# Thermal Denaturation of Myosin B in the Presence of Urea

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# Thermal Denaturation of Myosin B in the Presence of Urea

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*Keywords* : Urea, myosin B, thermal denaturation, aggregation

**Abstract**

The effect of urea on the thermal denaturation of myosin B was investigated from the viewpoints of aggregation process, sulfhydryl, and hydrophobic behaviors. The degrees of turbidity and light scattering intensity decreased with increasing the urea concentration on heating from 20 to 80℃, compared with myosin B in the absence of urea. These phenomena suggested that the aggregation process of myosin B was inhibited by thepresence of urea. The surface sulfhydryl groups of myosin B were oxidized readilyby heating myosin B in the presence of urea. 8-Anilino-1-naphthalenesulfonate fluorescence intensity indicated that the amount of hydrophobic group decreased with the increase of the oxidized SH group. These results suggested that the configuration of myosin B molecule consequently changed to be compact within a single myosin B on the treatment of urea and heating.

The muscles of shark which are classified in marine elasmobranch fish contain urea in the range of about 250-350 mM[1]. It is well known that the hydrophobic group and the hydrogen bond of protein are severed in the presence of urea[2]. The bonding among the constituent proteins of myosin B is considered to be influenced by urea. Niwa *et al.*[3] reported that the hydrophobic groups were hardly affected in the case of elasmobranch myosin B at urea concentrations up to 1.3 M, and increased above this concentration.

Myosin B molecules were also aggregated with thermal denaturation. Deng *et al.*[4] reported that the turbidity and light scattering intensity increased with the aggregation of myosin B. This results in the such protein-protein interaction, as disulfyde, hydrophobic group, and the hydrogen bonds[5].

However, the basic knowledge on the thermal denaturation of myosin B in the presence of urea is very limited, and this restricts the further utilization of shark muscle. In the present paper, the effect of urea concentration on the thermal denaturation of shark muscle myosin B was investigated from the viewpoints of aggregation process, sulfhydyl (SH), and hydrophobic group behaviors.

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## Material and Methods

*Material*

Smooth dogfish *Mustelus manazo* were caught off Ichiki in Kagoshima Prefecture. They were frozen immediately after landing, and kept at −25℃ until used. Myosin B was prepared from the ordinary muscle after thawing in flowing water of about 15℃.

*Preparation of myosin B*

Myosin B was obtained by the method of Takashi *et al.*[6] with some modification[7], and was dissolved with 0.45 M KCl - phosphate buffer (pH 6.8, ionic strength 0.5). Myosin B solution was adjusted to a final protein concentration of 0.4 mg/mℓ except for the measurement of SH group content. The protein concentration was determined by the biuret method of Gornall *et al.*[8]

After urea was added to myosin B solution from 0 to 3.0 M, the mixture was kept in an ice-water for 10 min, heated in the range of 20 to 80℃ for 10 min, and then cooled in an ice-water for 10 min.

*Turbidity*

The turbidity was measured at the absorbance of 350 nm by the method of the previous paper[7], using a HITACHI 100-20 spectrophotometer. Since the liner relationship between the turbidity and the protein concentration was found in the range of 0.1 to 1.0 mg/mℓ, the turbidity measurement was done at 0.4 mg/mℓ of myosin B concentration. This concentration was not interfered with myosin B coagulation, (preliminary experiment). The spectrum of heat-induced myosin B solution from 300 to 800 nm indicated the maximal change at 350 nm.

*Light scattering intensity*

The light scattering analysis was carried out using a FP-777 fluorescence spectrophotometer (Japan Spectroscopic Co., Ltd.). The light intensity scattered through an angle of 90° was estimated at 550 nm.

*SH group content*

The SH group contents of myosin B were determined using 5,5'-dithiobis (2-nitrobenzonic acid) (DTNB) by the method of Ellman[9]. To 8 mℓ of 0.45 M KCl-phosphate buffer (pH 8.0) was added 2 mℓ of myosin B solution (1.0 mg/mℓ) treated with urea and heating. Three amount of the mixture were added to 0.02 mℓ of 10 mM DTNB solution, stood for 30 min at a room temperature, and then the absorbance of reaction mixture was measured at 412 nm. The relative SH group contents Co (M) were calculated using the equation, $Co = A \times D/e$, where A is the absorbance at 412 nm, e is the extinction coefficient of TNB of 13,600 M/cm at 412 nm, and D the diluted rate. The amounts of disulfide (SS) bonds, inner SH and surface SH group were calculated by the method of Itoh *et al.*[10]

*8-Anilino-1-naphthalenesulfonate (ANS) fluorescence intensity*

The ANS fluorescence intensity was measured by a slightly modified method of Niwa *et al.*[3] To 2 mℓ of myosin B solution treated with urea and heating was added 0.02 mℓ of 2 mM ANS in 0.45 M KCl - phosphate buffer (pH 7.5), and the mixture was allowed to stand at 25℃ for 30 min. The fluorescence intensity was measured at 470 nm with 379 nm excitation, using a FP-777 fluorescence spectrophotometer.

## Results and Discussion

*Effects of urea and heating temperature on the aggregation of myosin B*

The turbidity measurement is available as the indicator of the aggregation process of myosin B molecules. The turbidity of myosin B incubated at 20℃ for 10 min decreased with increasing urea concentration and the addition of urea inhibited the aggregation of myosin B as shown in Fig. 1. When heated myosin B in the absence of

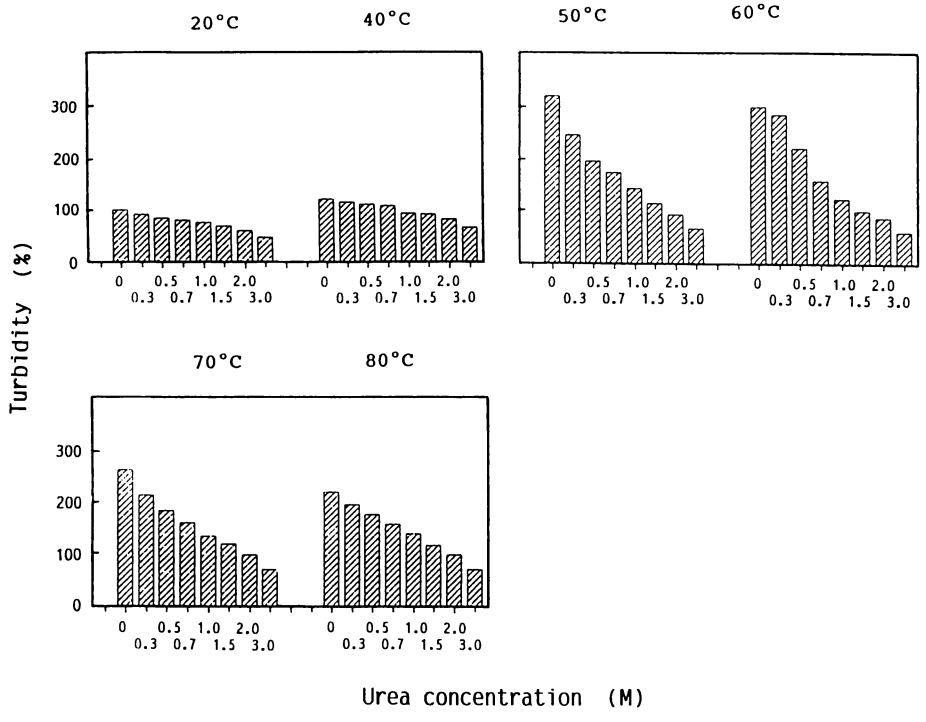


**Fig. 1.** Changes in turbidity of myosin B solution on the treatment of urea and heating.
The turbidity of samples was compared with that of myosin B heated at 20℃ in the absence of urea.

urea, the turbidity unchanged until the temperature reached at 40℃ and then increased markedly from 40 to 50℃, turning into a peak at around 50℃. The relative turbidity of myosin B heated at 50℃ was about three time as intense as at 20℃.

When heated at 50℃, the turbidity of myosin B in the presence of 0.3 M urea was about 75% of that in the absence of urea, and the aggregation degree of myosin B decreased in the presence of urea. The increase of urea concentration was reduced to the aggregation degree of myosin B on heating above 50℃, compared with myosin B in the absence of urea. On further increase of urea concentration, the turbidity almost unchanged with rising the temperature. It was suggested that the aggregation of myosin B with rising the heating temperature was inhibited by the increase of urea concentration.

When the myosin B solution containing various concentration of urea was heated at given temperatures, a good correlation was found between the light scattering intensity and the degree of the turbidity (Fig. 2). The light scattering intensity revealed the degree of the aggregation of myosin B, similar to the turbidity measurement.

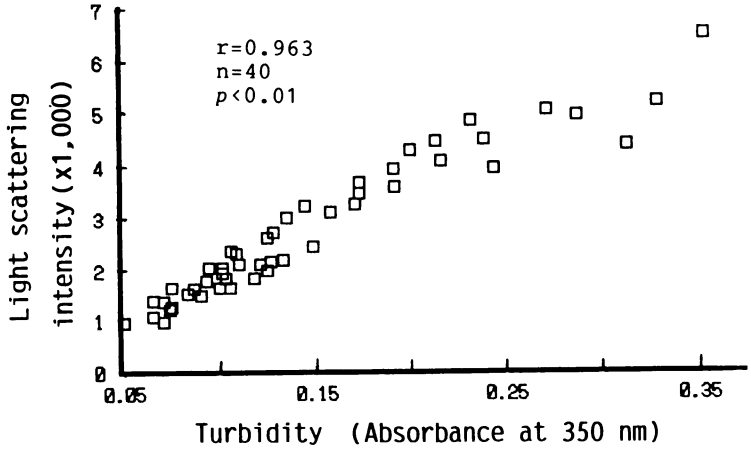


**Fig. 2.** Relationship between turbidity and light scattering intensity of myosin B solution treated with urea and heating.
r is the coefficient of correlation, n is sample number, and $p$ the level of significance, respectively.

*Effects of urea and heating temperature on SH and hydrophobic behaviors of myosin B*

It was reported that the surface SH group of protein reacted readily with DTNB, and the surface SH group was oxidized readily into SS bonds between the myosin B molecules during heating[10]. The total SH group contents consist of inner and surface SH groups, and the aggregation process of myosin B is considered to give rise to the reaction of the surface SH groups.

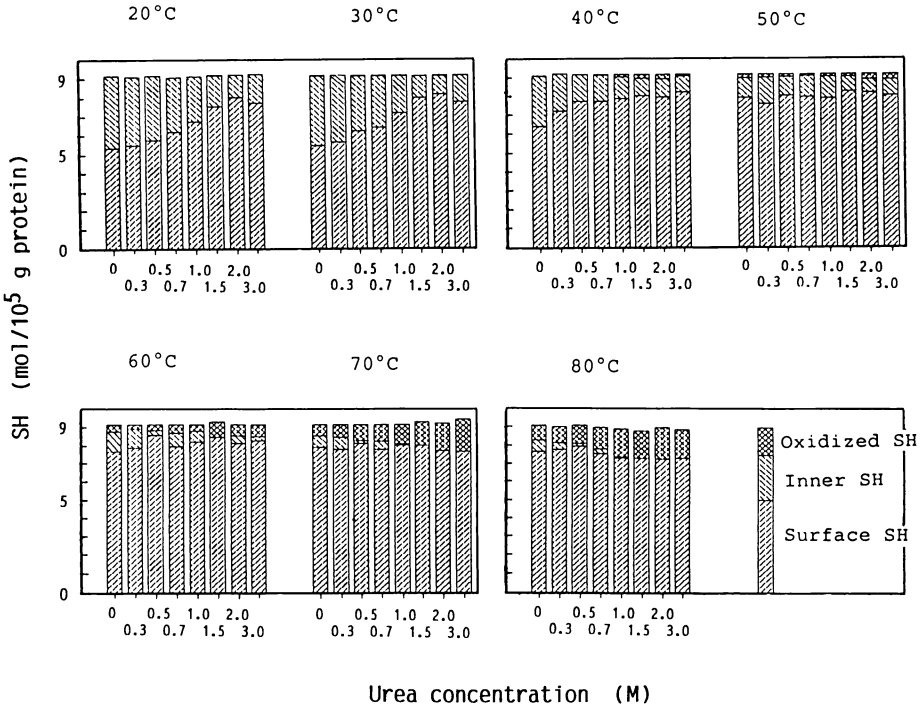


**Fig. 3.** Changes in surface SH, inner SH and oxidized SH group contents of myosin B on the treatment of urea and heating.

As shown in Fig. 3, when myosin B was incubated at 20 and 30℃, the surface SH group contents increased with increasing urea concentration, indicating the unfolding of myosin B molecules. The total SH group contents were approximately constant and SS bonds were not detected, irrespective of urea concentration. This result suggested that myosin B molecule was unfolded with increasing urea concentration, and was dissociated by urea, indicating the decrease in the degree of both turbidity and light scattering intensity. The surface SH group of myosin B in the absence of urea markedly increased from 40 to 50℃, suggesting the unfolding of protein molecules. The unfolded structure of myosin B by heating was different from that by the treatment of urea, since the degree of turbidity increased by heating and decreased by urea.

When heated at 40℃, the SH group was oxidized in the presence of urea above 1.0 M. On further rise of the heating temperature, the SH group was oxidized readily, and the oxidation of SH group was accelerated above the concentration of 1.0 M urea. Myosin B was caused to dissociate to myosin and actin in the presence of urea above 1.0 M (in our experiments). It was suggested that the oxidation of SH group was promoted by the increase of the mobility of myosin dissociated from myosin B.

ANS is a specific probe for the hydrophobic region which was formed by hydrophobic group of protein molecules. As shown in Fig. 4, when incubated at 20 and 30℃,

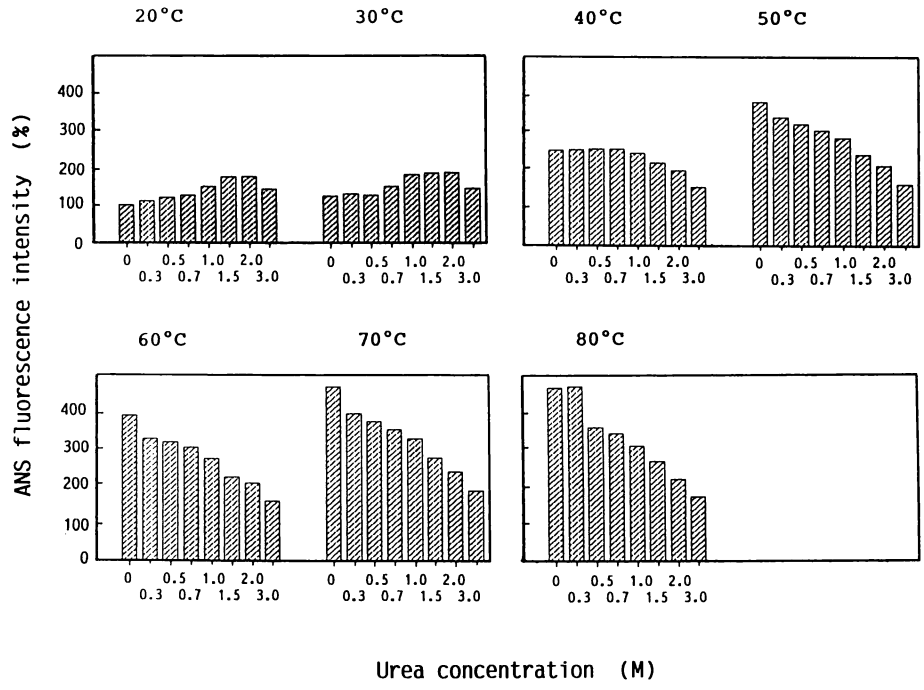


**Fig. 4.** Changes in ANS fluorescence intensity of myosin B on the treatment of urea and heating.
ANS fluorescence intensity of samples was compared with that of myosin B heated at 20℃ in the absence of urea.

ANS fluorescence intensity of myosin B increased with increasing urea concentration. It was supported that the structure of myosin B molecules was unfolded with increasing urea concentration. When heated at 40℃, ANS fluorescence decreased in the presence of urea above 1.0 M. This phenomenon was different from the result in the increase of SS bonds.

When heated above 50 ℃, ANS fluorescence intensity decreased with increasing urea concentration. Especially, in the presence of 3.0 M urea, ANS fluorescence intensity unchanged on rising the temperature. These results were similar to those of the turbidity and light scattering intensity of myosin B heated above 50 ℃. ANS fluorescence intensity was in fair agreement with the degrees of the turbidity and the light scattering intensity.

From the results of aggregation process, SH, and hydrophobic behavior of myosin B on the treatment of urea and heating, in the absence of urea the protein-protein interaction occurs between the myosin B molecules, and the aggregation increases with rising the temperature. In the presence of urea myosin B - urea interaction is stronger than that between the myosin B molecules, and the SH groups of myosin B molecules unfolded by urea give rise to the formation of intramolecular SS bonds on rising the

heating temperature. The oxidation of SH groups to intramolecular SS bonds leads to the decrease of the surface hydrophobic group in myosin B. The configuration of myosin B molecules consequently changes to be compact within a single myosin B on the treatment of urea and heating and the aggregation of myosin B molecules decreases.

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