Transactions of the Materials Research Society of Japan 26 [2] 605-608 (2001)

Sol-Gel Transition of Myosin Solution

Kazuko Ooi, Sawa Kouchi, Toshiaki Dobashi, Shingo Kondo and Hisashi Ichikawa*

Department of Biological and Chemical Engineering, Faculty of Engineering,
Gunma University, Kiryu, Gunma 376-8515
Fax: 0277-30-1477, e-mail: dobashi@bce.gunma-u.ac.jp
*Division of Marine Biological Resources, Graduate School of Marine Science and Engineering,
Nagasaki University, Nagasaki 852-8521
Fax: 095-844-3516, e-mail: ichinon@net.nagasaki-u.ac.jp

The concentration dependence of the shear modulus of fish myosin aqueous solutions has been measured as a function of temperature both in heating process from 25°C to 70°C and in cooling process from 70°C to 0°C. The curve for shear modulus vs. temperature has a sharp peak around $T_1 \approx 36$°C in the heating process, while a sol to gel phase transition occurs at $T_2 \approx 31$°C in the cooling process independently of myosin concentration. This characteristic behavior indicates that a temperature-induced conformation change of a single myosin molecule causes a structural change of the solution, yielding the characteristic temperature dependence of viscoelasticity of the myosin solution.

Key words: sol-gel transition, fish myosin, shear modulus, temperature dependence

1. INTRODUCTION

Kamaboko is made of *Surimi* which is the product obtained by bleaching fish meat in water and mixing them vigorously at high ionic strength (*Shiozuri*), by two-step heating around 30°C and around 100°C. Most of the conditions in the process have been determined from manufacturer's experience and the mechanism of the sol to gel transition of the fish meat proteins has not been clarified yet [1]. The terms used in Fisheries Science, for example, *Ashi*; the unique texture of *Kamaboko*, *Suwari*; the increase in gel strength at low temperatures around 30°C and *Modori*; the decrease in gel strength caused by heating the *Suwari* gel around 60°C, should be expressed by a certain number of rheological parameters. In Fisheries and Agricultural Science most mechanical studies are performed at room temperature as for breaking strength measurement which is known as correlating to the sensory evaluation, but a few rheological test during the heating has been reported [2]. It is interesting to compare the rheological parameters measured *in situ* at the real temperatures in the heating treatment. It is also hoped to measure both in heating and cooling processes to discriminate reversible and irreversible processes since there may be both physical cross-linking due to hydrogen bonding and hydrophobic bonding and chemical cross-linking due to S-S bonding.

Fish meat consists of muscle proteins such as myosin, actin and other decorating proteins. *Surimi* gel is known to be produced by cross-linking the myosin molecules [3]. Low breaking strength of *Surimi* found for some fish species is attributed to inhibition of cross-linking of myosin molecules [4]. Therefore it is required to study the gelation process of purified myosin and then compare with that of *Surimi*. Several studies attributed the complex cross-linking reactions in the gelation process of fish meat to the temperature dependence of transglutaminase activity which catalyzes the polymerization of myosin molecules but it is not yet clarified [4].

In this study, to verify the hypothesis that heat-set myosin gel is caused by conformation change of a single myosin molecule we measure the temperature dependence of shear modulus of

myosin solutions at various concentrations both in heating and cooling processes.

2. EXPERIMENT

2.1 Sample

The back muscle of Shiroguchi (*Pennahia argentata*, white croaker) obtained at Mogi harbor in Nagasaki prefecture was minced in seven-fold 19mM sodium phosphate buffer (pH7.5), homogenized for 60 min and centrifuged at 500×g for 10min. This process was repeated twice and the supernatant was removed. Then three fold buffer solution containing 2mM ATP, 2mM $MgCl_2$, 0.45M KCl, and 67mM sodium phosphate(pH6.4) was added to the precipitates. The solution was gently stirred for 10 min and centrifuged at 6,000 ×g for 15min, and then the supernatant was filtered with 2-3 layers of nylon gauze. The filtrate was poured in fourteen-fold cold deionized water and settled at 5°C for 10 min. Proteins were collected by centrifuging the precipitates appeared at the bottom of the vessel at 6,000×g for 10min (Dilution precipitation). The proteins were dissolved in 1/3-fold solution containing 3M KCl and 0.25M Tris-HCl(pH7.0). KCl concentration of the protein solution was adjusted to 0.6M by adding desired amount of cold deionized water. Then the solution was added by 2mM ATP and 50mM $MgCl_2$ and ultracentrifuged at 100,000×g for 60min to remove the precipitates. Final myosin solution was obtained by repeating the same dissolution and dilution precipitation process. From electrophoresis with sodium dodecyl sulfate-contained 10% polyacrylamide, the weight percents of myosin, actin, tropomyosin, troponin and others were obtained as 92%, 3%, ND, ND and 5%, respectively.

Myosin solutions with the concentrations 13, 18.5, 24, 30, and 40mg/ml were prepared. The salt concentration of them was fixed at 0.6M KCl and 50mM Tris-HCl(pH7.0).

2.2 Measurement

Rheological measurement was performed with a Rhesca Model RD-100 AD coaxial cylinder-type torsional viscoelastometer. 3 ml of myosin solution was poured between outer cylinder (Pyrex tube with 14.4 mm i.d.) and inner cylinder (stainless tube with 10 mm i.d. or 8 mm i.d. covered with Pyrex tube) and set in a air bath of the apparatus. Rotating the inner cylinder up-to the shear angle at 0.25° or 1.5°, damped oscillation was induced by elastic restoring force of the sample and piano wire supporting the inner cylinder. From the damped oscillation curve after the shear angle became less than 0.2° or 1°, the shear modulus G and the viscosity coefficient η was calculated by

$$\alpha_T = \ln\theta_0/\theta_1 = \ln\theta_1/\theta_2 = \cdots = (1/n)\ln\theta_0/\theta_n$$
$$\kappa = 4\pi^2 I/T_d^2 - k_0$$
$$R = (2\alpha_T/T_d)\, I$$
$$G = \kappa/4\pi l\,(1/r_1^2 - 1/r_2^2)$$
$$\eta = R/4\pi l\,(1/r_1^2 - 1/r_2^2)$$

where α_T: logarithmic decrement, θ_i: amplitude of i th wave, κ : rigidity coefficient, I: moment of inertia, T_d : period, k_0 : piano wire constant, R : viscosity constant, G : shear modulus, l : length of the region where the sample and inner cylinder are in contact, r_1 : outer diameter of inner cylinder, r_2 : inner diameter of outer diameter, η : viscosity coefficient, n : order of vibration.

The viscoelasticity of myosin solution with 13mg/ml was measured using the inner cylinder with 10mm o.d. at shear angle 1° and that of the solution with 18.5mg/ml was measured by using the inner cylinder with 10 mm o.d. at 0.2°. The inner cylinder with 8 mm o.d. at 0.2° was used for other solutions. The temperature of the solutions was raised from the room temperature to 70°C at 0.35°C/min, and then lowered to 0°C at the same rate.

3. RESULTS

Figures 1 and 2 show the temperature dependence of G in heating process and cooling process, respectively. In the heating process a clear peak is observed for the solutions except for 13 and 18.5mg/ml at nearly the same temperature independently of myosin concentration. The average temperature at the peak is 35.6°C. In the cooling process a peak is observed for all solutions. The average temperature at the peak is 31.3°C. G increases sharply near the peak in the cooling process. When the sample is heated again, G decreases and the sol state found in the first heating was recovered similarly to the usual gel to sol first-order phase transition[5].

Figure 3 shows the myosin concentration dependence of G at 25°C observed in the heating process (●) and cooling process (○). Considerable concentration dependence of G is found in the cooling process.

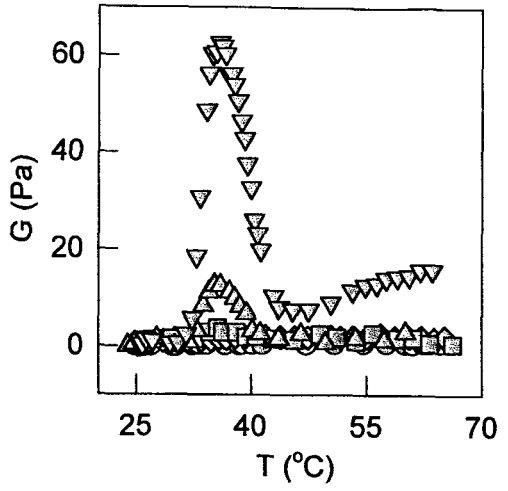

Fig.1 Shear modulus of myosin solution as a function of temperature in heating process. Myosin concentrations are (●)13mg/ml, (◆)18.5mg/ml, (■)24mg/ml, (▲)30mg/ml and (▼)40mg/ml, respectively.

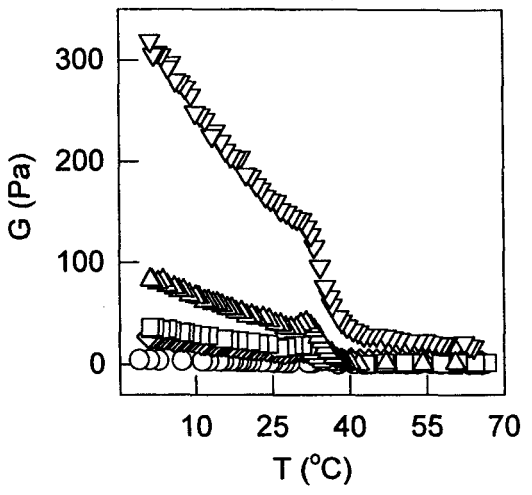

Fig.2 Shear modulus of myosin solution as a function of temperature in cooling process. Myosin concentrations are (○)13mg/ml, (◇)18.5mg/ml, (□)24mg/ml, (△)30mg/ml and (▽)40mg/ml, respectively.

Here we note that the outer diameter of the inner cylinder and the shear angle did not affect the value of G, and the characteristic behavior of η corresponds to that of G(not shown).

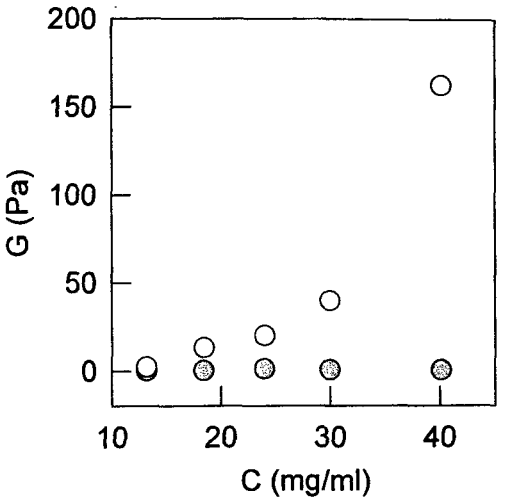

Fig.3 Shear modulus at 25°C measured in heating process (●) and cooling process (○).

4. DISCUSSION

Myosin consists of 6 subunits as shown in Fig. 4. Two among 6 peptides are named the heavy chains with high molecular weight and four are named the light chains with low molecular weight. Each heavy chain has a hydrophilic head portion and the two heavy chains are twisted together to form a neck region. Four light chains are connected to the neck of the head (tail side). Gelation of myosin has been generally considered to be resulted from aggregation of the myosin head and twisting of the myosin tail [1]. Heating myosin solution induces the oxidation of SH group and myosin dimer is formed at lower temperatures < 40°C and star-like multimer is formed between the head at higher temperatures >40°C [6, 7]. It was pointed out that S-S bridge is not produced between the head and the tail [6] and highly ordered water structure is important for the formation of myosin gel [8].

The difference of temperature dependence of G shown in heating process (Fig.1) and cooling process (Fig.2) indicates the irreversible change in myosin solution by heating. The significant increase of G in the cooling process (Fig.2) shows the sol to gel phase transition. The remarkable concentration dependence of G at the peak in the heating process (Fig.1) and G at lower temperatures in the cooling process (Fig.2) suggests a significant contribution of inter-molecular interaction of myosin molecules to G.

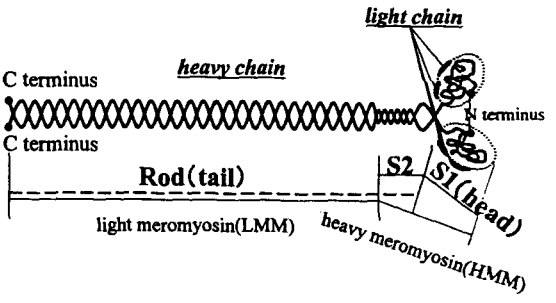

Fig.4 Schematic illustration of myosin molecule.

On the other hand, the experimental fact that the peak temperature and the sol to gel transition temperature do not depend on myosin concentration shows that this complex phenomenon is induced by a conformation change of a single myosin molecule. The large increase in G at lower temperatures as shown in Fig.2 is suggested to be related to "*Suwari*". From these results we speculated the mechanism of the sol to gel transition of myosin solution as follows:

Myosin molecules are dispersed with a certain amount of hydrated water at low temperatures. Hydrogen bondings break as temperature increases, resulting in conformation change of the myosin head and unwinding of the coiled-coil tail. This brings exposition of hydrophobic parts covered inside the hydrophilic part in myosin head at lower temperatures and S-S bondings are formed between myosin molecules. The increase in G around 35°C is attributed to the above conformation change of myosin molecules. At even higher temperatures two twisted heavy chains become unlaced and G decreases. Around 70°C most hydrogen bondings break up and the coiled-coil tails are almost unwound. With decreasing temperature, the hydrogen bondings are formed and the tails of different molecules twist again. This results in a considerable increase in G around 35°C. In the space between the twisting tails hydrated water can be accommodated. With increasing temperature, the same sol state is recovered. Thus the reversible sol to gel phase transition is mainly induced by hydrogen bonding formation on the myosin tail.

Recently, Ojima, et al. reported a reversible unfolding in the 2/3 part from the N-terminus and an irreversible unfolding in the 1/3 part from the C-terminus of Suketoudara (walleye pollack) light meromyosin by a heat treatment [9]. This finding is consistent with our speculation. It is required to determine the structure of a single myosin molecule by means of static and dynamic light scattering and NMR to confirm the gel-forming mechanism.

ACKNOWLEDGMENT

A part of this study was funded by a grant-in-aid for scientific research from the Ministry of Education, Science, Sports and Culture under grant numbers of 11555170 and 11640385.

REFERENCES

[1] K. Arai "Suisan syokuhin to tou alcohol", Towa Chem. Ind. Co., Ltd., Syokuzai Kaihatsu Kenkyu Center, (2000).

[2] T. Yasui, M. Ishioroshi and K. Samejima, "Syokuniku Kako Tokusei no Kettei Inshi", *Kagaku to Seibutsu* 19, 337-344 (1981).

[3] T. Numakura, N. Seki, I. Kimura, K. Toyoda, T. Fujita, K. Takama and K. Arai, "Cross-linking reaction of myosin in the fish paste during setting (*Suwari*)", *Nippon Suisan Gakkaishi*, 51(9), 1559-1565 (1985).

[4] J. Chang, H. Ichikawa, N. Noda, S. Goto, K. Osatomi and Y. Nozaki, "Effect of heating temperature and transglutaminase on gel-forming ability of salt-ground myofibrillar proteins from carp", *Nippon Suisan Gakkaishi* 64(4), 671-677 (1998).

[5] S. Kouchi, K. Ooi, T. Dobashi, H. Ichikawa and S. Kondo, to be published.

[6] Y. Itoh, Studies on the role of SS bonding in the formation of heat-induced gel of fish meat, *Nippon Suisan Gakkaishi*, 66(3), 380-383 (2000).

[7] K. Yamamoto; Electron Microscopy of Thermal Aggregation of Myosin, *J.Biochem.*108, 896-898 (1990).

[8] T. Tsuchiya and T. Sano, Gel-forming characteristics of fish meat proteins, *Nippon Shokuhin Kogyo Gakkaishi*, 35 (5) 367-376 (1988).

[9] T. Ojima, T. Higuchi, and K. Nishita ; Reversibility of Unfolding of Walleye Pollack Light Meromyosin by Heat-Treatment, *Fisheries Science* 65(3), 459-465 (1999).

(Received December 8, 2000; Accepted April 5, 2001)