Shaw, P. E. Use of a simple solvent optimization program to improve separation of limonin in citrus juices. *J. Chromatogr. Sci.* **1986**, *24*, 364.
Shaw, P. E.; Wilson, C. W., III Debittering Citrus Juices with β-Cyclodextrin Polymer. *J. Food Sci.* **1983**, *48*, 646.
Shaw, P. E.; Wilson, C. W., III Reduction in Bitterness in Grapefruit Juice with β-Cyclodextrin Polymer in a Continuous-Flow Process. *J. Food Sci.* **1985**, *50*, 1205.
Shaw, P. E.; Buslig, B. S. Selective Removal of Bitter Compounds from Grapefruit Juice and from Aqueous Solutions with Cyclodextrin Polymers and with Amberlite XAD-4. *J. Agric. Food Chem.* **1986**, *34*, 837.
Shaw, P. E.; Tatum, J. H.; Wilson, C. W., III. Improved Flavor of Navel Orange and Grapefruit Juices by Removal of Bitter Components with β-Cyclodextrin Polymer. *J. Agric. Food Chem.* **1984**, *32*, 832.
Wagner, C. J., Jr.; Wilson, C. W., III.; Shaw, P. E. Reduction of Grapefruit Bitter Components in a Fluidized β-Cyclodextrin Polymer Bed. *J. Food Sci.* **1988**, *53*, 516.

Received for review February 8, 1988. Accepted July 29, 1988. Mention of a trademark or proprietary product is for identification only and does not imply a guarantee or warranty of the product by the U.S. Department of Agriculture.

Influence of Various Salts on Heat-Induced ANS Fluorescence and Gel Rigidity Development of Tilapia (*Serotherodon aureus*) Myosin

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Changes induced by salts in thermal transition temperatures (T_r) observed in tilapia myosin with a polarity probe, 8-anilino-1-naphthalenesulfonate (ANS), and a thermal scanning rheology monitor (TSRM) followed the Hofmeister series. The T_r of myosin–ANS was easily decreased by salting-in salts, and only sodium sulfate markedly increased the T_r, indicating that ANS apparently probes for an extremely hydrophobic site on myosin. Ammonium acetate and sulfate diminished the initial (low-temperature) TSRM rigidity peak, increased the maximum rigidity attained, and increased T_r values. Salting-out salts had no effect on the second rigidity increase, indicating that nonhydrophobic interactions are primarily involved in stabilizing protein structure at higher temperatures. These studies to date suggest that, upon heating, solubilized fish myosin initially undergoes a subtle change in conformation that promotes aggregation of hydrophobic residues to form a soft but elastic gel structure. With further heating to higher temperatures, a more generalized denaturation and aggregation occurs imparting rigidity to the elastic gel.

It has been suggested that hydrophobic interactions are important in the unique setting phenomenon (gelation near 40 °C) and higher temperature gelation of fish muscle proteins (Niwa, 1975; Niwa et al., 1981a,b). In constant-rate heating studies, Wicker et al. (1986) observed a transition at 37 °C in dilute solutions of myosin–ANS and related the increase in fluorescence to a change in the hydrophobicity of myosin. Since the increase and subsequent decrease in fluorescence of myosin–ANS preceded the onset of gelation, the authors concluded that a change in conformation involving hydrophobic residues was prerequisite for gelation.

The addition of salts has proven useful in studying the role of hydrophobic interactions in the denaturation of protein (Robinson and Jencks, 1965; Schrier and Schrier, 1967; Von Hippel and Wong, 1964; Melander and Horvath, 1977; Asghar et al., 1985). Recently, Wicker and Knopp (1988) showed that the transition temperature detected by myosin–ANS fluorescence could be increased or decreased by selected salts in an order following the Hofmeister series. If a change in the effective hydrophobicity of myosin is a prerequisite for further myosin denaturation and gelation (Wicker et al., 1986), then transition temperatures of denaturation and gelation should also be affected by salts of the Hofmeister series. The objective of this study was to determine the effects of selected salts on the denaturation and gelation of fish myosin to aid in elucidating the mechanism of heat-induced gelation of fish myosin.

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MATERIALS AND METHODS

Myosin Preparation. Myosin was prepared from tilapia (*Serotherodon aureus*) by the method of Akahane (1982) as modified by Wicker et al. (1986). Myosin was clarified by centrifugation at 100000*g* for 45 min and dialyzed overnight against 0.6 M KCl, 50 mM potassium phosphate, pH 6.5. Myosin solutions were stored under refrigeration, and experiments were completed in less than 1 week to minimize aging effects. Protein concentration was determined by micro-Kjeldahl standardized Biuret or by A_{280} ($\epsilon^{\%}_{cm}$ = 5.5; Swenson and Ritchie, 1980).

Fluorescence Thermograms. Fluorescence of myosin–ANS was measured as reported by Wicker et al. (1986). The heating rate was maintained at 1 °C/min with a Neslab programmable water bath. Fluorescence intensity (FI) was recorded on an SLM Model 8000 spectrofluorometer equipped for photon counting. The wavelengths of excitation and emission were 380 and 475 nm, respectively. The excitation and emission slits were 0.5 and 16.0 nm, respectively. The fluorescence intensity represents the number of photons counted for a period of 10 s.

Rigidity Thermograms. Changes in modulus of rigidity (G) were measured with a thermal scanning rheology monitor (TSRM) as described by Wicker et al. (1986). The myosin solution was heated at a constant rate of 1 °C/min with the same Neslab programmable water bath used in fluorescence studies.

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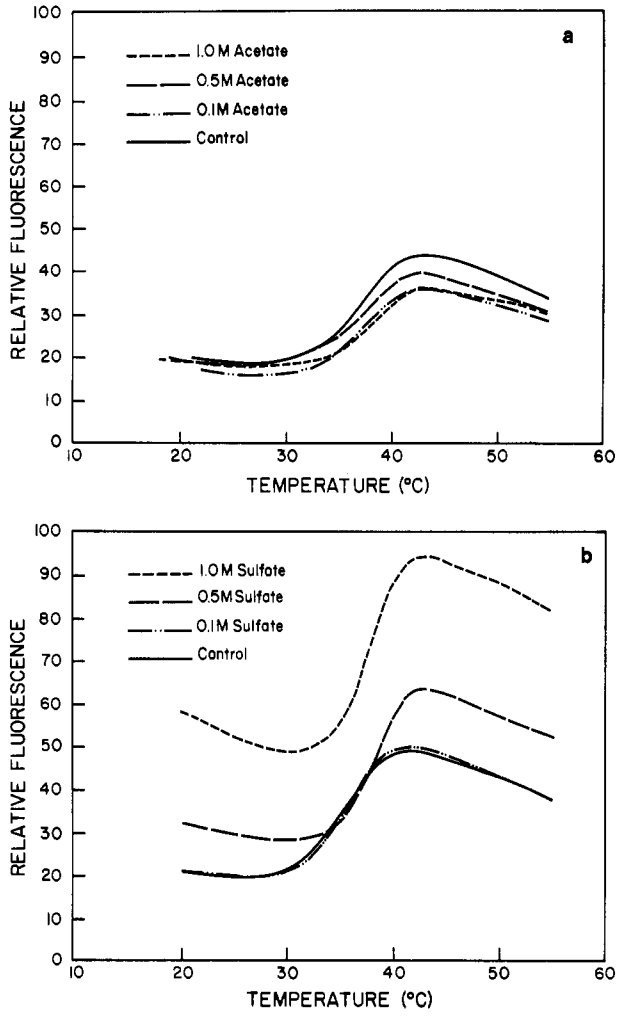

Figure 1. Fluorescence of myosin–ANS vs temperature (0.51 mg/mL myosin; 32 × 10^{-6} ANS; 0.6 M KCl; 50 mM potassium phosphate, pH 6.5): (a) (—) control, (-·-) 0.1 M ammonium acetate, (- - -) 0.5 M ammonium acetate, (···) 1.0 M ammonium acetate; (b) (—) control; (-·-) 0.1 M ammonium sulfate; (- - -) 0.5 M ammonium sulfate; (···) 1.0 M ammonium sulfate.

Addition of Salts. Ammonium salts of sulfate, acetate, chloride, nitrate, and thiocyanate were used to prepare 3.3 M stock solutions in 0.6 M KCl, 50 mM potassium phosphate, pH 6.5. By appropriate dilution, tilapia myosin, solubilized in 0.6 M KCl, 50 mM potassium phosphate, pH 6.5, was made to 0.1, 0.5, or 1.0 M in the appropriate salt and the pH adjusted to 6.5 if necessary with K_2HPO_4. To avoid localized denaturation of myosin by the anion and by the shearing forces of stirring, myosin was gently swirled during salt addition or pH adjustment. The final concentration of myosin was approximately 6.5 and 0.5 mg/mL for rigidity and fluorescence measurements, respectively; accurate protein concentrations were determined after dilution.

T_r values were determined as prominent maxima or minima of first-derivative plots (dFI/dT vs T or dG/dT vs T). The change in T_r values (ΔT_r) was determined from the difference in T_r values for the treatment and control run on the same day from the same myosin preparation. Mean values of the differences for five replications from a total of three myosin preparations are reported; plots shown are representative of replicate runs.

RESULTS

Effect of Ammonium Acetate and Ammonium Sulfate on Fluorescence Thermograms. The effects of added ammonium acetate and ammonium sulfate on the thermal scans of myosin–ANS are plotted in Figure 1. The T_r values did not change markedly from the control T_r (37 °C) due to the presence of either ammonium salt. However, a slight upward trend in ΔT_r values was evident with increasing concentration of each salt; ΔT_r values were −0.04, +0.11, and +0.48 °C for myosin in 0.1, 0.5, and 1.0 M ammonium acetate, respectively (Figure 1a) and −0.26, +1.26, and +1.42 °C for 0.1, 0.5, and 1.0 M sulfate, respectively (Figure 1b). An upward shift in the total relative fluorescence was noted with increasing ammonium sulfate concentration (Figure 1b), which likely arose from increased turbidity induced in the samples; however, there was less than a 10% change in light scattering between 20 and 65 °C, and the increase in turbidity did not influence the measurement of the T_r (Wicker and Knopp, 1988).

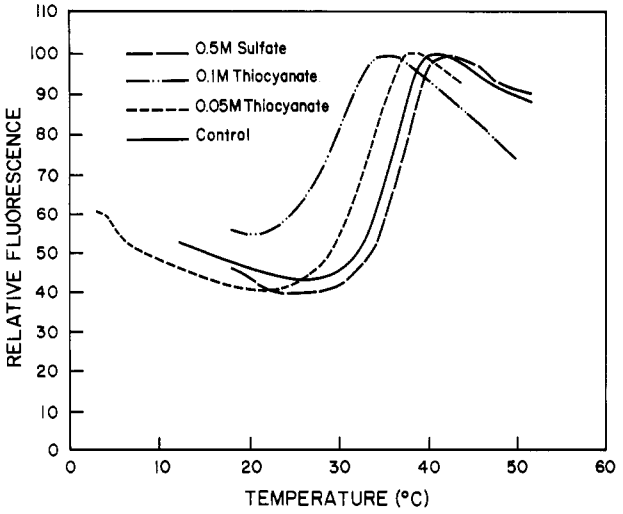

Figure 2. Fluorescence of myosin–ANS vs temperature (0.49 mg/mL myosin; 32 × 10^{-6} ANS; 0.6 M KCl; 50 mM potassium phosphate, pH 6.5): (—) control; (- - -) 0.05 M ammonium thiocyanate; (-·-) 0.1 M ammonium thiocyanate; (– –) 0.5 M sodium sulfate.

Effect of Sodium Thiocyanate and Sodium Sulfate on Fluorescence Thermograms. The effects of sodium thiocyanate and sodium sulfate on the transition temperature of myosin–ANS are shown in Figure 2. At 0.05 and 0.1 M sodium thiocyanate, mean ΔT_r values were −1.06 and −4.03 °C, respectively. Alternately, 0.5 M sodium sulfate addition induced an increase in the transition temperature, ΔT_r = +2.25 °C (addition of sodium sulfate at lower concentrations had no effect, data not shown). Lower concentrations of the salting-in salt sodium thiocyanate effected a larger change in the transition temperature than the salting-out sodium sulfate.

Effect of Ammonium Acetate on Rigidity Thermograms. The effect of ammonium acetate on the gelation profile of tilapia myosin (6.5 mg/mL) is given in Figure 3. In the control sample, the customary thermal profile was observed (Wicker et al., 1986). Rigidity began to increase at 42 °C, peaking soon thereafter (T_r = 44 °C), followed by a decrease in rigidity (T_r = 49 °C) before again gradually increasing in rigidity (T_r = 55 °C).

With increasing concentration, ammonium acetate generally effected increases in the first T_r and reduced the rigidity drop immediately following the initial rigidity rise (Figure 3). At 0.1 M ammonium acetate the ΔT_r values for the initial rigidity rise, decrease in rigidity, and final increase in rigidity were +1, +2, and 0 °C, respectively. For 0.5 M ammonium acetate, these ΔT_r values were +2, +2, and 0 °C. For 1.0 M ammonium acetate, the ΔT_r value for the first transition was +3 °C; only one additional

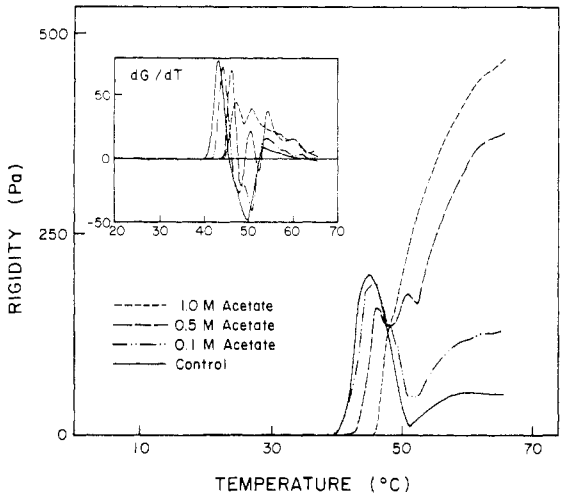

Figure 3. Rigidity vs temperature thermograms of fish myosin (7.7 mg/mL myosin; 0.6 M KCl; 50 mM potassium phosphate, pH 6.5): (—) control; (-·-) 0.1 (···) ammonium acetate; (- - -) 0.5 M ammonium acetate; (···) 1.0 M ammonium acetate. Inset: first-derivative plots of rigidity vs temperature.

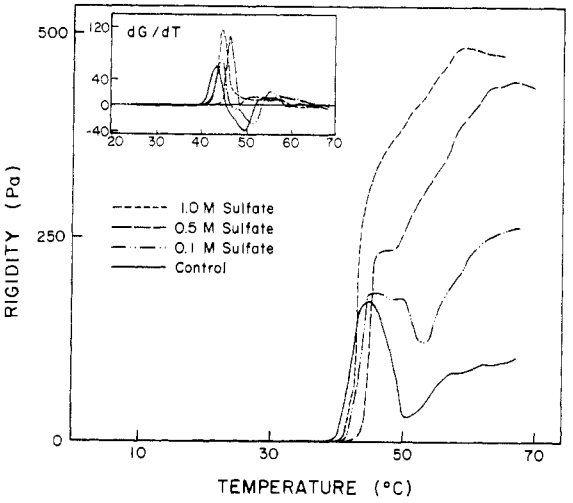

Figure 4. Rigidity vs temperature thermograms of fish myosin (6.4 mg/mL myosin; 0.6 M KCl; 50 mM potassium phosphate, pH 6.5): (—) control; (-·-) 0.1 M ammonium sulfate; (- - -) 0.5 M ammonium acetate; (···) 1.0 M ammonium sulfate. Inset: first-derivative plots of rigidity vs temperature.

transition (rise in rigidity) was observed (T_r = 52 °C) (inset, Figure 3). Final rigidity increased with increasing ammonium acetate concentration.

Effect of Ammonium Sulfate on Rigidity Thermograms. The TSRM rigidity thermograms of tilapia myosin were altered more by addition of ammonium sulfate (Figure 4) than by ammonium acetate (Figure 3). At concentrations up to 0.5 M ammonium sulfate, the initial T_r (rigidity peak) was shifted to slightly higher temperatures. At the same molar concentration, sulfate more effectively minimized the rigidity decrease following the initial rise, and substantially higher final rigidity values were observed with increasing ammonium sulfate concentration.

Addition of 1.5 and 2.0 M ammonium sulfate prevented the thermal gelation of tilapia myosin at the protein concentration of these experiments.

Effects of Ammonium Thiocyanate, Chloride, and Nitrate on Rigidity Thermograms. Myosin in the presence of 0.1 M ammonium thiocyanate, 0.5 M ammonium nitrate, or 0.5 M ammonium chloride did not form a gel upon thermal treatment. At lower concentrations (0.1 M) of ammonium nitrate or chloride, the myosin did initially show a rise in rigidity but failed to develop into a rigid gel at higher temperatures.

DISCUSSION

Hydrophobic interactions of proteins are strengthened in the presence of salts that are effective in "salting-out" proteins, the so-called "naturing" salts. Von Hippel and Wong (1964) noted that the effectiveness with which salts alter the stability of proteins is dependent primarily upon the anion, and secondarily upon the cation. They listed the relative effectiveness of various anions in salting-out of proteins as: sulfate > acetate > chloride > nitrate > thiocyanate. Anions to the left of this series tend to stabilize native structure of proteins while those on the right destabilize protein structure. As for cation effectiveness, Melander and Horvath (1977) noted that sodium was slightly more effective than ammonium with respect to stabilization of proteins.

The influence of salts on myosin–ANS fluorescence appears to follow the Hofmeister series with respect to the ability to alter myosin stability. Thiocyanate was very effective at decreasing the T_r value of myosin–ANS fluorescence. However, only those salts most effective at stabilizing proteins (sodium sulfate and acetate) caused an increase in the T_r value. The failure of either ammonium acetate or sulfate to substantially increase the fluorescence T_r value was surprising since the relative molal surface tension of these salts and their ability to salt out proteins is high (Melander and Horvath, 1977; Von Hippel and Wong, 1964). Apparently, ANS probes an extremely hydrophobic site of myosin that cannot be further enhanced in its stability by other than the most naturing of salts (i.e., sodium sulfate; Figure 2).

Kato and Nakai (1980) and others from the same laboratory (Voutsinas et al., 1983; Li-Chan et al., 1984) have questioned the reliability of ANS as a polarity probe, indicating that *cis*-parinaric acid is a better probe for hydrophobicity of proteins. However, later reports from the same laboratory noted the importance of considering aromatic (measured with ANS) as well as aliphatic (measured with *cis*-parinaric acid) hydrophobicity when studying protein structure–function (Hayakawa and Nakai, 1985; Li-Chan et al., 1985; Tsutsui et al., 1986).

In contrast to the negligible effect of ammonium acetate and sulfate on myosin–ANS thermograms, these same salts dramatically influenced the TSRM rigidity thermogram of concentrated myosin solutions. In each case, three similar effects were observed: The temperature of initial rigidity rise and the first T_r were progressively increased, the rigidity decrease following this initial rise in rigidity was progressively diminished, and final rigidity was progressively increased with increasing salt concentration. It is interesting that these effects of ammonium acetate and sulfate on TSRM thermograms of myosin are quite similar to the effects on TSRM rigidity thermograms of surimi noted by Wu et al. (1988) resulting from addition of the aromatic compounds *p*-toluenesulfonyl chloride (TSCl) and ANS to surimi (but at much higher concentrations than in the present study). The latter compounds ostensibly contribute to strengthening of hydrophobic interactions involved in formation of the protein gel. It would thus appear that hydrophobic interactions are essential to stabilization of the myosin molecule prior to denaturation but also likewise contribute to gel structure formation following partial or complete unfolding of myosin.

T_r values for the second rigidity increase of myosin were not markedly affected by addition of these salts. This may indicate that hydrophobic interactions are not the primary

intramolecular forces stabilizing myosin conformation at temperatures just below that required to induce the second rigidity increase (near 55 °C).

Thermally induced gelation of myosin at any temperature is almost certainly preceded by some conformational change of the molecules exposing sites of attachment or attraction among neighboring molecules (Ferry, 1948). During constant-rate heating, this conformational change is delayed by intramolecular protein–protein interactions (enhanced by naturing salts) and hastened by protein–solvent interactions (enhanced by denaturing salts). Thus, the temperature at which a change in conformation and subsequent gelation occurs depends on the balance of these counteracting forces. At 6–8 mg/mL, tilapia myosin did not form a gel in the presence of 0.5 M ammonium nitrate or chloride. It is likely that these denaturing salts enhanced protein–solvent interactions to the extent that intermolecular protein–protein interactions were insufficient for gel formation. However, the interrelationship of time, temperature, and protein concentration affects gelation; Nakayama et al. (1983) reported that rabbit myosin formed a gel at 15.5 mg/mL in 16 h at 4 °C in the presence of 4.0 M potassium thiocyanate.

At the opposite extreme, extensive enhancement of protein–protein interactions may also prevent gel formation, favoring instead the formation of a precipitate or coagulum. No gel was formed from tilapia myosin in the presence of 1.5 or 2.0 M ammonium sulfate, yet a coagulum was observed in the reaction vessel at the end of the heating period.

It appears that the mechanism of fish myosin denaturation and subsequent gelation involves a minimum of three steps. The first involves a change in the conformation of myosin involving hydrophobic residues represented by the increase in fluorescence of myosin–ANS (Wicker et al., 1986). Wicker and Knopp (1988) postulated that this increase in fluorescence resulted from a small enthalpic change in the conformation of myosin. Following this conformational change, aggregation and (under correct conditions of protein concentration, time, temperature, and solvent composition) gelation of myosin occur due to intermolecular hydrophobic interactions at the newly exposed sites. Such interactions of myosin may be head to head as proposed by Samejima et al. (1981), but tail to tail or tail to head interactions cannot be excluded based on the present data.

This initial conformational change may be due to exposure of the hydrophobic pocket described in rabbit myosin by Borejdo (1983), which is formed by part of the heavy chain and alkali Al light chain. The light chains of rabbit myosin dissociate between 30 and 40 °C (Dreizen and Richards, 1972). Alternately, the conformational change may be due to a subtle rotation of the helical tail to expose the repeating hydrophobic crevice described by McLachlan and Karn (1983). Using DSC, Wright and Wilding (1984) observed three transitions at 39, 44, and 50 °C in rabbit myosin at pH 6.0 and high ionic strength. Although the T_r at 39 °C was attributed to light meromyosin (LMM), the denaturation enthalpy for the DSC T_r at 30 °C is much higher than that estimated by Wicker and Knopp (1988) for the myosin–ANS T_r at 37 °C. Until ANS fluorescence measurements of myosin fragments have been made, it is premature to support or refute the hypothesis that myosin heads interact first, followed by unfolding and interaction of the tail region.

Gelation of fish proteins at low temperature (i.e., 40 °C or below) resulting from hydrophobic interactions constitutes the "setting" phenomenon, evidenced by the rapid development of a soft but elastic and cohesive gel (Lanier et al., 1982). While the initial rise in rigidity has previously been attributed to initiation of gelation (Wu et al., 1985b), measurements of elasticity development (decreased mechanical energy loss) of pollack surimi made on the TSRM in recent studies (Hamann et al., 1989) indicate that gelation (elasticity development) actually is not initiated until the peak of the first rigidity rise is reached. Thus, it is more likely that the initial rise in rigidity represents a rise in viscosity of the sol as the proteins unfold and begin to interact. That the rigidity actually decreases during the initial stages of elasticity development may be attributed to decreasing viscosity of the entrapped water as successively more protein comes out of the sol to build a soft but elastic gel network. Interestingly, it has been previously noted with croaker actomyosin that the optical properties of the sol change very little during "setting", as the gel formed at 40 °C retains virtually the same degree of translucency as the unheated protein sol (Lanier et al., 1982).

A third distinct event in the gelation of fish myosin and surimi is evidenced by the second rapid and more extensive rise in rigidity, usually occurring near 55 °C in TSRM thermograms of fish proteins. This rise in rigidity follows the completion of elasticity development (Hamann et al., 1989). Differential scanning calorimetry also shows a transition near this temperature for surimi and (fish) actomyosin (Wu et al., 1985a). It is in this temperature range that the muscle protein sol first loses its translucency, taking on a more white, opaque appearance (Lanier et al., 1982). Very little additional cohesiveness is imparted to the gel by this gelling transition (Montejano et al., 1984), but the already elastic gel becomes much more rigid as heating proceeds at this or higher temperatures (Lanier et al., 1982).

As shown in this study, neither naturing or denaturing salts have much effect upon the temperature at which this second rigidity transition occurs, indicating that intramolecular hydrophobic associations likely play a lesser role in stabilizing what remains of the native protein conformation at temperatures just below the occurrence of this transition. However, in the presence of 1.0 M ammonium sulfate and acetate, the second transition is obscured on TSRM rigidity thermograms. A single rigidity transition seems to occur that is initiated at a temperature intermediate to that of the first and second conformational transitions (represented by the first and third rigidity transitions of the present TSRM thermograms) of myosin in 0.6 M KCl alone. A similar disappearance of the second rigidity increase was noted by Wu et al. (1988) to be introduced by addition of aromatic compounds that ostensibly enhance protein–protein hydrophobic interactions. It is doubtful that this seemingly uninterrupted rise in rigidity induced by added salts or aromatic compounds is the result of a single, more generalized conformational change occurring in myosin or surimi. Rather, when intermolecular hydrophobic interactions are enhanced by naturing salts or aromatic compounds, the normal decrease in rigidity modulus may be superceded by increased hydrophobic interactions stabilizing the gel structure being formed. With increasing temperature, these intermolecular hydrophobic interactions are strengthened, at least up to the temperature required to induce the second conformational transition (Tanford, 1968). More generalized unfolding occurs above this temperature, and various types of intermolecular bonds are formed that evidently induce further increases in rigidity. In the presence of naturing salts, intermolecular hydrophobic interactions are en-

hanced and effect a higher gel rigidity than would have otherwise been attained (Figures 3 and 4).

The three steps involved in fish myosin gelation may thus be summarized as consisting of (1) localized denaturation and exposure of hydrophobic sites, (2) localized aggregation at these sites leading to formation of a weak but elastic gel structure, and (3) generalized denaturation and aggregation imparting rigidity to the gel. Rapid heating of fish muscle proteins to 80 °C or more obscures the effect of the initial localized aggregation on the gel structure; gels prepared in this way from surimi sols possess less strength than gels allowed to "set" for a short period of time in the 35–50 °C range before being processed at higher temperature (Lanier et al., 1982). The data presented here support a major role of hydrophobic interactions in the localized aggregation (i.e., "setting") step, while the more generalized aggregation likely involves additional types of bonding that add rigidity to the gel, notably disulfide linkages (Liu et al., 1982; Kim, 1987; Samejima et al., 1981) and electrostatic linkages (Wicker et al., 1986).

LITERATURE CITED

Akahane, T. Freeze denaturation of fish muscle proteins. Ph.D. Dissertation, Sophia University, Tokyo, Japan, 1982.

Asghar, A.; Samejima, K.; Yasui, T. Functionality of muscle proteins in gelation mechanisms of structured meat products. *CRC Crit. Rev. Food Sci. Nutr.* **1985**, 27–107.

Borejdo, J. Mapping of hydrophobic salts in the surface of myosin and its fragments. *Biochemistry* **1983**, *22*, 1182–1187.

Dreizen, P.; Richards, D. H. Studies on the role of light and heavy chains in myosin adenosine triphosphatase. *Cold Spring Harbor Symp. Quant. Biol.* **1972**, *37*, 29–45.

Ferry, J. D. Protein gels. *Adv. Protein Chem.* **1948**, *4*, 1–78.

Hamann, D. D.; Purkayastha, S.; Lanier, T. C. Applications of thermal scanning rheology to the study of food gels. In *Thermal Analysis of Foods*; Harwalker, V. R., Ma, C. Y., Eds.; Elsevier Applied Science: Barking, Essex, England, 1989; accepted for publication.

Hayakawa, S.; Nakai, S. Relationships of hydrophobicity and net charge to the solubility of milk and soy proteins. *J. Food Sci.* **1985**, *50*, 486–491.

Kato, A.; Nakai, S. Hydrophobicity determined by fluorescence probe method and its correlation with surface properties of proteins. *Biochem. Biophys. Acta* **1980**, *624*, 13–20.

Kim, B. Y. Rheological investigation of gel structure formation by fish proteins during setting and heat processing. Ph.D. Dissertation, North Carolina State University, Raleigh, NC, 1987.

Lanier, T. C.; Lin, T. S.; Lui, Y. M.; Hamann, D. D. Heat gelation properties of actomyosin and surimi prepared from Atlantic croaker. *J. Food Sci.* **1982**, *47*, 1921–1925.

Li-Chan, E.; Nakai, S.; Wood, D. F. Hydrophobicity and solubility of meat proteins and their relationship to emulsifying properties. *J. Food Sci.* **1984**, *49*, 345–350.

Li-Chan, E.; Nakai, S.; Wood, D. F. Relationship between functional (fat binding, emulsifying) and physicochemical properties of muscle proteins, effects of heating, freezing, pH and species. *J. Food Sci.* **1985**, *50*, 1034–1040.

Liu, Y. M.; Lin, T. S.; Lanier, T. C. Thermal denaturation and aggregation of actomyosin from Atlantic croaker. *J. Food Sci.* **1982**, *47*, 1916–1920.

McLachlan, A. D.; Karn, J. Periodic features in the amino acid sequence of nematode myosin rod. *J. Mol. Biol.* **1983**, *164*, 605–626.

Melander, W.; Horvath, C. Salt effects on hydrophobic interaction in precipitation and chromatography of proteins; interpretation of the lyotropic series. *Arch. Biochem. Biophys.* **1977**, *183*, 200–215.

Montejano, J. G.; Hamann, D. D.; Lanier, T. C. Thermally induced gelation of selected comminuted muscle systems—rheological changes during processing, final strengths and microstructure. *J. Food Sci.* **1984**, *49*, 1496–1505.

Nakayama, T.; Niwa, E.; Hamada, I. New type of myosin gel induced by salt. *Agric. Biol. Chem.* **1983**, *47*, 227–233.

Niwa, E. Role of hydrophobic bonding in gelation of fish flesh paste. *Bull. Jpn. Soc. Sci. Fish.* **1975**, *41*, 907–910.

Niwa, E.; Nakayama, T.; Hamada, I. Arylsulfonyl chloride induced setting of dolphinfish flesh sol. *Bull Jpn. Soc. Sci. Fish.* **1981a**, *47*, 179–182.

Niwa, E.; Sato, K.; Suzuki, R.; Nakayama, T. Fluorometric study of setting properties of fish flesh sol. *Bull. Jpn. Soc. Sci. Fish.* **1981b**, *47*, 817–821.

Robinson, D. R.; Jencks, W. P. The effect of compounds of the urea-guanidinium class on the activity coefficient of acetyltetraglycine ethyl ester and related compounds. *J. Am. Chem. Soc.* **1965**, *87*, 2462–2470.

Samejima, K.; Ishioroshi, M.; Yasui, T. Relative roles of the head and tail portions of the molecule in heat-induced gelation of myosin. *J. Food Sci.* **1981**, *46*, 1412–1418.

Schrier, E. E.; Schrier, E. B. The salting-out behavior of amides and its relation to the denaturation of proteins by salts. *J. Phys. Chem.* **1967**, *71*, 1851–1860.

Swenson, C. A.; Ritchie, P. A. Conformational transitions in the subfragment-II region of myosin. *Biochemistry* **1980**, *19*, 5371–5375.

Tanford, C. Protein Denaturation. Part B. The transition from native to denatured state. *Advances in Protein Chemistry*; Academic Press: New York, 1968; p 251.

Tsutsui, T.; Li-Chan, E.; Nakai, S. A simple fluorometric method for fatbinding capacity as an index of hydrophobicity of proteins. *J. Food Sci.* **1986**, *51*, 1268–1272.

Von Hippel, P. H.; Wong, K.-Y. Natural salts. The generality of their effects on the stability of macromolecular conformations. *Science (Washington, D.C.)* **1964**, *145*, 577–580.

Voutsinas, L. P.; Cheung, E.; Nakai, S. Relationships of hydrophobicity to emulsifying properties of heat denatured proteins. *J. Food Sci.* **1983**, *48*, 26–32.

Wicker, L.; Knopp, J. A. The binding of 8-anilino-1-naphthalene sulfonate (ANS) to fish myosin and the effect of salts on the thermal transitions of fish myosin-ANS complex. *Arch. Biochem. Biophys.* **1988**, *266*, 452–461.

Wicker, L.; Lanier, T. C.; Hamann, D. D.; Akahane, T. Thermal transitions in myosin-ANS fluorescence and gel rigidity. *J. Food Sci.* **1986**, *51*, 1540–1543, 1562.

Wright, D. J.; Wilding, P. Study of muscle and its proteins: myosin and its subfragments. *J. Sci. Food Agric.* **1984**, *35*, 357–372.

Wu, M. C.; Akahane, T.; Lanier, T. C.; Hamann, D. D. Thermal transitions of actomyosin and surimi prepared from Atlantic croaker as studied by differential scanning calorimetry. *J. Food Sci.* **1985a**, *50*, 10–13.

Wu, M. C.; Lanier, T.; Hamann, D. D. Rigidity and viscosity changes of croaker actomyosin during thermal gelation. *J. Food Sci.* **1985b**, *50*, 14–19, 25.

Wu, M. C.; Lanier, T. C.; Hamann, D. D. Effects of urea, P-toluenesulfonyl chloride and 1-anilino-naphthalene-8-sulfonate on rheological properties of fish proteins during thermal processing. Submitted for publication in *J. Food Sci.* **1988**.

Received for review May 19, 1987. Accepted August 1, 1988. Paper No. 11059 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27695-7601. The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service nor criticism of similar ones not mentioned. This work is the result of research sponsored, in part, by NOAA—Office of Sea Grant, Department of Commerce, under Grant No. NA-83AA-D-00012, and the North Carolina Department of Administration. The U.S. Government is authorized to produce and distribute reprints for governmental purposes not withstanding any copyright that may appear hereon.