加熱による魚肉ミオシンB Mg2+-ATPaseの変化と坐り

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Heat Changes of Myosin B Mg^{2+}-ATPase and "Setting" of Fish Meat Paste

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To clarify the "setting" mechanism of fish muscle paste, the effect of heat on fish muscle myosin B Mg^{2+}-ATPases was examined in connection with the "setting" ability of the meat paste. By heat treatment, the Mg^{2+}-ATPase activity of myosins B showed some increment. This varied by fish species as indicated in the following decreasing order: Alaska pollack and sardine>white croaker, horse mackerel, mackerel, and carp>crucian carp and marlin groups. The myosin-poor fish pastes which were prepared by washing with GUBA-STRAUB solution gave "set" gels (jelly formation) of lower jelly strength. Addition of pyrophosphate promoted the "setting" of black marlin paste, but not of sardine paste. From these results, it is suggested that the "setting" of fish paste is associated with the interaction between myosin and actin.

In the processing of fish jelly, the gelation procedure of meat paste at a temperature such as 30°C is useful for attaining a strong network structure in the cooked jelly. Such a gelation is called "setting" (suwari in Japanese). When the pastes obtained from different fish species were made "set", there are appreciable differences in "setting" ability among fish species.

So far, these differences have been attributed to the differences in heat-denaturation velocity of actomyosin[1,2], or in the composition of the protein extractable at a low ionic strength from muscle tissue[3]. However, the exact mechanism of "setting" remains for the most part to be elucidated.

To gain further information about the "setting" mechanism of fish muscle paste, the present investigation was carried out by observing the effect of heating on fish muscle myosin B Mg^{2+}-ATPase. Also, the "setting" ability of the pastes of some fishes was studied in connection with the activity of myosin B Mg^{+2}-ATPase.

Materials and Methods

The following fish species were selected as materials to cover the meat samples with various degree of "setting" ability: Alaska pollack *Pleurogrammus azonus*, sardine *Sardinops melanosticta*, white croaker *Argyrosomus argentatus*, horse mackerel *Trachurus japonicus*, mackerel *Scomber japonicus*, carp *Cyprinus carpio*, crucian carp *Carassius cuvieri*, Pacific marlin *Makaira mitsukurii*, and black marlin *Makaira mazara*.

As an index of the biochemical properties of meat paste, myosin B Mg^{2+}-ATPase was estimated. Myosin B was prepared by the dilution-precipitation method after extracting each fish meat with WEBER-EDSALL solution. Each myosin B preparation suspended in 0.1 M KCl-10 mM tris-maleate buffer of pH 7.0 was treated at a definite constant temperature ranging from 15°C to 44°C for 30 min. The resulting myosin B was submitted to ATPase assay. ATPase assay was done in a reaction mixture consisting of 35 mM KCl, 25 mM tris-maleate buffer of pH 7.0, 1 mM $MgCl_2$, 5 mM ATP, and 0.4–0.5 mg/m*l* of the protein. The mixture was incubated at 25°C for 2 min. The activity was expressed as percentage of that of intact myosin B by estimating the amount of Pi liberated per mg of protein.

For the preparation of fish meat paste, the minced meat was washed two or three times with cold water. By centrifugation, the excess moisture was removed from the meat. The moisture and pH in the system were adjusted to 83–84% and 6.8, respectively. To obtain the myosin-poor meat, the meat washed with 3 vol. of GUBA-STRAUB solution (0.09 M KH_2PO_4, 0.06 M K_2HPO_4, 0.3 M KCl, pH 6.5) for 10 min, prior to washing with water. This procedure was able to remove a large amount of myosin from the minced meat, though

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the losses of small amount of action and the other proteins were observed at the same time. Each paste was obtained by grinding the meat with 3% salt unless otherwise stated. Each sample heated in water bath (28°, 30°, or 40°C) was submitted to the jelly (breaking) strength measurement by a rheometer (Type NRM-2001J, Nihonseimitsu Co. Ltd. with a plunger diameter, 0.5 cm).

Results and Discussion

Heat Changes of Myosin B Mg^{2+}-ATPase

To know the differences in the property of paste among fish species, the change in myosin B Mg^{2+}-ATPase activity on heat treatment was examined. The ATPase assays were done at 25°C. The activity curves of myosin B Mg^{2+}-ATPase from nine fish species by plotting the variation of activity versus heating temperature are shown in Fig. 1. As seen in Fig. 1, the shape of the curves is characterized by a peak-height expressing the increase of the activity and its corresponding temperature. From these curves, it was found that the height and the temperature were different among fish species. The temperature of the peak-height could be approximately divided into three groups: Alaska pollack and mackerel at temperatures below 30°C, horse mackerel, sardine, white croaker,

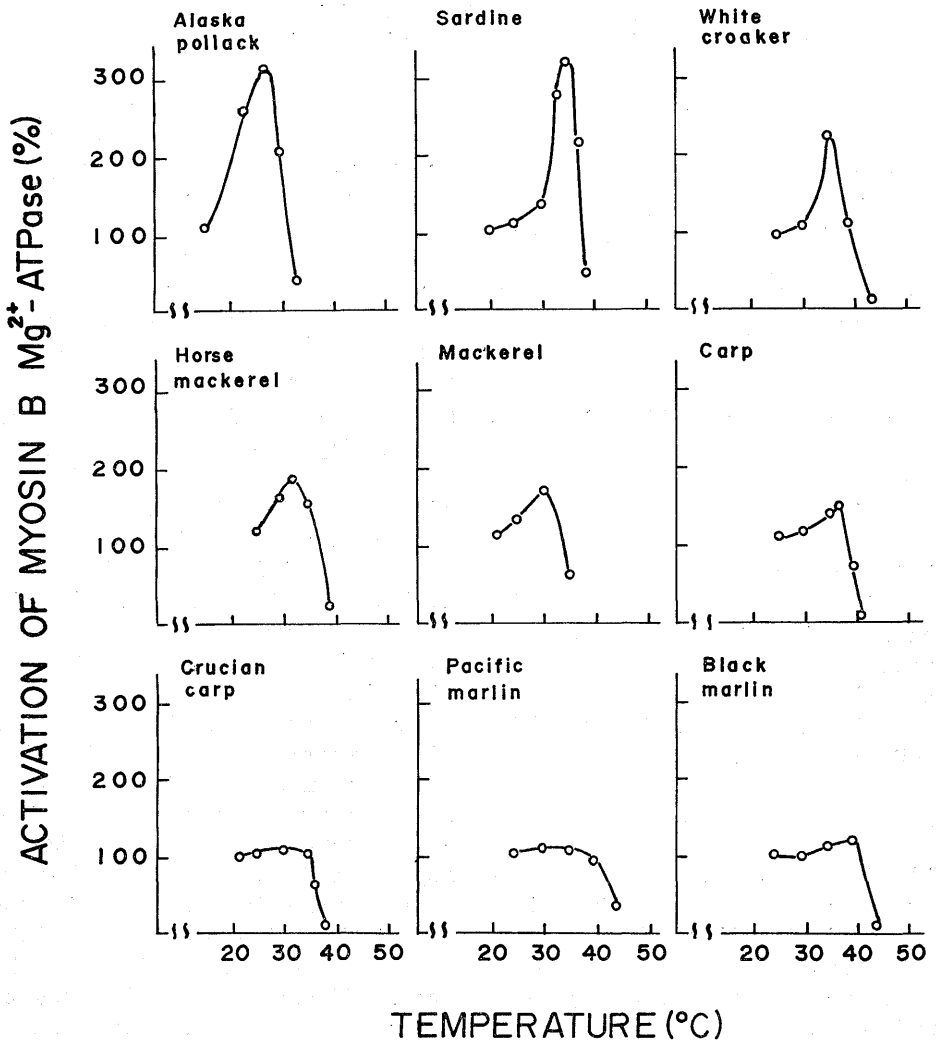

Fig. 1. Changes in the activity of fish myosin B Mg^{2+}-ATPases by heat treatment.
Each myosin B was treated in the presence of 0.1 M KCl-10 mM tris-maleate buffer (pH 7.0) at a definite temperature for 30 min. After the treatment the activity of Mg^{2+}-ATPase was measured at 25°C.

crucian carp, and carp between 31° and 36°C, and Pacific marlin and black marlin above 37°C, respectively. Besides the above difference, serious differences were observed in the level of peak-height. The levels of the activity increment were arranged in the decreasing order: Alaska pollack and sardine>white croaker, horse mackerel, mackerel, and carp>crucian carp, Pacific marlin, and black marlin.

It is worth noting that the "setting" ability of fish meat paste corresponds to the activity curve of their myosin B Mg^{2+}-ATPase by heating, as previously pointed out[5]. The "setting" phenomenon may be in association with the properties of heat change of myosin B Mg^{2+}-ATPase. Though the reaction mechanism of myosin B Mg^{2+}-ATPase is of really complicating features, the heat-activity increment of myosin B Mg^{2+}-ATPase may be due to some change in the interaction between myosin and actin.

"Setting" of Meat Paste

From the point of view mentioned above, the "setting" abilities of the myosin-poor meat paste and the normal meat paste were compared to know whether "setting" is affected by the interaction between myosin and actin. When the minced meat of horse mackerel or sardine was washed with GUBA-STRAUB solution, it was confirmed by SDS-polyacrylamide gel electrophoresis that a fair amount of myosin was removed from the meat. The effect of myosin on the strength of "set" gel was examined. These results are given in Fig. 2. Fig. 2 shows that the jelly strength of the washed paste is inferior to that of the unwashed paste over the range measured. It has been reported that myosin paste from mackerel or red sea bream muscle was turned into a very elastic gel by heating at 80°C[6]. From these results, it seems that myosin plays an important role in the "setting" ability of the paste.

However, it is well-known that marlin meat paste is not liable to "set". To bring upon the change in the interaction between myosin and actin, the effect of added pyrophosphate on "setting" of black marlin and sardine pastes was examined. The results are shown in Fig. 3. Fig. 3 shows that the addition of pyrophosphate promotes "setting" of black marlin paste, while it does not influence that of sardine paste. As generally accepted, pyrophosphate enhances the hydration and dissociation of actomyosin. These two effects may take place simultaneously, on the basis of the results shown in Figs. 1 and 3. From the standpoint of the interaction between myosin and actin, it is suggested that the "setting" of marlin meat having the unchangeable activity curve (Fig. 1) needs the addition of pyrophosphate to result in a change of the interaction between myosin and actin. On the other hand, sardine

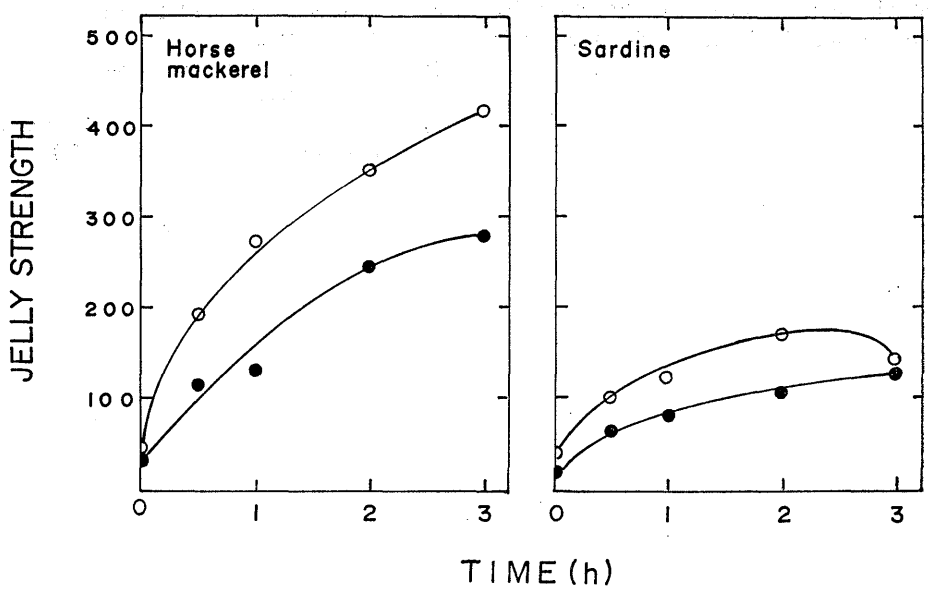

Fig. 2. "Setting" of fish meat pastes washed with GUBA-STRAUB solution. The breaking strength of gel at 30°C was expressed as jelly strength.
○: Unwashed, ●: Washed with GUBA-STRAUB solution.

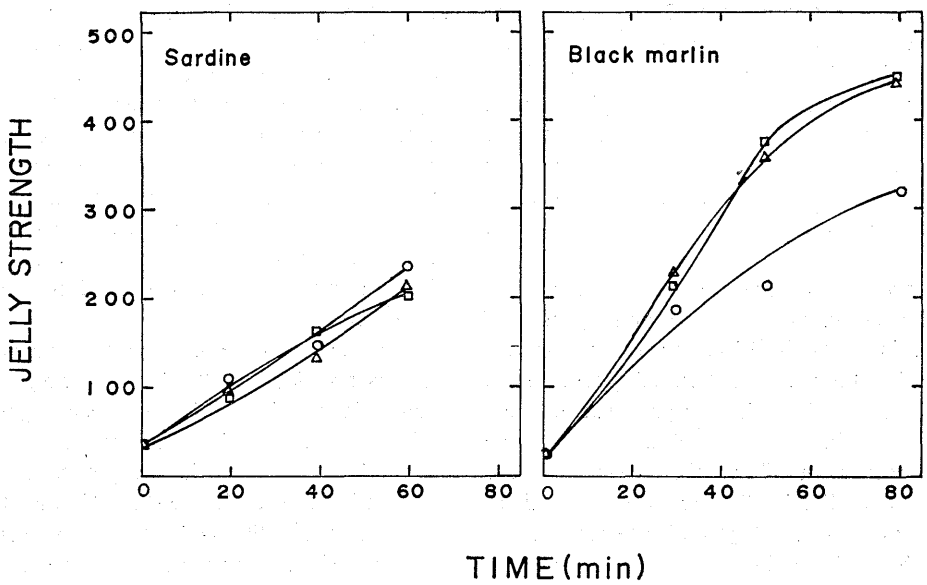

Fig. 3. Effect of pyrophosphate on "setting" of fish meat pastes. The meat pastes of sardine and black marlin were heated at 28°C and 40°C, respectively.
○: Without pyrophosphate, □: 1 mM Pyrophosphate, △: 5 mM Pyrophosphate.

meat is liable to undergo a heat change in the interaction regardless of the addition of pyrophosphate. With the above suggestion, it can be considered that the effect of myosin on the "setting" ability (Fig. 2) is due to that the myosin in the paste is free from the modification by actin.

As to whether "setting" of fish meat paste is associated with the change in the interaction between myosin and actin, further studies are now in progress.

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