE and Western blotting patterns of supernatant fraction separated from the heated myofibril. A: all proteins on the nitrocellulose membrane were stained by amidoblack, B: nitrocllulose membrane was treated with myosin antibody.