Thermally Induced Gelation of Squid (*Illex argentinus*) Actomyosin. Influence of Sexual Maturation Stage

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The biochemical properties and the characteristics of heat-induced gelation of actomyosin from mature and immature squid were investigated. Both Mg^{2+}-ATPase activity and reduced viscosity values of actomyosin showed no significant differences between mature and immature squids. A lower content of myosin heavy chain and a higher content of a 160 kDa component were observed in the SDS−PAGE 10% pattern of actomyosin from immature specimens. Gelation of both actomyosins at 10 mg mL^{-1} protein concentration was optimal at 80 °C and pH 6.0. The highest rigidity was reached at 0.25 M KCl with actomyosin from both mature and immature squids. Irrespective of the heating temperature, ionic strength, and pH condition, the rigidity of mature squid actomyosin gel was greater than that of immature squid. Scanning electron micrographs of gels obtained with actomyosin from mature squids showed a better tridimensional structure than those of immature squids.

Keywords: *Squid; myofibrillar proteins; thermally induced gelation*

INTRODUCTION

Gelation of myofibrillar proteins (particularly of myosin and actomyosin) is considered to contribute substantially to the binding between meat particles and comminuted or sectioned muscles (Fukazawa et al., 1961; Samejima et al., 1969; Siegel and Schmidt, 1979; Acton et al., 1983). Myosin is indispensable for the heat-induced gelation of model systems (Samejima et al., 1969; Ishioroshi et al., 1980). F-Actin enhanced the heat-induced gel-forming capacity of myosin (Yasui et al., 1980, 1982; Ishioroshi et al., 1980). Modifications in the myosin/actin weight ratio in reconstituted and natural actomyosin resulted in a substantial change in the rigidity of the formed gel (Yasui et al., 1980; Samejima et al., 1982). Crupkin et al. (1988) had reported variations in the myosin/actin ratio in hake actomyosin related to the reproductive cycle of the fish. It had also been reported that these variations in the actomyosin composition of mature hake influenced the gelling properties of the major myofibrillar protein (Beas et al., 1988). In addition, it was also reported that heat-induced gel strength of chicken red myosin is influenced by nutritional stress (Asghar et al., 1984).

The meat of squid has a low gel-forming capacity (Nagashima et al., 1992). This behavior was attributed to high protease activities present in the mantle of some squid species that led to myosin degradation (Rodger et al., 1984; Nagashima et al., 1992). Most of these studies were made on squid meat paste (Rodger et al., 1984; Nagashima et al., 1992; Gomez-Guillén et al., 1996a,b). Only a few papers are related to heat-induced gelation of squid myofibrillar proteins in model systems (Cheng and Chung, 1989). The purpose of this paper was to characterize the heat-induced gelation of squid actomyosin. In addition, the possible influence of the sexual maturation stage of specimens on the gelling properties of this protein was also investigated.

MATERIALS AND METHODS

Specimens of squid (*Illex argentinus*) (de Castellanos) were caught on the Patagonian shelf by the research ship Capitan Oca Balda. Capture was done at latitude 45−52° in the southwestern Atlantic Ocean. The sexual maturation stage of the specimens was determined according to the classification of Brunetti (1990). Female squids in sexual maturation stage 2−3 (immature) or 5 (mature) were used. Squids were divided according to sexual maturation stage in two lots of 12 samples each, immediately frozen at −25°C, and stored frozen at −30 °C for up to 1 month. Frozen samples were thawed at 10 °C and then used for analysis. Four or six specimens from both mature and immature squids were taken for each experiment.

Actomyosin Preparation. Actomyosin was obtained from the mantles peeled off the epidermis according to the methods described by Paredi et al. (1990). All of the solutions were 0.1 mM phenylmethanesulfonyl fluoride (PMSF). The procedure was performed at 2−4 °C. The final partially purified pellet of actomyosin was solubilized in 20 mM Tris−maleate buffer (pH 6.8) containing 0.6 M KCl, and aliquots were taken to measure protein concentration, Mg^{2+}-ATPase activity, reduced viscosity, and sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) (10%). Gelling properties were measured on the final partially purified actomyosin solubilized in 50 mM phosphate buffer at different ionic strengths.

Reduced Viscosity. Reduced viscosity of actomyosin was measured at 20 ± 0.1 °C using a Ubbelodhe viscometer according to the procedure described by Crupkin et al. (1979).

Protein Determination. Protein concentration was determined by using the Lowry method, with serum bovine albumin as standard (Lowry et al., 1951).

Mg^{2+}-ATPase activity was measured in 0.20 mg mL^{-1} squid actomyosin in Tris−maleate solution (pH 6.8) according to the procedure described by Paredi and Crupkin (1997).

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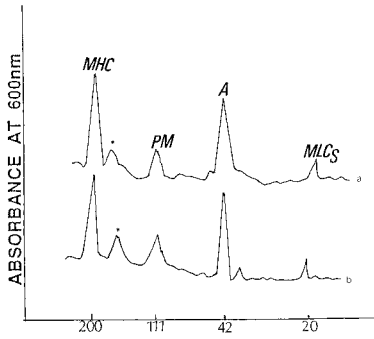

Figure 1. Densitometric patterns of SDS−PAGE 10% gels of actomyosin from mantle of female squid: (a) stage 5 (mature); (b) stage 2−3 (immature). Abbreviations: MHC, myosin heavy chain (200 kDa); PM, paramyosin (111 kDa); A, actin (42 kDa); MLCs, myosin light chains (20 kDa). The asterisk indicates MHC degraded (160 kDa).

SDS−PAGE of actomyosin was performed in 10% gels using a Shandon vertical gel apparatus, as previously reported (Porzio and Pearson, 1977). The protein loaded on the gel was varied to check linearity of myosin heavy chain, actin, paramyosin, and myosin light chains. A linear response was obtained with 30 μg of protein. The mobility−molecular weight curve was calibrated with standards of molecular weights (MW-SDS-70L and MW-SDS-200) (Sigma Chemical Co., St. Louis, MO). Quantitative actomyosin composition was determined by scanning gels at 600 nm with a Shimadzu dual-wavelength chromatogram scanner (Model CS 910) equipped with a gel scanning accessory (Kyoto, Japan). Myosin/actin and myosin/paramyosin ratios were calculated by dividing myosin heavy chain plus myosin light chains areas by actin and paramyosin, respectively.

Gelation was measured with a Yasui device (Yasui et al., 1979) with the modification described by Beas et al. (1988).

Scanning Electron Microscopy (SEM). The samples were prepared according to the procedure described by Beas et al. (1988), with slight modifications. The gels were prepared under the following conditions: protein concentration, 10 mg mL^{-1}; 0.25 M KCl, pH 6.0; heating at 80 °C for 20 min. The samples were fixed in 2% glutaraldehyde in phosphate buffer (pH 7.2) and dehydrated in serially increasing concentrations of acetone (from 40 to 100%). After that they were dried in a critical point dryer and mounted on brass studs. The dried samples were coated with a thin layer of gold in a JEOL JEF-4X vacuum evaporator and observed by SEM (JEOL J. S.M 35 CF) at an accelerating voltage of 15 kV.

Statistical Analysis. Analysis of variance, Student's *t* test, and Duncan's new multiple-range test were performed using the statistical analysis package Statistica/MAC (Statistica MAC, 1994).

RESULTS AND DISCUSSION

Actomyosin from mantles of both mature and immature squids was characterized by its SDS−PAGE profile and biochemical properties. Figure 1 shows the densitometric profiles of SDS−PAGE 10% gels of actomyosin from the mantle of squids at different stages of maturation. The characteristic polypeptidic bands corresponding to the major myofibrillar complex were observed in both profiles. Actomyosin from immature specimens showed a lower content of the myosin heavy chain (MHC) and a higher 160 kDa component than that from mature ones (Figure 1). Rodger et al. (1984) demonstrated that a protease in squid muscles caused myosin degradation with a concomitant increment in a 155 kDa component. No degradation products were observed in the SDS−PAGE 10% gels of frozen stored isolated mantles from either mature or immature squids (Paredi and Crupkin, 1997). The major myofibrillar protein from frozen mature and immature specimens showed slightly lower Mg^{2+}-ATPase activity and reduced viscosity values in comparison to those from unfrozen squid (Table 1; Paredi and Crupkin, 1997). No significant differences were detected in the ATPase activity, reduced viscosity, and myosin/paramyosin ratio of actomyosin from specimens at different sexual maturation stages (Table 1). However, the myosin/actin ratio in actomyosin from mature squids was higher ($P < 0.05$) than that from immature specimens. These results are in agreement with those previously reported on isolated squid mantles (Paredi and Crupkin, 1997).

Table 1. Biochemical Properties of Actomyosin from Squid at Different Sexual Maturation Stages

biochemical properties	mature (5)[a]	immature (2−3)[a]
Mg^{2+}-ATPase activities (μmol of P_i/min·mg of AM)	0.86 ± 0.03	0.81 ± 0.08
reduced viscosity	3.84 ± 0.30	3.76 ± 0.50
myosin/actin ratio	1.25 ± 0.05[b]	1.10 ± 0.08[b]
myosin/paramyosin ratio	3.78 ± 0.20	3.72 ± 0.16

[a] Each value represents a mean of six determinations ± SD. Means with the same superscripts are significantly different ($p < 0.05$).

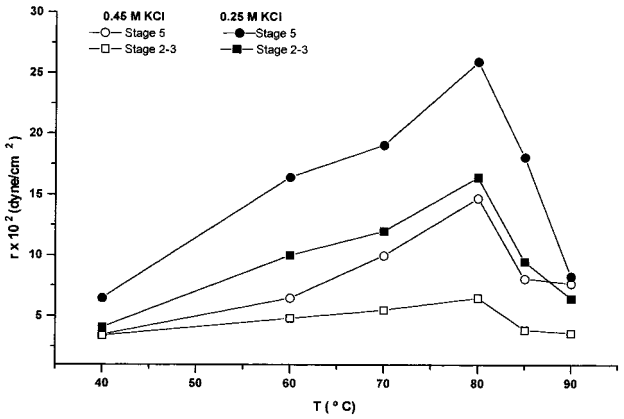

Figure 2. Changes in rigidity of actomyosin gels from mantle of mature and immature squids with heating temperature. Actomyosin solutions (10 mg mL^{-1}) in 50 mM phosphate buffer (pH 6.0) in 0.45 and 0.25 M KCl, respectively, were incubated for 20 min at constant temperature between 40 and 90 °C. Each point represents a mean of four to six determinations.

Effect of Temperature on Gelation. The partially purified actomyosin solution from both mature and immature squids (10 mg mL^{-1}) in 0.25 or 0.45 M KCl and 50 mM phosphate buffer (pH 6.0) was incubated for 20 min at a constant temperature between 40 and 90 °C. The changes in the rigidity of gels of squid actomyosin are shown in Figure 2. Irrespective of the ionic strength, the rigidity of actomyosin gels from mature squids was higher than that from immature squids. A previous work showed that actomyosin from postspawned hake has higher values of both myosin/actin ratio and rigidity modulus than that from prespawned hake (Beas et al., 1988). In this way, the higher rigidity observed in actomyosin from mature squids could be explained on the basis of a higher MHC content in actomyosin from mature squids, which is a consequence of the minor presence of the 160 kDa degradation product in contrast to immature squids (Figure 1). Rodger et al. (1984) and Nagashima et al. (1992) reported that proteolytic activity in squid muscles produces myosin degradation that influences the textural quality of cooked squid meat. As Figure 2 also shows, a considerable increment in modulus rigidity

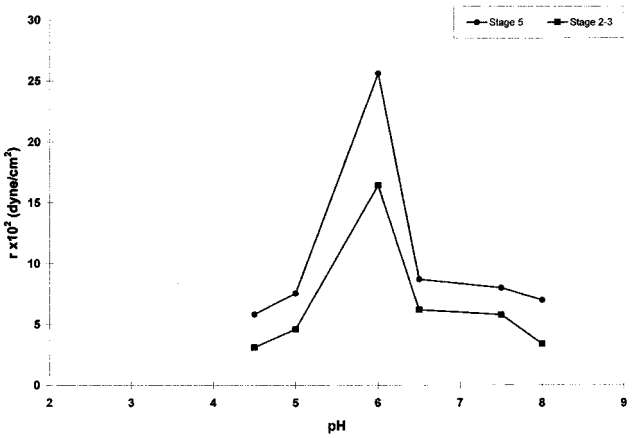

Figure 3. Changes in rigidity of actomyosin gels from mantle of both mature and immature squids with pH. Actomyosin solutions (10 mg mL^{-1}) in 0.25 M KCl, 50 mM citrate (pH 4.5−5.5), phosphate (pH 6.0−7.0), or Tris-HCl (pH 7.5−8.0) buffer were incubated for 20 min at 80 °C. Each point represents the mean of measurements of three to four samples.

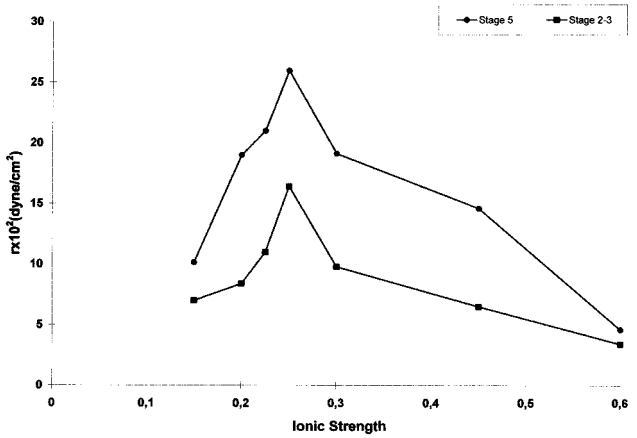

Figure 4. Changes in rigidity of actomyosin gels from mature and immature squid mantles with ionic strength. Actomyosin solutions (10 mg mL^{-1}) in 50 mM phosphate buffer (pH 6.0) and different concentrations of KCl (ionic strength) were incubated for 20 min at 80 °C. Each point represents the mean of measurements of three to four samples.

took place from 60 to 80 °C, irrespective of the ionic strength. The optimal gelation temperature of both actomyosins was 80 °C. A similar optimal temperature was obtained during the heat-induced gelation of squid actomyosin at a higher ionic strength (Cheng and Chung, 1989). Higher heating temperatures caused a dramatic decrease in rigidity values (Figure 2).

Because the highest modulus of the rigidity of actomyosin in both samples was obtained at 80 °C, this temperature was used in the following experiments.

Effect of pH and Ionic Strength on Gelation. The influence of pH on the rigidity of squid actomyosin is shown in Figure 3. An increase of the pH up to 6.0 produces higher rigidity values in actomyosin from both mature and immature squids. Independent of the maturation stage of the specimens, the highest rigidity value of actomyosin was obtained at pH 6.0. A similar optimum pH value for the maximum rigidity of actomyosin from squid was reported (Cheng and Chung, 1989). It has been reported that depending on the mole myosin/actin ratio in reconstituted actomyosin, maximum gel strength occurred in the pH range of 5.5−6.0 (Yasui et al., 1980). A similar optimal pH value was also reported in hake actomyosin gelation (Beas et al., 1988). Gel strength in both actomyosins underwent a sharp decrease at pH values >6.0.

The changes in the modulus of rigidity of both actomyosins at different KCl concentrations are shown in Figure 4. The maximum rigidity value in actomyosin from both mature and immature squids occurred at 0.25 M KCl. In agreement with these results it had been reported that the maximal heat-induced gel rigidity for cod actomyosin in a model system was obtained at 0.15 M KCl and pH 6.0 (Careche et al., 1991). In addition, it was also reported that freshly prepared rabbit myosin formed gels with an extremely high rigidity in 0.1−0.3 M KCl at pH 6.0 (Ishioroshi et al., 1983). Higher increments in ionic strength up to 0.6 M produce a decrease in the rigidity of both actomyosins. No changes in rigidity were observed at KCl concentrations between 0.6 and 0.8 M (data not shown).

Effect of Protein Concentration on Gelation. The effects of protein concentration from 5 to 20 mg mL^{-1} on the quantitative gelling capacity of squid actomyosin are shown in Figure 5. A linear correlation was obtained

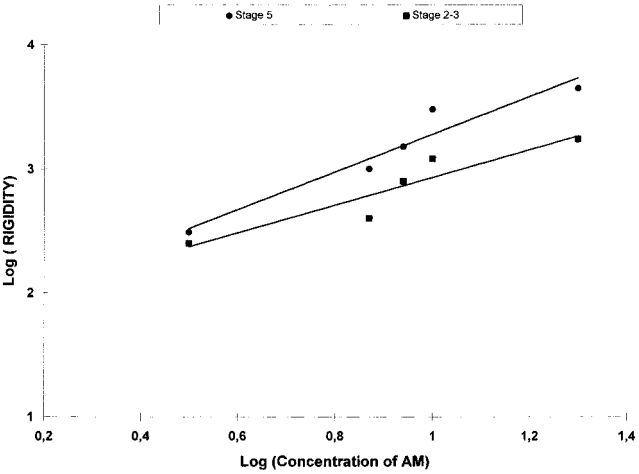

Figure 5. Log of rigidity vs log of actomyosin concentration from both mature and immature squid mantles. Different concentrations of actomyosin in 50 mM phosphate (pH 6.0) and 0.25 M KCl buffer were incubated for 20 min at 80 °C.

when the log of rigidity was plotted against a log of protein concentration. The slope was 1.50 ($r = 0.97$) for actomyosin from mature specimens and 1.12 ($r = 0.94$) for that from immature squids. There were no significant differences between the slopes of both conditions. As a consequence of that, rigidity of squid actomyosin heat-induced gelation increases proportionally to the protein concentration, irrespective of the sexual maturation stage of the specimens. Similar results were reported for reconstituted red actomyosin and hake actomyosin (Yasui et al., 1980; Beas et al., 1988).

Ultrastructure of Gel. The scanning electron micrographs of heat-induced gels of squid actomyosin obtained with 10 mg mL^{-1} protein at 80 °C in 50 mM phosphate buffer (pH 6.0) and 0.25 M KCl are shown in Figure 6. Gels of actomyosin from immature squids show globular structures with small spaces of water retention (Figure 6A). Conversely, the ultrastructure of mature squid actomyosin shows uniform spaces for water retention and a definite tridimensional structure (Figure 6B).

In agreement with this result it was observed in model systems that an increased myosin/actin ratio produced actomyosin gels with more uniform tridimen-

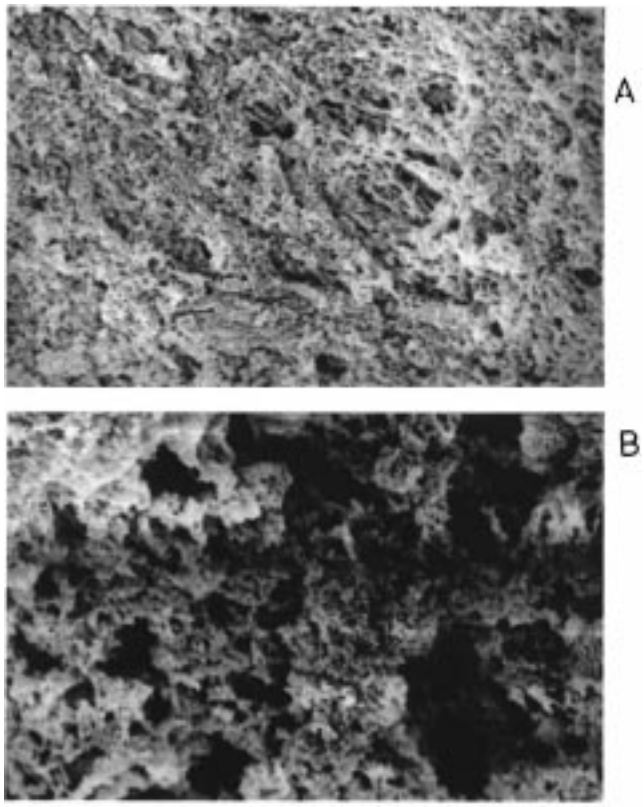

Figure 6. Scanning electron micrographs of actomyosin gels (10 mg mL^{-1}) from mantle of both immature (A) and mature (B) squids. Gels were formed at 80 °C and 0.25 M KCl. Magnification = 5400× (figure is reproduced here at 67% of the original).

sional network (Yasui et al., 1980). Similar scanning electron micrographs were obtained with natural postspawned hake actomyosin (Beas et al., 1988).

Conclusions. Irrespective of the sexual maturation stages of the specimens, optimal thermally induced gelation of squid actomyosin was observed at 80 °C, pH 6.0, and 0.25 M KCl. Actomyosin from mature squids had better gelling properties than that from immature specimens.

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