JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2005,** *53,* 9193−9198 **9193**

Rheological Properties of Fast Skeletal Myosin Rod and Light Meromyosin from Walleye Pollack and White Croaker: Contribution of Myosin Fragments to Thermal Gel Formation

HIDETO FUKUSHIMA,[†] YOSHIE SATOH,[†] SUNG HO YOON,[‡] MARIE TOGASHI,[§] MISAKO NAKAYA,[†] AND SHUGO WATABE*,[†]

Laboratory of Aquatic Molecular Biology and Biotechnology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo, Tokyo 113-8657, Japan, Department of Food Science and Technology, Pukyong National University, Busan 608-737, Korea, and Diabetes Center, University of California at San Francisco, San Francisco, California 94143

Myosin rod and light meromyosin (LMM) of walleye pollack and white croaker were examined for their rheological properties by measuring dynamic viscoelastic parameters. Rods from walleye pollack and white croaker increased their storage moduli (G') in the ranges of 29−43 °C and 31−38 °C, respectively, in temperature sweep analysis. Walleye pollack LMM showed no peak of G' upon heating, whereas the white croaker counterpart exhibited a single sharp peak of G' at 35 °C. Loss modulus (G'') showed similar temperature-dependent changes for the two fish species as the case of G', irrespective of rod and LMM, although G'' values were lower than those of G'. Thus, rheological properties of rod and LMM were different between walleye pollack and white croaker. Taken together with data previously reported for myosin, it was considered that both myosin rods from walleye pollack and white croaker are attributed to thermal gel formation of myosin in a low-temperature range, though in a species-specific manner.

KEYWORDS: Light meromyosin; myosin rod; thermostability; viscoelasticity; walleye pollack; white croaker

INTRODUCTION

Rheological properties of fish meat gel products are affected by various factors such as myofibrillar protein contents (*1*), processing conditions (*2*−*4*), and additives (*5*, *6*). The most abundant myofibrillar component (*7*), myosin, is particularly important for the heat-induced gel formation of fish meat (*8*, *9*), because myosin unfolds and subsequently forms a gel network upon heating, which determines the quality of thermal meat gel products (*9*−*11*).

The myosin molecule is composed of two heavy chains of about 200 kDa and four light chains of about 20 kDa (*12*). Myosin is cleaved by limited proteolysis into two structural and functional parts. A globular N-terminal half composed of both heavy and light chains is called subfragment 1 (S1), which contains the ATP-hydrolysis and actin-binding sites (*13*). A C-terminal half, the rod, consisting of only heavy chain, has a coiled-coil structure of α-helices. Myosin rod is further cleaved into a C-terminal half, light meromyosin (LMM), which has an assembly competence domain responsible for thick filament formation (*14*, *15*), and an N-terminal half, subfragment 2 (S2), which connects S1 and LMM (*16*).

It has been reported that fish myosin forms a thermal gel in which rod reacts at lower temperatures and S1 does at higher temperatures (*17*, *18*). The first step in development of gel formation is induced by hydrophobic interaction in association with unfolding of rod upon heating at low temperatures (*17*, *19*). The gel formed at this first step is usually weakened at subsequent higher temperatures, which is partially attributed to dissociation of myosin aggregates (*20*). Upon further heating, gel networks become ordered and irreversible, which is ultimately responsible for the formation of the three-dimensional gel structure and rigidity of protein matrix (*20*).

We previously demonstrated differences in the thermal gel formation between walleye pollack and white croaker myosins by measuring dynamic viscoelastic parameters (*21*). Gel formation of walleye pollack myosin proceeded mainly in two steps: the first step at low temperatures of 29−46 °C with the increase of storage modulus (G') and a few peaks of loss modulus (G''), followed by the second step over 58 °C with the decrease of both G' and G''. On the other hand, the progress of gel formation of white croaker myosin was revealed by three steps upon heating: the first step at low temperatures of 30−36 °C with the increase of G' and G'', the second step at intermediate temperatures of 40−52 °C with the decrease of G' and G'', and

* Corresponding author: telephone +81-3-5841-7520; fax +81-3-5841-8166; e-mail awatabe@mail.ecc.u-tokyo.ac.jp.
[†] The University of Tokyo.
[‡] Pukyong National University.
[§] University of California at San Francisco.

10.1021/jf051223h CCC: $30.25
Published on Web 10/13/2005

the last step at high temperatures over 60 °C, again with the decrease of G' and G''. Thus, thermal gel-formation patterns of walleye pollack and white croaker myosin were different from each other, especially at lower temperatures. In addition, we showed that white croaker LMM, but not walleye pollack counterpart, had a polymer-forming ability through a disulfide bridge of cysteine residues (*22*). Hence, our previous observation suggests that the differences in myosin gel formation between walleye pollack and white croaker are possibly due to the species-specific structural properties of the rod region (*21*). However, the contribution of rod and LMM to myosin gel formation upon heating, except disulfide bridges in white croaker LMM, has not been examined directly.

The objective of this study was to investigate rheological properties by measuring dynamic viscoelastic parameters on temperature sweep analysis of rod and LMM regions of walleye pollack and white croaker myosins in order to clarify the mechanisms involved in their species-specific thermal gel formation. Thermodynamic properties of myosin rod were also determined for reference by differential scanning calorimetry (DSC).

MATERIALS AND METHODS

Materials. Frozen surimi (FA grade) of walleye pollack (*Theragra chalcogramma*) containing 4% sorbitol, 5% sugar, and 0.3% polyphosphates was kindly supplied by Nippon Suisan Kaisha Co., Ltd. (Tokyo, Japan). It was cut into 100 g blocks while frozen, packed into polyethylene bags, and stored at −80 °C until use. Live specimens of white croaker (*Pennahia argentata*) in an average body weight of about 300 g were collected in Tokyo Bay. Dorsal fast skeletal muscle of fish was immediately dissected, minced, mixed in 50% glycerol, and stored at −30 °C until use.

The expression vector plasmid pET-11a and *Escherichia coli* strain BL21 (DE3) pLysS were purchased from Novagen (Madison, WI). The expression vector pET-wpLMM containing walleye pollack LMM cDNA (*23*) and pET-wcLMM containing white croaker LMM cDNA (*22*) were previously constructed.

Preparation of Myosin Rod. Walleye pollack and white croaker fast skeletal myosins were prepared according to Hwang et al. (*24*). Myosin rod was prepared by the method of Stafford et al. (*25*) as modified by Hwang et al. (*24*). Myosin at a concentration of 10−15 mg/mL was digested at 10 °C for 30 min with *N*-tosyl-L-lysine chloromethyl ketone treated α-chymotrypsin (Sigma, St. Louis, MO) at an enzyme-to-myosin weight ratio of 1:130 in 20 mM potassium phosphate (pH 7.0) containing 0.12 M NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), and 0.1 mM dithiothreitol (DTT). Digestion was stopped by the addition of phenylmethanesulfonyl fluoride to a final concentration of 0.5 mM. The digest thus obtained was subjected to centrifugation at 100000*g* for 1 h, and the resulting pellet was dissolved in and dialyzed against 20 mM Tris-HCl (pH 8.0) containing 0.6 M KC1, 5 mM $MgCl_2$, and 0.1 mM DTT.

Preparation of LMM. LMM was prepared as described in our previous report (*26*). Briefly, LMM was expressed in *E. coli* by induction with isopropyl[26] β-D-thiogalactopyranoside. Cells were sonicated with an ultrasonic disrupter, and crude LMM was extracted. Crude LMM treated with low and high ionic strength buffers was dissolved in and dialyzed against 20 mM sodium pyrophosphate (pH 7.5) containing 30 mM KCl, 1 mM DTT, and 1 mM EDTA. After dialysis, the solution was applied to a DEAE-Toyopearl 650 M ion-exchange column (1.4 × 26 cm) equilibrated with the same buffer, and proteins adsorbed were eluted with a linear gradient of 30−600 mM KCl.

Protein Concentration. Protein concentrations were determined by the biuret method of Gornall et al. (*27*) with bovine serum albumin as the standard.

SDS−PAGE. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis was performed by the method of Laemmli (*28*) with 12.5% polyacrylamide gels containing 0.1% SDS. The gel was stained with a solution containing 0.05% Coomassie Brilliant Blue R-250, 50% methanol, and 10% acetic acid and destained with a solution containing 25% methanol and 7% acetic acid. The percent purity was calculated by using National Institutes of Health image software.

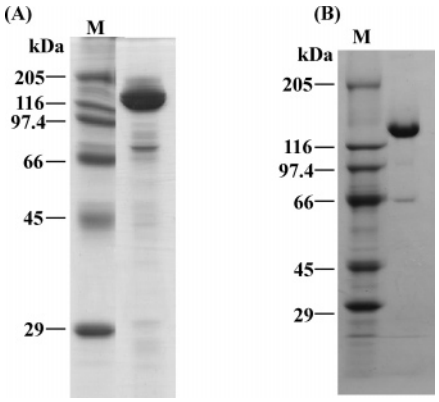

Figure 1. SDS–PAGE patterns of myosin rods from walleye pollack (**A**, 10 μg/lane) and white croaker (**B**, 3 μg/lane). M, molecular weight markers.

Dynamic Viscoelastic Measurement. Dynamic viscoelastic parameters of myosin rod and LMM were determined with a MCR300 rheometer (Physica, Stuttgart, Germany) by use of a 25 mm parallel plate geometry with a gap of 1.5 mm according to our previous report (*21*). Samples adjusted to 30 mg/mL in 20 mM potassium phosphate (pH 6.4) containing 0.6 M KCl were heated with an increasing rate of 1 °C/min from 5 to 80 °C. The storage moduli (G') of samples thus heated were determined by frequency sweep analysis at 80 °C under a constant amplitude strain of 1% in an angular frequency range of 0.1−10 s^{-1}. Dynamic viscoelastic parameters including G', loss moduli (G''), and damping factors (tan δ) were also determined in a temperature sweep mode according to our previous report (*21*) at a constant frequency of 1.0 Hz and amplitude strain of 1%. Samples prepared as in the case of frequency sweep analysis were heated from 5 to 80 °C at an increasing rate of 1 °C/min, and data were collected every 60 s.

DSC Analysis. DSC was performed with a MicroCal microcalorimeter model VP-DSC (MicroCal, Northampton, MA) equipped with a personal computer system. The solvent used was 50 mM Tris-HCl (pH 8.0) containing 0.6 M KCl, 5 mM $MgCl_2$, and 1 mM DTT, where the protein concentration was adjusted to about 1 mg/mL. DSC scans were performed at an increasing rate of 60 °C/h in a temperature range of 5−70 °C under air pressure at 2.7 Pa. DSC data were analyzed by use of a software package, Origin, developed by MicroCal according to Nakaya et al. (*29*). The heat capacity data were fit by using nonlinear least-squares, initially assuming that $\Delta H_{cal}/\Delta H_{vh} = 1$, where ΔH_{cal} and ΔH_{vh} are calorimetric and van't Hoff enthalpy, respectively. When data were not fit satisfactorily, heat capacity curves were subsequently fit by allowing ΔH_{cal} and ΔH_{vh} to float. After minimization by appropriate computer programs, the values for the thermal transition, including the transition temperature (T_m), ΔH_{cal}, and ΔH_{vh}, and the molar excess heat capacity (ΔC_p) were obtained. The cooperative ratio was determined as $\Delta H_{vh}/\Delta H_{cal}$.

RESULTS AND DISCUSSION

Rheological Properties of Rods. SDS−PAGE for purified myosin rods gave a major band of approximately 130 kDa and a few minor bands of possible degradation products (**Figure 1**). The percent purities of walleye pollack and white croaker rods were calculated to be over 90% and 95%, respectively, suggesting these preparations are pure enough for dynamic viscoelastic measurement and DSC analysis (*21*, *30*).

We previously conducted frequency sweep analysis for white croaker and walleye pollack myosins at 30 mg/mL and found that this protein concentration facilitated gel formation by myosins (*21*). Myosin rods at 30 mg/mL were also demonstrated by frequency sweep analysis to form gels upon heating at 80 °C in the present study, since both rods showed constant G'

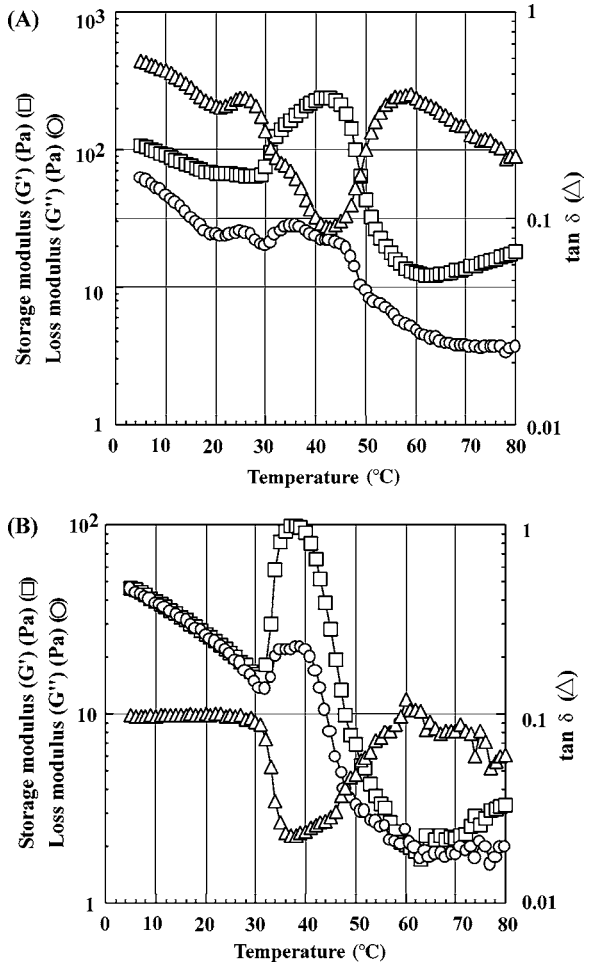

Figure 2. Changes in storage modulus (G'), loss modulus (G''), and tan δ during thermal gelation of myosin rods from walleye pollack (**A**) and white croaker (**B**) at 5–80 °C.

values in an angular frequency range of 0.1−10 s^{-1} at 1% amplitude strain (data not shown). Thus, temperature sweep analyses were performed at 5−80 °C for walleye pollack and white croaker rods at 30 mg/mL (**Figure 2**). As a result, two quite distinct patterns in gelation were observed with the two myosin rods. The changes in rheological properties are summarized in **Table 1**, together with those of LMMs described in the following section. The storage modulus, G', representing the elastic component, of walleye pollack rod decreased gradually until 20 °C, maintained a constant value until 29 °C, and then increased, reaching a peak at 43 °C, indicating that walleye pollack rod forms a gel network between 29 and 43 °C (**Figure 2A**). Upon further heating, G' decreased rapidly until 60 °C, followed by a gradual increase until 80 °C. The loss modulus, G'', showing the viscous component, decreased gradually until 19 °C. Thereafter, two peaks appeared at 25 and 36 °C with a shoulder at 44 °C, followed by a gradual decrease until 80 °C. Consequently, tan δ, the ratio of G'' to G', first decreased gradually until 20 °C, increased to 26 °C, and then decreased rapidly to 43 °C with a shoulder at 34 °C, indicating the occurrence of sol−gel transition in the range of 26−43 °C (*31*, *32*). Taken together, the changes of rheological properties for walleye pollack rod were very similar to those of myosin in a lower temperature range, although the increase of G' in the range of 43−46 °C and G'' in the range of 51−54 °C for myosin previously reported (*21*) were not found in the present rod (**Table 1**). These results suggest that walleye pollack rod is attributed to myosin gel formation in a lower temperature range.

Table 1. Major Changes of Dynamic Viscoelastic Parameters upon Heating at 5–80 °C of Rod and LMM from Walleye Pollack and White Croaker

	temperature		
protein	G' [a]	G'' [b]	tan δ [c]
	Walleye Pollack		
rod[d]	5–20 (decrease)	5–19 (decrease)	26–43 (decrease)
	29–43 (increase)	21–25 (increase)	58–80 (decrease)
	43–60 (decrease)	30–36 (increase)	
LMM[e]	5–20 (decrease)	5–30 (decrease)	14–80 (decrease)
	20–80 (increase)		
	White Croaker		
rod[d]	5–31 (decrease)	5–31 (decrease)	31–38 (decrease)
	31–38 (increase)	31–39 (increase)	60–80 (decrease)
	39–60 (decrease)	39–60 (decrease)	
LMM[e]	8–28 (decrease)	5–32 (decrease)	32–35 (decrease)
	32–35 (increase)	32–35 (increase)	
	35–38 (decrease)	35–39 (decrease)	

[a] Storage modulus. [b] Loss modulus. [c] Ratio of G'' to G'. [d] Values obtained from **Figure 2**. [e] Values obtained from **Figure 5**.

The G' values of white croaker rod decreased until 31 °C and then markedly increased, showing a peak at 38 °C (**Figure 2B**, **Table 1**). G' again decreased in the range of 39−60 °C and gradually increased until 80 °C. Rod G'' decreased gradually until 31 °C and then increased sharply, reaching a peak at 39 °C with a short lag phase and a marginal peak at 35 °C. Thereafter, G'' rapidly decreased until 60 °C, followed by a gradual decrease until 80 °C. Thus G'' showed temperature-dependent changes similar to those of G', although the peak height at about 40 °C was much larger in the former. Consequently, tan δ decreased markedly in the range of 31−38 °C, which agreed well with the changes in G' and G'', suggesting the development of gel elasticity of white croaker rod in this temperature range. It was noted that the increase of G' in the range of 47−54 °C and G'' in the range of 53−60 °C for myosin previously reported (*21*) were not found in the present rod (**Table 1**). These results suggest that white croaker rod is subject to myosin gel formation in a lower temperature range as in the case of walleye pollack.

DSC Analyses of Myosin Rods. DSC patterns of walleye pollack and white croaker rods are shown in **Figure 3** and their thermodynamic parameters are shown in **Table 2** together with those previously reported for LMMs (*22*). Walleye pollack rod showed two major endothermic peaks for unfolding with three T_ms at 28.3, 32.6, and 42.1 °C. On the other hand, white croaker myosin rod showed two major endothermic peaks with T_m at 32.2 and 38.2 °C. The lowest T_m of walleye pollack rod at 28.3 °C was lower than that of white croaker at 32.2 °C, whereas the highest T_m at 42.1 °C was rather higher than that of white croaker at 38.2 °C. Walleye pollack and white croaker rods unfolded in the temperature ranges of 18−50 °C and 26−60 °C, respectively (**Figure 3**), suggesting lower thermal stability in the former rod.

Thermodynamic properties of walleye pollack and white croaker rods are compared with those of their LMMs (*22*) in **Table 2**. Walleye pollack LMM unfolds in the temperature range of 18−50 °C (*22*), showing four endothermic peaks as shown in **Table 2**. Both DSC patterns of walleye pollack rod and LMM showed major endothermic peaks around 30 °C and minor ones in the range of 40−50 °C, suggesting that thermodynamic properties of walleye pollack rod are dominated by those of LMM. In the case of white croaker, T_m of rod at 32.2 °C was almost the same as that of a single endothermic peak of LMM at 32.1 °C (*22*). Thus, T_m at 38.2 °C of myosin rod seems to

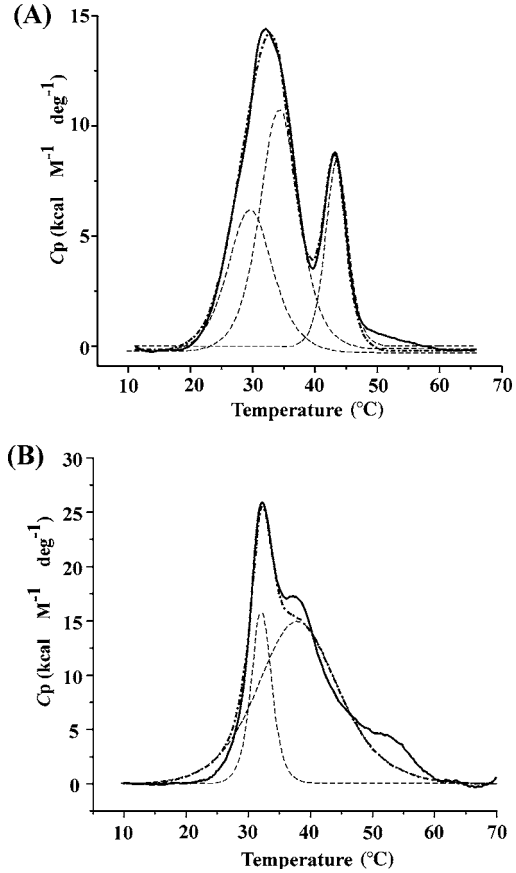

Figure 3. DSC scans of myosin rods from walleye pollack (**A**) and white croaker (**B**). The observed DSC patterns (solid lines) were subjected to smoothing treatment (bold dashed lines), together with their computer-calculated differential endotherms by the convolution analysis using three and two peaks for walleye pollack and white croaker, respectively (dotted lines).

Table 2. Thermodynamic Parameters on Thermal Unfolding of Rod and LMM from Walleye Pollack and White Croaker

protein	T_m[a] (°C)	ΔH_{cal}[b] (kcal/ mol)
	Walleye Pollack	
rod[c]	28.3	59.8 (0.4)
	32.6	87.5 (0.3)
	42.1	38.9 (1.4)
LMM[d]	27.7	58.4 (1.5)
	30.5	58.4 (1.3)
	35.8	61.4 (1.4)
	43.9	35.6 (5.7)
	White Croaker	
rod[c]	32.2	35.0 (2.4)
	38.2	129 (0.2)
LMM[d]	32.1	217

[a] T_m represents transition temperature. [b] The values in parentheses are ratio of ΔHvh to ΔHcal. ΔHvh and ΔHcal are calorimetric and van't Hoff enthalpies, respectively. [c] Values obtained from **Figure 3**. [d] Cited from ref *22*.

correspond to that of S2. However, it has been generally accepted that the thermostability of LMM is higher than that of S2 (*33*). Therefore, it is also possible to assume that both S2 and LMM contribute to each T_m value for rod.

Fibrous proteins of coiled-coil α-helices such as myosin rod consist of a series of 28-amino acid repeats, which themselves are composed of a unit of seven amino acid residues, *a*−*g* (*34*−*36*). While hydrophobic residues predominate at the interface between two α-helices, their hydrophobic interactions form the basis for the coiled-coil structure of two α-helices. Changes in CD spectra for walleye pollack and white croaker rods upon heating roughly reflected DSC patterns (data not shown), suggesting that endothermic peaks in DSC closely related to unfolding of α-helical structure as reported previously for carp myosin isoforms (*29*, *30*) and their LMMs (*37*, *38*).

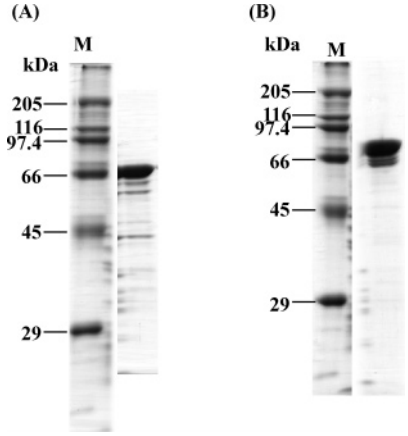

Figure 4. SDS–PAGE patterns of LMMs from walleye pollack (**A**) and white croaker (**B**). Proteins (10 mg/lane) were applied. M, molecular weight markers.

Rheological Properties of LMMs. LMMs were prepared by use of the *E. coli* expression system according to our previous papers (*22*, *23*, *26*, *37*). Although walleye pollack and white croaker LMM showed some minor bands of possible degradation products (**Figure 4**), their percent purities were calculated to be over 90% and 95%, respectively. Therefore, the purity was judged to be enough for dynamic viscoelastic measurement, referring to our previous paper (*22*).

Frequency sweep analysis showed constant G' values in the angular frequency range of 0.1−10 s^{-1} at 1% amplitude strain (data not shown) for walleye pollack and white croaker LMMs at 30 mg/mL upon heating at 80 °C, demonstrating that both LMMs formed gel in the case of their myosins (*21*) and rods. Thus, temperature sweep analyses were performed at 5−80 °C for LMMs at 30 mg/mL (**Figure 5**). For the results obtained in temperature sweep analysis on two LMMs, the *y* axis presented dynamic viscoelastic parameters in linear scale (**Figure 5**), because their changes were small compared to those of rods (see **Figure 2**). Walleye pollack LMM showed no apparent peak for G' and G'' (**Figure 5A**). G' decreased from 5 to 20 °C and increased gradually in a temperature range of 20−80 °C, whereas G'' decreased gradually until 30 °C and maintained almost constant levels until 80 °C. Consequently, tan δ maintained almost a constant value until 14 °C and then decreased gradually from 14 to 80 °C, suggesting that walleye pollack LMM gradually formed a gel upon heating.

On the other hand, G' of white croaker LMM increased until 8 °C, decreased until 28 °C, and then maintained a constant value until 32 °C. G' began to increase at 32 °C with a peak at 35 °C and thereafter decreased rapidly until 38 °C. G' maintained a constant value from near 40 to 70 °C and increased rapidly up to 80 °C. G'' decreased until 32 °C with a shoulder at 20 °C. G'' showed a peak at 35 °C, then decreased rapidly until 39 °C, and decreased gradually until 80 °C with a shoulder at 55 °C. It was noted that tan δ decreased rapidly in a temperature range of 32−35 °C and gradually in other temperature ranges during heating. These results suggest that white croaker LMM formed thermal gel predominantly in the former temperature range.

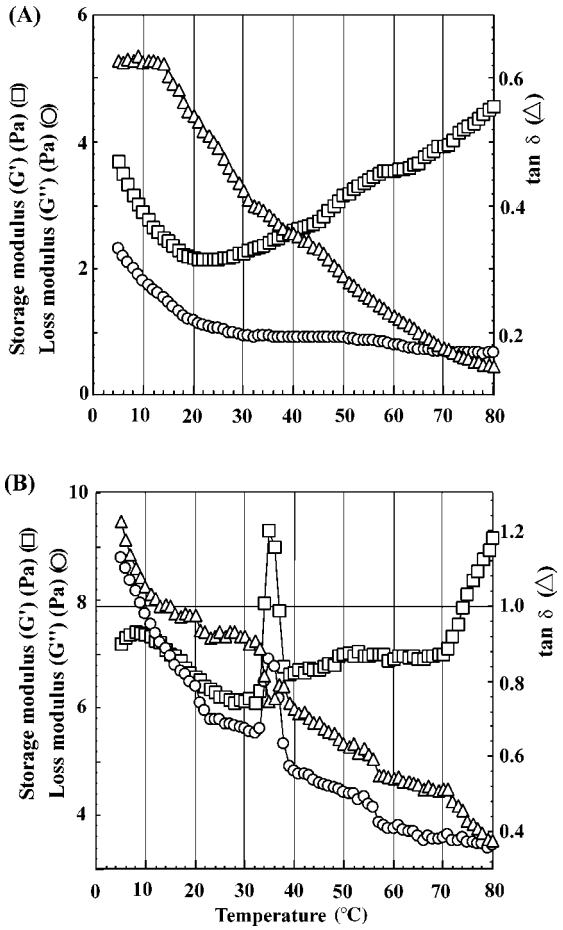

Figure 5. Changes in storage modulus (G'), loss modulus (G''), and tan δ during thermal gelation of LMMs from walleye pollack (**A**) and white croaker (**B**) at 5–80 °C.

Possible Mechanisms Involved in Gel Formation of Walleye Pollack and White Croaker Myosins. Dynamic viscoelastic measurements revealed that rheological properties of walleye pollack rod were almost similar to those of myosin, although rod showed no increase of G'' at higher temperatures. On the other hand, white croaker rod did not show any increase of G' and G'' and decrease of tan δ at higher temperatures in contrast to the myosin counterparts. Therefore, it is suggested that myosin rods from both white croaker and walleye pollack contributed to myosin gel formation in a lower temperature range. However, the temperature ranges of thermal unfolding and gel formation of walleye pollack and white croaker rods were different from each other. The temperature range of 29−43 °C in which G' of walleye pollack myosin rod increased was wider than that of white croaker, from 32 to 38 °C. This difference possibly reflects species-specific thermal myosin gel formation for the two myosins. As reported previously, G' of walleye pollack myosin did not increase in the higher temperature range over 47 °C, whereas G'' showed a peak at 54 °C. In the case of carp skeletal myosin, it is strongly suggested that the rod gel formed in a lower temperature range is strengthened by S1 aggregates to be formed in a higher temperature range (*17*). Walleye pollack rod forms the thermal gel, but subsequent S1 contribution to gel formation of myosin may be weak compared with white croaker counterpart.

Carp myosin forms thermal gel in which rod reacts at lower temperatures and S1 does at higher temperatures (*17*). It has been reported that walleye pollack myosin exposed irreversibly a heavy meromyosin (HMM)/LMM junction to the surface of the myosin molecule upon heating (*39*). The present DSC analysis on rod from walleye pollack and white croaker showed several T_m values in the temperature range of 28−42 °C and 32−38 °C, corresponding to lower temperature ranges of their myosin gel formation, respectively (**Table 2**). Therefore, it is suggested that walleye pollack and white croaker myosins unfold in the order of rod and S1 upon heating.

It has been reported that carp fast skeletal LMM forms an elastic gel by heat treatment, whereas HMM containing S1 and S2 does not (*17*). In the present study, frequency analysis indicated that walleye pollack and white croaker LMMs heated at 80 °C formed a thermal gel as described above. Meanwhile, rheological behaviors of the two LMMs were different from each other. In contrast to white croaker LMM, walleye pollack counterpart formed thermal gel gradually upon heating (see **Figure 5**). In our previous paper (*22*), DSC analysis showed that walleye pollack LMM unfolded in several steps, whereas white croaker counterpart unfolded in one step (**Table 2**). Thus, the temperature ranges in which LMMs unfolded almost corresponded to those of thermal gel formation, indicating that thermal gel formation of LMMs is induced by hydrophobic interaction accompanying with structural unfolding upon heating. Furthermore, the different thermodynamic properties of walleye pollack and white croaker LMMs possibly are attributed to their species-specific thermal gel formation in lower temperature ranges. Such species-specific differences between walleye pollack and white croaker LMMs were similar to those of their rods. Therefore, it is likely that thermodynamic and structural properties of rods are dominated by those of LMM. There are 51 amino acid substitutions between walleye pollack and white croaker LMMs (*35*, *36*). Thus, it seems that the different properties between the two LMMs are determined by such amino acid substitutions.

In this study, myosin rod was prepared by limited proteolysis of α-chymotrypsin. Therefore, a tail piece, locating in a C-terminal part and nonhelical region of myosin, was possibly cleaved away, resulting in changes of rheological and thermodynamic properties. The changes of the latter properties have been demonstrated for LMM preparation (*23*, *26*, *29*). Besides, myosin rod of walleye pollack contains a few isoforms (*40*). However, their composition has not been determined yet. On the other hand, LMM prepared by use of the *E. coli* expression system is homogeneous; thus the recombinant protein is considered to be a useful model for rheological and thermodynamic studies.

The present study determined thermodynamic properties and changes in dynamic viscoelastic parameters of myosin rods and LMMs from walleye pollack and white croaker. The two rods formed gel in a low temperature range which almost corresponded to a lower temperature range of the gel formation of myosin. It was found that walleye pollack rod formed gel in a temperature range wider than that of white croaker. Walleye pollack and white croaker LMMs also showed similar differences in terms of temperature-dependent gel formation. These differences are probably attributed to the species-specific myosin gel formation for walleye pollack and white croaker.

LITERATURE CITED

(1) Nakayama, T.; Sato, Y. Relationship between binding quality of meat and myofibrillar proteins. *J. Texture Stud.* **1971**, *2*, 475−488.

(2) Okada, M. Effect of washing on the jelly forming ability of fish meat. *Bull. Jpn. Soc. Sci. Fish.* **1964**, *30*, 255−261.

(3) Morioka, K.; Shimizu, Y. Contribution of sarcoplasmic proteins to gel formation of fish meat. *Nippon Suisan Gakkaishi* **1990**, *56*, 929−933.
(4) Lin, T. M.; Park, J. W. Extraction of proteins from Pacific whiting mince at various washing conditions. *J. Food Sci*. **1996**, *61*, 432−438.
(5) Akahane, T.; Chihara, S.; Yoshida, Y.; Tsuchiya, T.; Noguchi, S.; Ookami, H.; Matsumono, J. J. Application of differential scanning calorimetry to food technological study of fish meat gels. *Bull. Jpn. Soc. Sci. Fish.* **1981**, *47*, 105−111.
(6) Nakamura, S.; Ogawa, M.; Saito, M.; Nakai, S. Application of polymannosylated cysteine to surimi from roe-herring to prevent gel weakening. *FEBS Lett*. **1998**, *427*, 252−254.
(7) Bechtel, P. J. Muscle development and contractile proteins. *Muscle as Food*; Academic Press: Orlando, FL, 1986; pp 1−35.
(8) Iwata, K.; Kanna, K.; Okada, M. Kamaboko formation of mackerel and red sea bream myosins. *Bull. Jpn. Soc. Sci. Fish.* **1977**, *43*, 237.
(9) Akahane, T.; Chihara, S.; Yoshida, Y.; Tsuchiya, T.; Noguchi, S.; Ookami, H.; Matsumono, J. J. Roles of constituent proteins in gel properties of cooked meat gels. *Bull. Jpn. Soc. Sci. Fish.* **1984**, *50*, 1029−1033.
(10) Satoh, S.; Tsuchiya, T.; Matsumoto, J. J. Electron microscopic study of fine structure of kamaboko fish jellies. *Bull. Jpn. Soc. Sci. Fish.* **1984**, *50*, 1869−1876.
(11) Satoh, S.; Tsuchiya, T.; Matsumoto, J. J. Electron microscopic study of dispersion profiles of proteins in frozen surimi. *Bull. Jpn. Soc. Sci. Fish.* **1984**, *50*, 2117−2126.
(12) Harrington, W. F.; Rogers, M. E. Myosin. *Annu. Rev. Biochem.* **1984**, *53*, 35−73.
(13) Lowey, S.; Slayter, H. S.; Weeds, A. G.; Baker, H. Substructure of the myosin molecule. I. Subfragments of myosin by enzymic degradation. *J. Mol. Biol.* **1969**, *42*, 1−29.
(14) Sohn, R.; Vikstrom, K. L.; Strauss, M.; Cohen, C.; Szent-Gyorgyi, A. G.; Leinwand, L. A. A 29 residues region of the sarcomeric myosin rod is necessary for filament formation. *J. Mol. Biol*. **1997**, *266*, 317−330.
(15) Cohen, C.; Parry, D. A. D. A conserved C-terminal assembly region in paramyosin and myosin rods. *J. Struct. Biol*. **1998**, *122*, 180−187.
(16) Rattrie, N. W.; Regenstein, J. M. Action of crude papain on actin and myosin heavy chains isolated from chicken breast muscle. *J. Food Sci*. **1977**, *42*, 1159−1163.
(17) Sano, T.; Noguchi, S. F.; Matsumoto, J. J.; Tsuchiya, T. Thermal gelation characteristics of myosin subfragments. *J. Food Sci*. **1990**, *55*, 55−58.
(18) Visessanguan, W.; Ogawa, M.; Nakai, S.; An, H. Physicochemical changes and mechanism of heat-induced gelation of arrowtooth flounder myosin. *J. Agric. Food Chem*. **2000**, *48*, 1016−1023.
(19) Li-Chan, E.; Nakai, S.; Wood, D. Relationship between functional and physicochemical properties of muscle proteins. *J. Food Sci*. **1985**, *50*, 1034−1040.
(20) Benjakul, S.; Visessanguan, W.; Ishizaki, S.; Tanaka, M. Differences in gelation characteristics of natural actomyosin from two species of bigeye snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*. *J. Food Sci*. **2001**, *66*, 1311−1318.
(21) Fukushima, H.; Satoh, Y.; Nakaya, M.; Ishizaki, S.; Watabe, S. Thermal effects on fast skeletal myosins from Alaska pollock, white croaker, and rabbit in relation to gel formation. *J. Food Sci*. **2003**, *68*, 1573−1577.
(22) Fukushima, H.; Yoon, S. H.; Watabe, S. Differences in polymer formation through disulfide bonding of recombinant light meromyosin between white croaker and walleye pollack and their possible relation to species-specific differences in thermal unfolding. *J. Agric. Food Chem*. **2003**, *51*, 4089−4095.
(23) Togashi, M.; Kakinuma, M.; Nakaya, M.; Ooi, T.; Watabe, S. Differential scanning calorimetry and circular dichroism spectrometry of walleye pollack myosin and light meromyosin. *J. Agric. Food Chem*. **2002**, *50*, 4803−4811.
(24) Hwang, G.-C.; Watabe, S.; Hashimoto, K. Changes of carp myosin subfragment-1 induced by temperature acclimation. *J. Comp. Physiol. B* **1990**, *160*, 141−146.
(25) Stafford, W. F.; Szentkiralyi, E. M.; Szent-Gyorgyi, A. G. Regulatory properties of single-headed fragments of scallop myosin. *Biochemistry* **1979**, *18*, 5273−5280.
(26) Kakinuma, M.; Nakaya, M.; Hirayama, Y.; Watabe, S.; Maeda, K.; Ooi, T. Thermal unfolding of the cold acclimation-type isoform of carp light meromyosin expressed by recombinant DNAs. *Fish. Sci*. **1997**, *63*, 1008−1013.
(27) Gornall, A. G.; Bardawill, C. J.; David, M. M. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **1949**, *177*, 751−765.
(28) Laemmli, U. K. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680−685.
(29) Nakaya, M.; Kakinuma, M.; Watabe, S.; Ooi, T. Differential scanning calorimetry and CD spectrometry of acclimation temperature-associated types of carp light meromyosin. *Biochemistry* **1997**, *30*, 99179−9184.
(30) Nakaya, M.; Watabe, S.; Ooi, T. Differences in the thermal stability of acclimation temperature-associated types of carp myosin and its rod on differential scanning calorimetry. *Biochemistry* **1995**, *34*, 3114−3120.
(31) Egelandsdal, B.; Martinsen, B.; Autio, K. Rheological parameters as predictor of protein functionality: a model study using myofibrils of different fiber-type composition. *Meat Sci*. **1995**, *39*, 97−111.
(32) Arntfild, S. D.; Murray, E. D.; Ismond, M. A. H.; Bernatsky, A. M. Role of the thermal denaturation-aggregation relationship in determining the rheological properties of heat induced networks for ovalbumin and vicilin. *J. Food Sci*. **1989**, *54*, 1624−1631.
(33) Li, Y.; Brown, J. H.; Reshetnikova, L.; Blazsek, A.; Farkas, L.; Nyitray, L.; Cohen, C. Visualization of an unstable coiled coil from the scallop myosin rod. *Nature* **2003**, *424*, 341−345.
(34) Parry, D. A. D. Structure of rabbit skeletal myosin: analysis of the amino acid sequences of two fragments from the rod region. *J. Mol. Biol.* **1981**, *153*, 459−464.
(35) Yoon, S. H.; Kakinuma, M.; Hirayama, Y.; Yamamoto, T.; Watabe, S. cDNA cloning and characterization of the complete primary structure for myosin heavy chain of white croaker fast skeletal muscle. *Fish. Sci*. **2000**, *66*, 1158−1162.
(36) Togashi, M.; Kakinuma, M.; Hirayama, Y.; Fukushima, H.; Watabe, S.; Ojima, T.; Nishita, K. cDNA cloning of myosin rod and the complete amino acid sequence of myosin heavy chain of walleye pollack fast skeletal myosin. *Fish. Sci*. **2000**, *66*, 349−357.
(37) Kakinuma, M.; Nakaya, M.; Hatanaka, A.; Hirayama, Y.; Watabe, S.; Maeda, K.; Ooi, T.; Suzuki, S. Thermal unfolding of three acclimation temperature-associated isoforms of carp light meromyosin expressed by recombinant DNAs. *Biochemistry* **1998**, *37*, 6606−6613.
(38) Kakinuma, M.; Hatanaka, A.; Fukushima, H.; Nakaya, M.; Maeda, K.; Doi, Y.; Ooi, T.; Watabe, S. Differential scanning calorimetry of light meromyosin fragments having various lengths of carp fast skeletal muscle isoforms. *J. Biochem. (Tokyo)* **2000**, *128*, 11−20.
(39) Higuchi, T.; Ojima, T.; Nishita, K. Heat-induced structural changes and aggregation of walleye pollack myosin in the light meromyosin region. *Fish. Sci*. **2002**, *68*, 1145−1150.
(40) Ojima, T.; Kawashima, N.; Inoue, A.; Amauchi, A.; Togashi, M.; Watabe, S.; Nishita, K. Determination of primary structure of heavy meromyosin region of walleye pollack myosin heavy chain by cDNA cloning. *Fish. Sci*. **1998**, *64*, 812−819.

Received for review May 25, 2005. Revised manuscript received August 24, 2005. Accepted September 6, 2005.

JF051223H