Dynamic Rheological Properties and Secondary Structure of Chicken Breast Myosin As Influenced by Isothermal Heating

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Gelation of chicken breast myosin in 0.6 M NaCl, pH or pD 6.5, was studied by monitoring changes in rheological properties and secondary structure using dynamic testing and Fourier transform infrared spectroscopy, respectively. Myosin heated at 75 °C had a higher loss tangent (more viscous), while myosin at 55 and 65 °C formed more elastic gels. After cooling, storage modulus (G'), loss modulus (G''), and loss tangent of myosin heated at 65 and 75 °C increased, but few changes were observed at 55 °C. Spectra of native myosin showed protein absorption bands for α-helix (1652 cm^{-1}) and β-sheet (1636 cm^{-1} paired with 1676 cm^{-1}) that decreased when heated above 45 °C. Intensity of a band at 1683 cm^{-1} increased and a new band at 1613 cm^{-1} appeared at 55 °C; these changes were attributed to formation of intermolecularly hydrogen-bonded β-structure.

Keywords: *Chicken breast, myosin, gelation, FTIR, dynamic testing*

INTRODUCTION

Because of the importance of myosin in the texture of comminuted meat products, myosin unfolding and aggregation during heating have been investigated to understand the mechanism of protein gelation and to manipulate protein gel functionality (Wicker et al., 1986; Dudziak et al., 1988). Heat-induced protein gelation was defined as a two-step process involving unfolding of proteins followed by aggregation into a three-dimensional network. Protein unfolding and orientation of unfolded molecules during aggregation influence the development of a gel network (Ferry, 1948; Hermansson, 1978).

During isothermal heating, the gel strength of porcine myosin increased up to 55 °C but decreased at 60 and 70 °C (Foegeding et al., 1986). Wu et al. (1991) also reported that chicken myosin started to gel at 43 °C and formed a weaker gel structure when heated at temperatures above 55 °C. These results implied that unfolding and interactions of myosin domains below 55 °C might be important to gel properties. Structural changes of rabbit myosin subfragment 1 (S-1) and hinge region occurred in the temperature range 37–45 °C (Sutoh et al., 1978; Swenson and Ritchie, 1980; Burke et al., 1987). Head-to-head aggregation of rabbit myosin was observed using electron microscopy when heated isothermally at 40 °C (Yamamoto, 1990; Sharp and Offer, 1992). From the results of differential scanning calorimetry (DSC), the domain with the lowest stability in rabbit myosin was assigned to the hinge region; the denaturation peaks between 48 and 60 °C were assigned to light meromyosin (LMM) (Potekhin et al., 1979; Bertazzon and Tsong, 1990). Myosin S-1 had a melting temperature around 46 °C under high-salt conditions (Bertazzon and Tsong, 1989; Shriver and Kamath, 1990). In contrast, the helical content of turkey breast myosin (Arteaga and Nakai, 1992) and rabbit LMM (Morita and Yasui, 1991), as measured by circular dichroism, decreased at temperatures as low as 30 °C. On the basis of the above observations, the mechanism of myosin denaturation is still unclear.

Infrared absorption spectroscopy has been recognized as an important tool for estimating the secondary structure of polypeptides and proteins (Krimm, 1962). The thermal unfolding of α-helix, accompanied by an increase of β-structure, has been reported in several proteins using Raman spectroscopy (Lin and Koenig, 1976; Painter and Koenig, 1976; Clark et al., 1981; Li-Chan and Nakai, 1991) and Fourier transform infrared spectroscopy (FTIR) (Byler and Purcell, 1989; Herald and Smith, 1992). Koenig and co-workers suggested that β-structure was formed by intermolecular hydrogen bonding (Lin and Koenig, 1976; Painter and Koenig, 1976). Clark et al. (1981), using IR and laser–Raman spectroscopy to examine globular protein gels, also observed that formation of β-sheet correlated with the aggregation process. This finding was later supported by Byler and Purcell (1989), as evidenced by the appearance of new peaks near 1614 and 1684 cm^{-1} in β-lactoglobulin and bovine serum albumin after heat treatment. The band associated with α-helix decreased in intensity but was still observed after thermal denaturation, suggesting not all of the α-helices had uncoiled. Herald and Smith (1992) compared the changes in secondary structure with DSC transition temperatures and rheological properties of S-ovalbumin. The authors observed a decrease in β-sheet, α-helix, and 3_{10}-helix and increases in peaks of 1614 and 1684 cm^{-1} when heated. Increases in intensity of bands at 1614 and 1684 cm^{-1} were found to correspond to increases in G' of S-ovalbumin gels during heating.

Since the mechanism of myosin gelation is not clear, it is necessary to understand the relationship between myosin structural changes and gel development. In this study, we used small strain dynamic testing to follow the gelation of chicken breast myosin during isothermal heating and monitored changes in its secondary structure using FTIR.

MATERIALS AND METHODS

Extraction of Myosin. Breast muscle myosin from commercial broilers was extracted immediately after sacrifice as described by Nauss et al. (1969) at 4 °C. Muscles were ground twice through a 4 mm plate (KitchenAid, Hobart Corp., Troy, OH), and the minced meat was extracted with 3 volumes of modified Guba-Straub solution (0.3 M KCl, 0.1 M KH_2PO_4, 50 mM K_2HPO_4, 1 mM EDTA, 4 mM sodium pyrophosphate, pH 6.5) for 10 min with vigorous stirring but without foaming. The extract was diluted with 3 volumes of distilled water, and the

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muscle residue was filtered through three layers of cheesecloth. The filtrate was diluted with 6.5 volumes of 1 mM EDTA with rapid stirring and allowed to precipitate overnight. The supernatant was removed by siphoning and precipitated protein was collected by centrifugation at 1000g for 45 min at 4 °C. The precipitate was resuspended in 3 M KCl/25 mM PIPES buffer, pH 7.0, and subsequently diluted with distilled water to 0.6 M KCl/5 mM PIPES. Magnesium chloride and sodium pyrophosphate were added to final concentrations of 5 and 3 mM, respectively. The solution was stirred vigorously for 10 min without foaming and centrifuged at 78000g for 1 h at 4 °C (Beckman ultracentrifuge, Model L7-65, Beckman Instruments, Inc., Palo Alto, CA). Solid $(NH_4)_2SO_4$ was added slowly to 35% saturation with constant stirring, and the solution was centrifuged at 10000g for 15 min at 4 °C. The supernatant was brought to 48% saturation. The myosin pellet was collected by centrifugation at 10000g for 15 min at 4 °C and stored at −20 °C.

Prior to use, myosin was resuspended in 1 mM EDTA/0.6 M NaCl/50 mM sodium phosphate buffer, pH 6.5, and dialyzed against two changes of the same buffer. Myosin was dialyzed a third time against the same buffer but without EDTA. The dialyzed myosin solution was centrifuged at 78000g for 1 h at 4 °C. Myosin concentration was determined using an extinction coefficient of $E^{1\%} = 5.5$ at 280 nm (Swenson and Ritchie, 1980).

The deuterated myosin was prepared by concentrating 2 mL of a 2 mg/mL myosin solution in microconcentrators (Centricon 10, Amicon, Danvers, MA) at 5000g for 4 h and replacing with 0.6 M NaCl/50 mM KD_2PO_4 buffer, pD 6.5 (measured as pH + 0.4). The deuterium exchange process was repeated twice. The microconcentrators were purged with dry nitrogen and capped prior to centrifugation.

Dynamic Rheological Measurements. Dynamic rheological testing was used to monitor gel development of myosin (10 mg/mL) at 55, 65, and 75 °C for 30 min. Measurements were performed using a Rheometrics fluid spectrometer (RFS-8400, Rheometrics, Inc., Piscataway, NJ) fitted with a 50 mm diameter parallel plate and 100 g-cm transducer. Temperature was controlled with a programmable circulating oil bath (MTP-6 microprocessor, Neslab Instruments, Inc., Newington, NH). Myosin solution was loaded in the sample cup and equilibrated at the desired temperature for 1 min prior to measurement. Temperature of the sample was verified using a thermocouple connected to the upper plate of the sample cup. The gap between upper and lower plates was controlled between 1 and 1.5 mm. Storage (G') and loss (G'') moduli were recorded continuously at a frequency of 10 rad/s (Wang et al., 1990) and a strain of 0.03. Strain was determined in preliminary experiments by conducting strain sweeps (0.0001–0.5) at each isothermal temperature. The strain selected was within the linear range of all conditions. Loss tangent ($\tan \delta = G''/G'$) was used to show the relative viscoelastic properties. It is zero for a pure solid and infinite for a pure liquid. The slopes of transitions were measured at selected points using the software provided by the manufacturer. The inflection points were defined by the intersection of two regression lines determined from the slopes.

Fourier Transform Infrared Spectroscopy. Deuterated myosin (2 mg/mL in 0.6 M NaCl/50 mM potassium dideuterium phosphate, pD 6.5) in capped glass vials was purged with nitrogen gas for 10 min, heated at 45, 55, 65, and 75 °C for 30 min in a water bath, and cooled in ice water. Both heated and unheated myosin solutions in capped vials were purged with dry nitrogen gas for 10 min prior to testing. Myosin solutions (100 μL) were loaded into a circular demountable cell (Model P-3 N930-1117, Perkin-Elmer, Norwalk, CT) with CaF_2 windows and Teflon spacers of 75 μm path length in a glovebox under an atmosphere of nitrogen which was dried by passing through $CaSO_4$ desiccant. Infrared spectra were collected at ambient temperature using a FTIR spectrometer (Model 1800, Perkin-Elmer) equipped with an incandescent wire source, a potassium bromide coated beam splitter, and a broad-range mercury/cadmium/telluride detector. All spectra were scanned 500 times at a resolution of 2 cm^{-1}. Second-derivative analysis (CDS-2 applications software, Perkin-Elmer) was performed to enhance resolution, using the Savitzky–Golay derivative routine (Savitzky and Golay, 1964) with a 13-data point (13 cm^{-1}) window. Spectra contributed from a deuterated buffer blank and residual water vapor were subtracted before analysis. Band frequencies were assigned to secondary structural features on the basis of published values (Byler and Susi, 1986, 1988; Prestrelski et al., 1991a,b; Susi and Byler, 1983, 1987).

Statistical Analysis. A completely randomized design containing six replications was used to study the influence of isothermal heating and cooling on dynamic rheological properties and FTIR studies of myosin. Two replicates were evaluated within each of three extractions. Tukey's test and analysis of variance (two-way ANOVA) were performed to test the significance between replications and treatments using MSTAT (version C, 1990, Michigan State University, East Lansing, MI).

RESULTS AND DISCUSSION

Dynamic Rheological Properties. Myosin heated isothermally at 45 °C did not gel as G' and G'' did not change. Myosin heated at 55 °C showed a sharp increase in both G' and G'' during the first 5 min (Figure 1a). Little change in G'' was found on further heating. A slight increase and then decrease in G' was observed when myosin was heated at 65 °C during the first 5 min, followed by a gradual increase toward equilibrium (Figure 1b). An initial transition was also observed in G''; however, prolonged heating showed little effect on G'' development. Isothermal heating at 75 °C caused an initial increase in G' which did not change on further heating. Little change was observed in G'' at this temperature (Figure 1c). Myosin at 65 and 55 °C had about 3–4-fold higher G' than myosin at 75 °C after 30 min of heating, while G'' values were not different (Table 1).

Loss tangent or tangent δ at all three isothermal temperatures showed a two-phase transition (Figure 2). Tangent δ decreased rapidly at 55 °C (slope −0.311), indicating sol-to-gel transitions. After 4.3 min of heating, tangent δ decreased at a slower rate (slope −0.014) and reached equilibrium, suggesting gel development was complete. The same trend was observed at 65 and 75 °C (Table 2). The low tangent δ and high G' at both 55 and 65 °C after 30 min of heating (Table 1) indicated the development of gel elasticity. Myosin at 75 °C had the highest loss tangent (the most viscous) after 30 min of heating. The high loss tangent and low G' of myosin at 75 °C suggested poorer gel quality.

Wu et al. (1991) reported that myosin gelation followed second-order kinetics with a maximum rate constant at 52 °C. The maximum equilibrium shear modulus was between 48 and 50 °C in 0.5 M NaCl/10 mM sodium phosphate, pH 7.0. Gels formed between 44 and 56 °C had a greater shear modulus and were more elastic than those formed between 58 and 70 °C. In our study, high-temperature heating (75 °C) produced poor myosin gels; however, no significant differences in G', G'' and loss tangent were observed between myosin gels formed at 55 and 65 °C. One difference was that Wu et al. (1991) reported shear modulus under equilibrium conditions that required longer heating times, while we recorded the moduli after 30 min of heating. The higher negative value of the second tangent δ slope at 55 °C might lead to a lower loss tangent (higher elasticity) than those at 65 and 75 °C during prolonged heating.

Storage moduli of myosin paralleled G'' during cooling after heating at all three temperatures (Figure 1d–f). Cooling of myosin heated at 65 and 75 °C for 40 min caused a significant increase in G', G'', and tangent δ (Table 1). Gel elasticity was increased (higher G'), but the gel had more liquid-like behavior due to cooling (higher tangent δ). In contrast, smaller changes in moduli were observed during cooling of myosin heated at 55 °C. Increases in G'

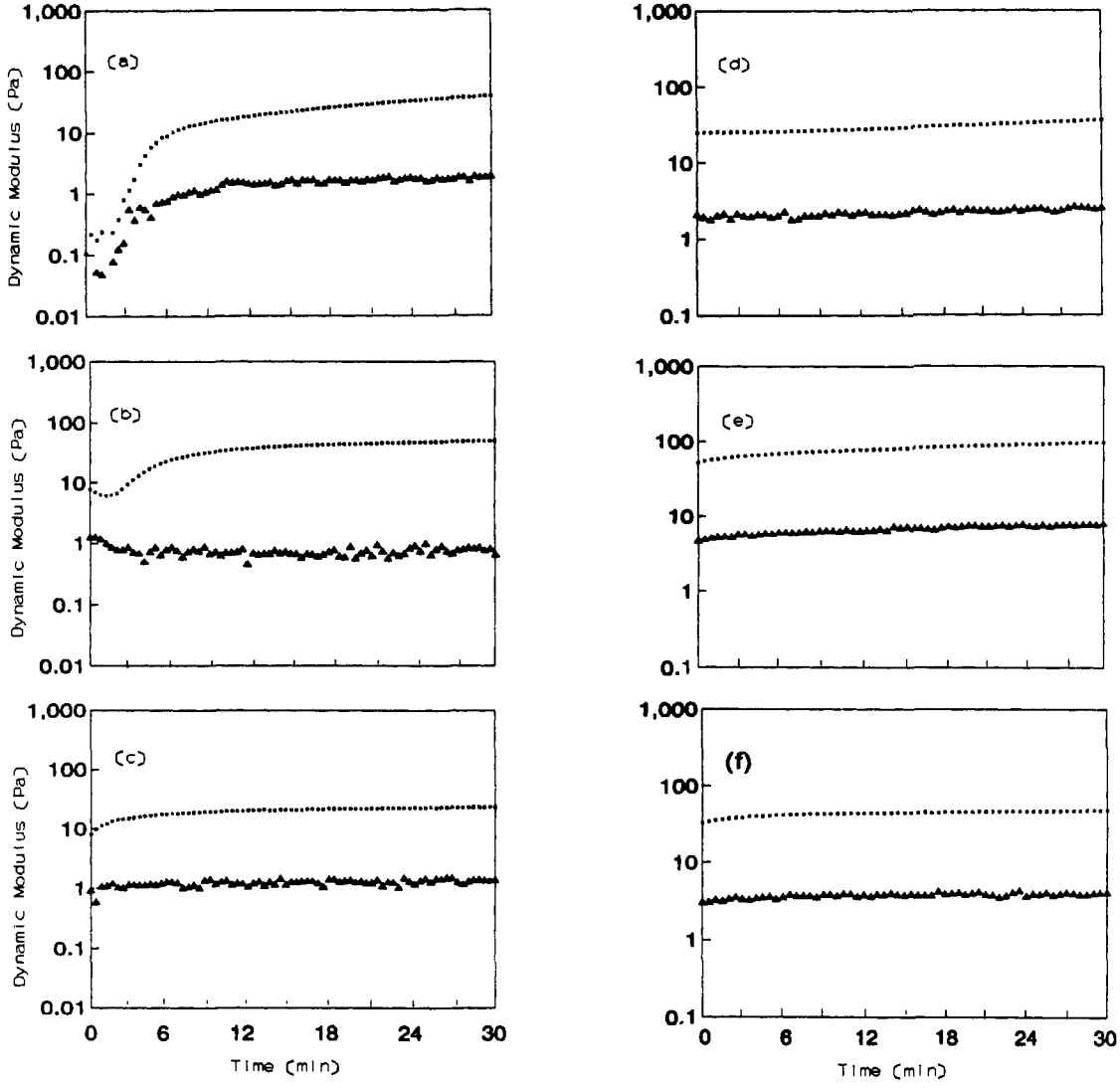

Figure 1. Effect of isothermal heating for 30 min and cooling on storage (dot) and loss (triangle) moduli of myosin in 0.6 M NaCl/50 mM sodium phosphate buffer, pH 6.5. Myosin was heated at (a) 55, (b) 65, and (c) 75 °C and cooled to 30 °C after heating at (d) 55, (e) 65, and (f) 75 °C.

Table 1. Dynamic Rheological Properties of 10 mg/mL Myosin in 0.6 M NaCl/50 mM Sodium Phosphate Buffer, pH 6.5, after Isothermal Heating and Cooling[a]

	temp		
moduli	55 °C	65 °C	75 °C
heating			
G' (Pa)	37.6^{cd}	50.2^{c}	13.7^{e}
G'' (Pa)	1.47^{c}	1.42^{c}	0.69^{c}
loss tangent	0.04^{ef}	0.03^{f}	0.06^{cd}
cooling			
G' (Pa)	24.7^{de}	84.7^{b}	45.5^{cd}
G'' (Pa)	1.23^{c}	5.57^{b}	3.90^{b}
loss tangent	0.05^{de}	0.07^{c}	0.09^{b}

[a] Myosin was heated at the indicated temperature for 30 min and cooled for 40 min to a final temperature of 27–30 °C. Means within the same modulus (both heating and cooling) followed by the same letter are not different ($P > 0.05$).

and loss tangent due to cooling were also reported by Hines and Foegeding (1993) using α-lactalbumin, β-lactoglobulin, bovine serum albumin (BSA), and whey protein isolate initially heated to 80 °C. According to our study, increases in moduli due to cooling occurred only for myosin heated above 55 °C. This might suggest that cooling promoted additional intra- or intermolecular protein–protein interactions in networks formed at higher heating temperatures.

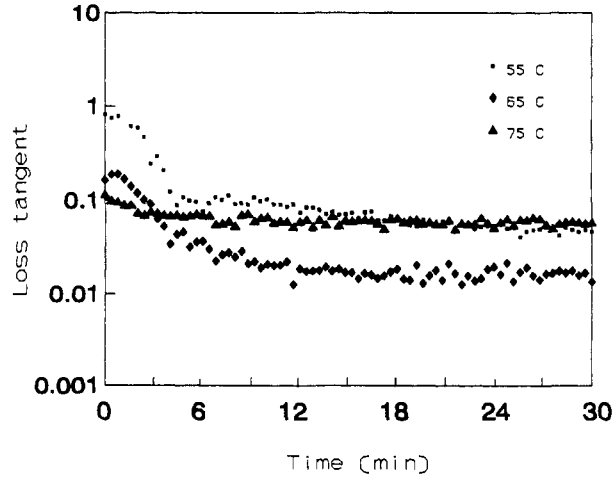

Figure 2. Effect of temperature on loss tangent of myosin (10 mg/mL) during isothermal heating in 0.6 M NaCl/50 mM sodium phosphate buffer, pH 6.5.

Secondary Structure by FTIR. Identification of peaks in second-derivative spectra of myosin is given in Table 3. The spectrum of unheated myosin showed two major peaks near 1636 and 1651 cm^{-1}, as well as a minor peak around 1676 cm^{-1} (Figure 3a). The bands absorbing between 1650 and 1657 cm^{-1} were identified as α-helix; a

Table 2. Effect of Temperature on Loss Tangent of Myosin in 0.6 M NaCl/50 mM Sodium Phosphate Buffer, pH 6.5, during Isothermal Heating for 30 min[a]

	temp		
	55 °C	65 °C	75 °C
slope 1	−0.311[b]	−0.124[a]	−0.090[a]
slope 2	−0.014[b]	−0.005[a]	−0.004[a]
inflection point (min)	4.3[b]	6.0[a]	4.1[b]

[a] Means within rows followed by the same letter are not different ($P > 0.01$).

Table 3. Band Identification of Myosin Second-Derivative Spectra (cm^{-1}) Obtained by Fourier Transform Infrared Spectroscopy[a]

band assignment[b]	unheated myosin	isothermal heating temp			
		45 °C	55 °C	65 °C	75 °C
turns; hydrogen-bonded β-sheet		1683	1683	1683	1683
β-sheet	1676	1675			
turns			1669	1669	1672
turns				1664	1666
α-helix; loops				1658	1657
α-helix	1652	1651	1650	1650	1651
irregular				1648	1646
irregular			1640	1641	1640
β-sheet	1636	1635	1635	1636	1636
extended strand		1629		1631	1627
hydrogen-bonded β-sheet			1614	1613	1614

[a] Myosin (2 mg/mL in 0.6 M NaCl/50 mM potassium dideuterium phosphate buffer, pD 6.5) was heated isothermally at the indicated temperature for 30 min. [b] Band assignments were based on the works of Prestrelski et al. (1991a,b), Byler and Susi (1988, 1986), and Susi and Byler (1987, 1983).

strong component at 1637 cm^{-1} paired with a minor peak in 1670–1680-cm^{-1} region was identified as β-structure (Byler and Susi, 1986; Krimm and Bandekar, 1986; Susi and Byler, 1987).

Myosin contains two globular heads and a rod region, which is a double-stranded coiled coil (Lowey et al., 1969). On the basis of the three-dimensional structure of chicken pectoralis myosin S-1 proposed by Rayment et al. (1993), myosin S-1 is mainly composed of α-helices and β-strands connected by turns and loops. The band at 1651 cm^{-1} was probably due to α-helix in the myosin S-1 and rod portions. The peak at 1636 cm^{-1} indicating β-structure was attributed to the globular head region of myosin. Since the band frequency of loops was near 1655 cm^{-1} (Prestrelski et al., 1991a), it is possible that the absorption by loops and α-helix overlapped.

The spectrum of myosin heated at 45 °C for 30 min was similar to that of native myosin, showing two major bands around 1635 and 1651 cm^{-1} (Figure 3b). The band of 1676 cm^{-1} in native myosin was split into two components of 1683 and 1675 cm^{-1} on heating. A weak band at 1683 cm^{-1} was assigned to type II β-turns according to the findings of Prestrelski et al. (1991a) or to type III turns on the basis of the results of Stein et al. (1991). A shoulder around 1629 cm^{-1} (extended strands) appeared. Circular dichroism (CD) studies revealed full reversibility of secondary structure of turkey breast myosin on cooling after heating at 40 °C for 5–30 min and at 50 °C for 5 min (Arteaga and Nakai, 1992). Morita and Yasui (1991) reported that more than 80% of the helical content of rabbit skeletal LMM could be restored by cooling after heating at 70 °C and above for 20 min. It is possible that myosin heated at 45 °C renatured during cooling because calorimetric studies have shown that chicken breast muscle myosin unfolds as low as 35 °C (Wang, 1993). As we evaluated myosin structure after cooling, FTIR results would not reveal changes in secondary structure at 45 °C.

When myosin was heated at 55 °C, the band at 1675 cm^{-1}, which was the high-frequency component of β-structure, disappeared (Figure 3c,d). A weak band near 1669 cm^{-1} (turns) appeared in some of the myosin spectra at 55 °C. Unresolved broad bands occurred between 1660 and 1620 cm^{-1} with distinguishable peaks around 1650, 1640, and 1635 cm^{-1}. Peaks that absorbed at 1651 (α-helix) and 1635 cm^{-1} (β-sheets) decreased in intensity. These changes indicated irreversible unfolding of helices and β-structures when heated at 55 °C. The new peak observed at 1640 cm^{-1} might indicate 3_{10}-helices (1639 cm^{-1}) or, more likely, the formation of irregular structures (1640–1648 cm^{-1}) (Prestrelski et al., 1991a,b). The peak at 1629 cm^{-1} first observed at 45 °C and other minor bands might be masked due to the broadness of bands in the frequency region 1660–1629 cm^{-1}. The spectra within the 1660–1620-cm^{-1} region were not constant at 55 °C, which might be due to sol-to-gel transitions, so myosin structures were highly variable after cooling.

The band around 1669 cm^{-1} observed at 55 °C did not change when myosin was heated at 65 and 75 °C, but a new peak around 1664–1666 cm^{-1} appeared (Figure 3e,f). These frequencies have been identified as turns (Byler and Susi, 1986). A decrease in intensity of the broad peaks in the 1660–1620-cm^{-1} region was observed when compared to myosin at 55 °C. A shoulder at 1658 cm^{-1} occurred in some of the spectra at 65 and 75 °C, which was within the frequency range of α-helix. This shoulder might be due to loops which absorb at 1655 cm^{-1} (Prestrelski et al., 1991a). Myosin bands below 1636 cm^{-1} were assigned to the low-frequency component of extended strands (Prestrelski et al., 1991a). Bands at 1650 and 1636 cm^{-1} were present in myosin heated at 65 and 75 °C, indicating the structure was not completely unfolded by heat. These observations are similar to those made by Casal et al. (1988), using β-lactoglobulin B, as well as Byler and Purcell (1989), using β-lactoglobulin, BSA, and α-lactalbumin. Morita and Yasui (1991) also reported the helical content of rabbit LMM determined by CD decreased to about 10% when heated at 70 °C and above for 20 min. However, most of the helix unfolded during the early stage of fish myosin denaturation (Chan et al., 1992).

Myosin heated to 55 °C showed an increase in band intensity near 1683 cm^{-1}. The intensity of this band increased when myosin was heated at 75 °C. A new band near 1613 cm^{-1} appeared when myosin was heated at 55 °C, increased at 65 °C, and remained relatively constant at 75 °C (Figure 3). Similar observations were reported by other researchers. Herald and Smith (1992) reported an intense, sharp peak at 1614 cm^{-1} paired with a weaker peak at 1684 cm^{-1} for S-ovalbumin heated at 90 °C. Byler and Purcell (1989) observed new peaks near 1614 and 1684 cm^{-1} in B-lactoglobulin and bsa after heating to 80 and 75 °C, respectively. Clark et al. (1981) reported a 1620-cm^{-1} shoulder paired with a band near 1680 cm^{-1} in the IR spectrum of BSA heated at 90 °C. The absorption band near 1683 cm^{-1} has been assigned to turns in native proteins (Prestrelski et al., 1991a; Stein et al., 1991). However, with the appearance of the intense band below 1620 cm^{-1} at high temperature, this observation was assigned to intermolecular hydrogen-bonded β-sheet (Painter and Koenig, 1976; Clark et al., 1981; Byler and Purcell, 1989; Herald and Smith, 1992).

Relationship between Gelation and Secondary Structure. Myosin formed gels when heated at 55 °C and above, and the bands assigned as hydrogen-bonded

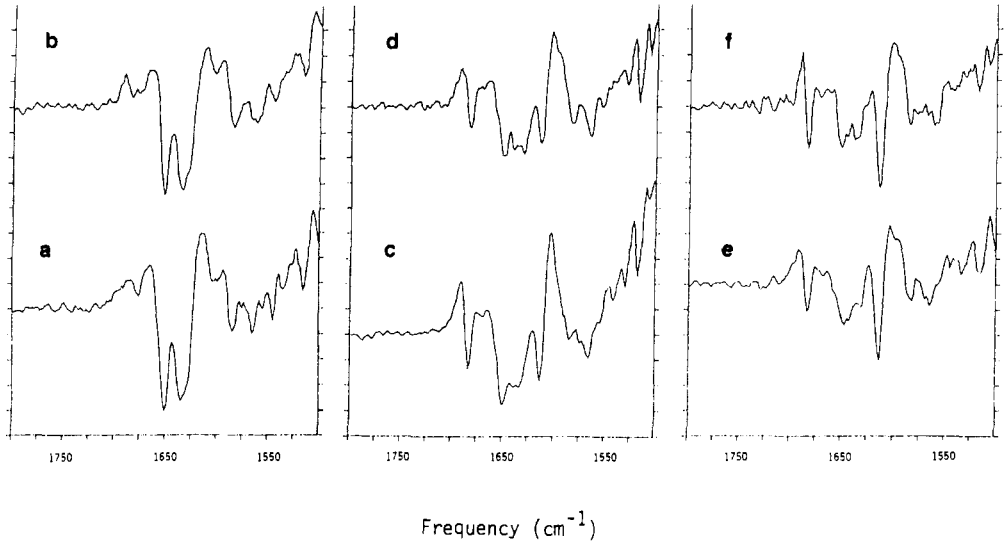

Figure 3. Effect of isothermal heating for 30 min on myosin spectra: (a) unheated; (b) 45 °C; (c and d) 55 °C; (e) 65 °C; (f) 75 °C. The spectra were recorded after myosin was cooled to ambient temperature.

β-sheet increased with temperature. No similar peaks were observed for myosin heated at 45 °C or for native myosin. These results suggested that hydrogen-bonded β-structure might be correlated with the formation of a gel network. Herald and Smith (1992) reported few changes in the secondary structure of S-ovalbumin before it was heated to the onset temperature determined by DSC. Decreases in β-sheet, α-helix, and 3_{10}-helix and increases in peaks of 1614 and 1684 cm^{-1} were observed between onset and denaturation temperatures at pD 3, 7, and 9. The authors also reported that increases in intensity of bands at 1614 and 1684 cm^{-1} corresponded to increases in G' of S-ovalbumin gels. Byler and Purcell (1989) observed intermolecularly hydrogen-bonded β-strands proceeded before thermal gelation of β-lactoglobulin and BSA. α-Lactalbumin, which did not gel, had no intense peak below 1620 cm^{-1}. On the contrary, Clark et al. (1981) reported gels or viscous solutions prepared using different concentrations of BSA all showed similar spectra. Therefore, the authors suggested that differences in protein properties (e.g., gel vs viscous solution) did not necessarily lead to widely different changes in secondary structures.

According to our results, myosin heated at 65 °C had the highest G' after cooling. No significant differences in G' were observed between myosin heated at 55 and 75 °C. The intensity of bands at 1613 and 1683 cm^{-1} was higher when myosin was heated to 65 and 75 °C than to 55 °C. Even though the hydrogen-bonded β-structure was only observed when myosin was heated above 55 °C (the sol-to-gel transition temperature), the intensity of these peaks did not follow the same trend with heating temperature as G'. There is a possibility that a certain intermediate proportion of hydrogen-bonded β-structure is optimal for gel properties; below or above this proportion, either no gel forms or coagulation occurs. The high proportion of intermolecular sheet structure and high loss tangent in myosin gel at 75 °C might be due to formation of locally strong interactions (aggregate formation) leading to nonhomogeneity, instead of an ordered gel network. The FTIR spectra showed changes in secondary structure for myosin at 55 °C and above, suggesting myosin unfolding and protein–protein interactions important to gel formation had occurred.

Conclusion. In this study, we evaluated the effect of isothermal heating and cooling on gel development of myosin. The sol-to-gel transition occurred at 55 °C, where α-helix and β-sheet decreased due to myosin unfolding. Unfolding of myosin continued when heated to a higher temperature. Myosin gels at 65 °C had the highest elasticity. Cooling of myosin caused an increase in G', G'', and loss tangent at 65 and 75 °C but had no effect on myosin at 55 °C. Bands assigned as hydrogen-bonded β-sheet appeared at 55 °C, and band intensity increased at 65 and 75 °C. The intensity of these bands did not correlate with increased G'. It was concluded that unfolding of myosin led to the formation of hydrogen-bonded β-sheet, which was not solely responsible for gel properties.

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