Biochemical Properties of Actomyosin from Frozen Stored Mantles of Squid (*Illex argentinus*) at Different Sexual Maturation Stages

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Mantles of squid at different sexual maturation stages were frozen and stored at −30 °C. Similar biochemical properties of actomyosin from unfrozen mantles of both immature and mature squid were found. A decrease in extractability, reduced viscosity, and Mg^{2+}-ATPase activity of actomyosin was observed during frozen storage. The decrease in the biochemical properties of actomyosin was independent of the sexual maturation stage of the specimens. The relative percentages of myosin and actin in actomyosin from unfrozen mantles of immature squid were significantly different from those of actomyosin of mature squid. Myosin significantly decreased ($p < 0.0$l) in frozen stored samples.

Keywords: *Myofibrillar proteins; biochemical properties; frozen storage; squid mantle*

INTRODUCTION

Several authors have reported some aspects related to handling, processing, and frozen storage of squid (Takahashi, 1965; Joseph et al., 1977; Botta et al., 1979; Moral et al., 1983). Joseph et al. (1985) reported that squid *Loligo duvauceli* frozen after 8 h of ice storage was in acceptable conditions up to 60 weeks while squid kept on ice for 1 day was in acceptable conditions up to 38 weeks of frozen storage. These authors also reported the gradual decrease in protein extractability during frozen storage. In other species of the squid such as *Ommaestrephes sloani pacificus*, protein extractability expressed as the percentage of the extractable actomyosin remains without major changes during frozen storage (Iguchi et al., 1981). The seasonal effect on the quality of raw material was studied by Takahashi (1965) who found that squid caught in summer spoiled faster than that captured in autumn. These seasonal variations in the spoilage rate of squid meat could be related with reproductive cycle of the specimens.

The purpose of the present paper was to investigate the effects of frozen storage for long term on the biochemical properties of actomyosin obtained from squid mantle. In addition, the posible influence of the sexual maturation stages of specimens on the frozen storage of squids was also analyzed.

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 Brunetti (1990). Female squids in sexual maturation stage 2−3 (immature) or 5 (mature) were used. Squids were gutted and cleaned and the mantles peeled off the epidermis. Then the mantles were divided according to their sexual maturation stage in two lots of 24 samples each, immediatly frozen on board at −25 °C, and stored thereafter at −30 °C up to 9 months. Six mantles of each lot were taken at 0 (unfrozen samples) and 3, 6, and 9 months of storage and then used for analysis. Frozen samples were thawed at 10 °C.

Actomyosin Preparation. Actomyosin was obtained from the mantles according to the method described by Paredi et al. (1990). All 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. Aliquots were taken to measure protein concentration, Mg^{2+}-ATPase activity, reduced viscosity, and SDS−PAGE (10%). Extractability of actomyosin is expressed as mg of extractable actomyosin/g of muscle.

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

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

Mg^{2+}-ATPase Activity. Mg^{2+}-ATPase activity was measured in 0.20 mg mL^{-1} actomyosin in 20 mM Tris-maleate solution (pH 6.8), 30 mM KCl, 2 mM $MgCl_2$, 2 mM ATP, 0.1 mM $CaCl_2$. The reaction was stopped after 1 min at 20 °C with TCA at a 10% final concentration. Phosphorus was determined by the Chen method (Chen et al., 1956).

SDS−Polyacrylamide Gel Electrophoresis (SDS−PAGE). SDS−PAGE of actomyosin was performed in 10% gels using a Shandon vertical gel apparatus, as previously reported (Portzio 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 actomyosin. 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.

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

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Table 1. Biochemical and Physicochemical Properties of Actomyosin from Squid Mantles[a]

stage	reduced viscosity	ATP response	Mg^{2+}-ATPase activity (μmol of P_i/min·mg of AM)
5	4.80 ± 0.9	70 ± 5.0	1.09 ± 0.07
2−3	4.60 ± 0.9	66 ± 8.0	1.04 ± 0.08

[a] Results are expressed as the means of 12 determinations ± standard deviations.

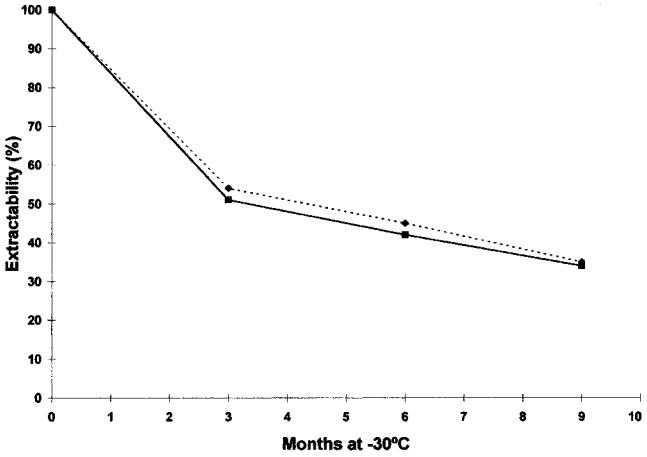

Figure 1. Changes in extractability of actomyosin of squid mantles during storage at −30 °C. The values are relative to those of original unfrozen samples (100%). The extractability of such unfrozen samples was 63 and 60 mg of actomyosin/g of muscle for specimens in stages 5 (dotted line, ◆) and 2−3 (solid line, ■),respectively. Results are expressed as the means of 12 determinations, average relative standard deviations less than ±3%.

RESULTS AND DISCUSSION

Purified actomyosin from unfrozen mantles of both immature and mature squid had the characteristic high reduced viscosity, Mg^{2+}-ATPase activity, and ATP response (Table 1).

Irrespective of the sexual maturation stage of specimens, the extractability of actomyosin fell ($p < 0.01$) about 50−55% during 3 months of frozen storage and decreased slowly after that (Figure 1). A gradual decrease in protein extractability during frozen storage of squid *L. duvauceli* was also reported (Joseph et al., 1985). However, extractable actomyosin from frozen squid *O. sloani pacificus* only decreased slightly even after a long freezing period (Iguchi et al., 1981). The discrepancy among the results obtained by Iguchi et al. (1981) with those obtained by Joseph et al. (1985) and us could be related to the difference in species, the biological condition of the specimens analyzed, freezing conditions, etc.

Figure 2 shows the changes in the reduced viscosity of actomyosin from frozen stored mantles. The viscosity fell ($p < 0.01$) about 55% during 3 months of frozen storage and decreased slowly after that up to the end of storage (Figure 2). Similar results were obtained with actomyosin from mantles of squids at either stage 2−3 or stage 5.

The changes in Mg^{2+}-ATPase activity of actomyosin from frozen stored squid are shown in Figure 3. It is widely accepted that modifications in the Mg^{2+}-ATPase activity occur when changes in the actin−myosin interaction take place. The Mg^{2+}-ATPase activity fell ($p < 0.01$) almost 60% in actomyosin from mantles of both immature and mature specimens during 3 months of frozen storage and decreased slowly after that up to the end of storage (Figure 3). Iguchi et al. (1981) reported

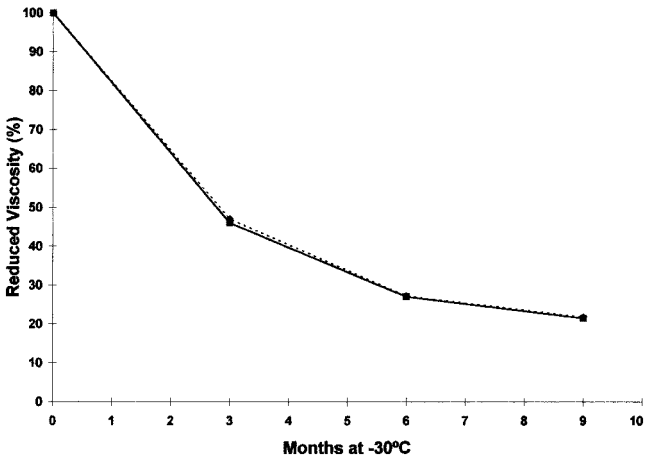

Figure 2. Changes in reduced viscosity of actomyosin from squid mantles during storage at −30 °C. The values are relative to those of the original unfrozen samples (100%). The reduced viscosity of such unfrozen samples was 4.8 and 4.6 dL/g for specimens in stages 5 (dotted line, ◆) and 2−3 (solid line ■), respectively. Results are expressed as the means of 12 determinations, average relative standard deviations less than ±3%.

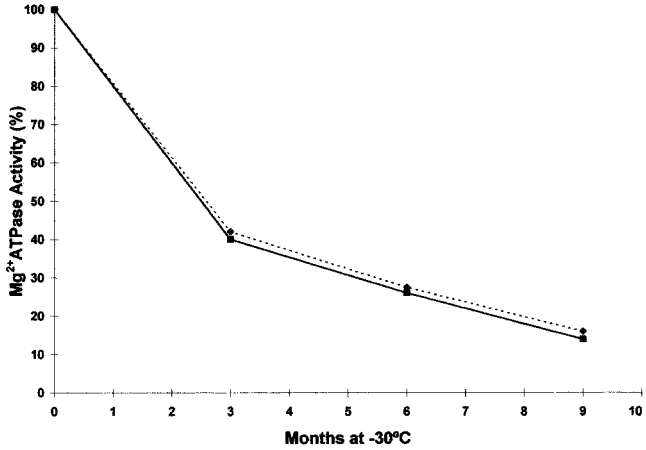

Figure 3. Changes in Mg^{2+}-ATPase activity of actomyosin from squid mantles during storage at −30 °C. Values are relative to those of the original unfrozen samples (100%). The activity of such unfrozen samples was 1.09 and 1.04 μmol of P_i/min·mg of actomyosin for specimens in stages 5 (dotted line, ◆) and 2−3 (solid line, ■), respectively. Results are expressed as the means of 12 determinations, average relative standard deviations less than ±3%.

a slight decrease in the Ca^{2+}-ATPase activity of actomyosin from frozen stored mantles of squid *O. sloani pacificus*. Our results and those reported by Iguchi et al. (1981) would suggest that the decreases in both Mg^{2+}- and Ca^{2+}-ATPase activity of actomyosin from frozen stored mantles are due to myosin denaturation.

The densitometric profiles of SDS−PAGE 10% gels of actomyosin from unfrozen (0 time) and frozen stored mantles of mature squid are shown in Figure 4. The characteristic polypeptidic bands of myosin heavy chain (MHC), paramyosin (PM), actin (A), and myosin light chains (MLCs) were also present in densitometric profiles of actomyosin from immature squid mantle (data not shown). These profiles are similar to that of actomyosin from other squid species (Iguchi et al., 1981).

A decrease of the MHC and MLCs areas in actomyosin was observed during the frozen storage of squid mantles. The relative percentages of myosin, actin, and paramyosin in actomyosin from unfrozen (0 time) and frozen stored mantles of both immature and mature squids are shown in Table 2.

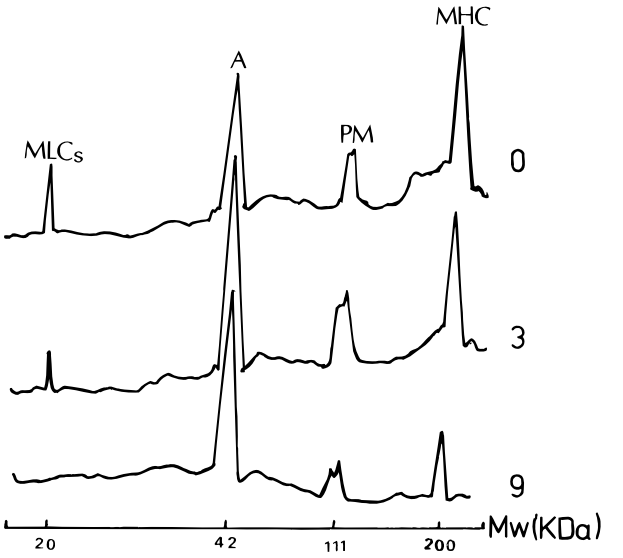

Figure 4. Densitometric analysis profiles of SDS−PAGE 10% gels of the actomyosin from squid mantles in stage 5 at zero time (unfrozen) and at 3 and 9 months of frozen storage. MHC, myosin heavy chain (200 kDa); PM, paramyosin (111 kDa); A, actin (42 kDa); MLCs, myosin light chains (20 kDa).

Significant differences were observed in relative percentage of myosin ($p < 0.05$), actin ($p < 0.01$), and myosin/actin ratios ($p < 0.05$) in actomyosin from both unfrozen mature and immature specimens. These differences remained during frozen storage. The relative percentage of myosin significantly decreased ($p < 0.01$) and that of actin increased during frozen storage. Paramyosin increased slightly ($p < 0.05$) during 3 months of frozen storage and then decreased ($p < 0.05$) up to the end of storage. A significant decrease ($p < 0.01$) in both myosin/actin and myosin/paramyosin ratios during frozen storage was observed (Table 2). These changes occur in specimens at both sexual maturation stages. The decrease of MHC and MLCs areas could be related to either proteolytic activity or denaturation−aggregation of the protein. No degradation products were observed in the SDS−PAGE 10% gels of actomyosin obtained from frozen stored mantles (Figure 4). These results could suggest that the decrease in myosin would not be caused by proteolytic activity. It is recognized that changes in texture because of long term frozen storage are due to severe alterations of muscle proteins usually termed denaturation−aggregation (Dyer, 1951; Sikorski, 1978; Shenouda, 1980; Matsumoto, 1980; Jiang and Lee, 1985). So, the decrease in myosin areas in SDS−PAGE gels of actomyosin of frozen stored mantles could be attributed to a denaturation−aggregation of the protein.

We reported in a previous work that a thick filament of the marine invertebrate *Aulacomya ater ater* (Molina) aggregates faster than a thin filament during frozen storage of striated adductor muscles (Paredi et al., 1996). The present work showed that the thick filament of squid mantle was also affected by frozen storage. In addition, our results indicate that myosin was more affected that paramyosin during the denaturation−aggregation modifications of this filament.

CONCLUSIONS

The extractability of actomyosin decreased during frozen storage. This change was accompanied by a simultaneous decrease in both reduced viscosity and Mg^{2+}-ATPase activity. Myosin was more affected than

Table 2. Relative Percentages of Myosin, Paramyosin, and Actin and Myosin/Actin and Myosin/Paramyosin Ratios in Actomyosin from Squid Mantles[a]

months at −30 °C	relative percentage (%)						ratio			
	M		PM		A		M/A		M/PM	
	5	2−3	5	2−3	5	2−3	5	2−3	5	2−3
0	52.50 ± 1.70[a]	48.90 ± 1.26[b]	11.50 ± 1.65	11.60 ± 1.64	34.40 ± 1.50[a]	38.80 ± 1.27[c]	1.44 ± 0.10[a]	1.24 ± 0.08[b]	4.67 ± 0.10	4.70 ± 0.13
3	29.70 ± 3.40[a]	23.70 ± 0.94[b]	19.30 ± 1.44	18.90 ± 0.80	50.82 ± 2.40[a]	56.12 ± 1.28[c]	0.60 ± 0.11[a]	0.42 ± 0.03[b]	1.35 ± 0.18	1.27 ± 0.07
9	12.80 ± 0.93[a]	9.00 ± 1.04[c]	14.50 ± 0.71	14.00 ± 1.10	69.60 ± 2.70[a]	77.40 ± 1.71[c]	0.19 ± 0.01[a]	0.12 ± 0.02[c]	0.81 ± 0.02[a]	0.64 ± 0.05[c]

[a] Each value represents the mean ± SD ($n = 6$). Means in each column were significantly different ($p < 0.01$), except for percentage of PM ($p < 0.05$). Means within each measurement in each row with different superscripts were significantly different: [a,b] $p < 0.05$; [a,c] $p < 0.01$.

other major myofibrillar proteins during frozen storage. These changes were independent of the sexual maturation stage of the specimens.

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