Biochemical Properties of Actomyosin and Expressible Moisture of Frozen Stored Striated Adductor Muscles of *Aulacomya ater ater* (Molina): Effects of Polyphosphates

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Aulacomya adductor muscles with and without prior immersion in polyphosphates were frozen and stored at −30 °C. Expressible moisture of control samples increased after freezing and showed the highest increment at the second week of storage. Actomyosin extractability was not affected by freezing but decreased during frozen storage in both untreated and treated muscles. The reduced viscosity and the Mg^{2+}-ATPase activity of actomyosin in dip-treatment free muscles fell with freezing and frozen storage. Polyphosphates reduced the amount of expressible moisture of the muscles and delayed the decrease in the enzymatic activity of actomyosin. Treatment with polyphosphates did not affect the viscosity of actomyosin. The relative percentages of myosin and paramyosin significantly decreased ($p < 0.01$) and those of actin significantly increased in actomyosin of both control and treated muscles at 4 weeks of frozen storage. These results would indicate that thin filaments in frozen striated adductor muscle are more stable than thick filaments.

Keywords: *Striated adductor muscles; myofibrillar proteins; biochemical properties; frozen storage; expressible moisture*

INTRODUCTION

Paredi et al. (1990) reported on the behavior of biochemical properties of myofibrillar proteins and the water-holding capacity of the meat of *Aulacomya* striated adductor muscles stored at 2−4 °C. Decreases of about 44% in both Mg^{2+}-ATPase activity and reduced viscosity of actomyosin were observed after the first day of storage. Paredi et al. (1992) found evidences of conformational changes in actomyosin from intrinsic viscosity and surface hydrophobicity determinations. The changes in the biochemical properties of actomyosin from muscles stored at 2−4 °C were accompanied by increases in the amounts of expressible juice. The shucked oyster (*Crassostrea virginica*) meat also loses liquid during ice storage (Cook et al., 1988).

Weight loss is of great economic importance in shellfish handling and shipping. Weight losses are attributed to water losses in meat during processing and frozen storage which are related to the denaturation of myofibrillar protein (Hamm, 1960, 1986; Fennema, 1990). Jiang et al. (1977, 1985) reported weight losses and the presence of drip in shucked and frozen oyster (*Crassostrea gigas*) meat stored at low temperatures. There is scarce information available on the stability of scallops during frozen storage (Hardy and Smith, 1970; Dyer and Hiltz, 1974; Hiltz and Dyer, 1973; Maxwell-Miller et al., 1982; Chung and Merrit, 1991). Hardy and Smith (1970) reported that on thawing frozen scallop meats, water is lost as drip and the weight of meat after freezing and thawing is lower than the weight before freezing. The thawed meat of sea scallop (*Placopecten magellanicus*) is more tender and produces less drip if it is originally frozen in prerigor rather than in postrigor (Dyer and Hiltz, 1974; Chung and Merritt, 1991). There is no direct evidence now relating water loss and myofibrillar protein denaturation during freezing and frozen storage of bivalve adductor muscles.

The purpose of this work is to investigate the water-holding capacity of the meat and the biochemical properties of actomyosin in striated adductor muscles of *Aulacomya*, during freezing and frozen storage at −30 °C.

MATERIALS AND METHODS

Samples. Specimens of *Aulacomya ater ater* (Molina) were collected from the Gulf of San Jose, Chubut, Argentina, from October 1993 to July 1995. The specimens arrived alive at our laboratory within the first 24 h after collection. Mature individuals of 60−70 mm length were selected. Maturity of gonads was determined by macroscopic observation, and the histological analysis of the mantle was performed according to the procedure described by Vinuesa and Tortorelli (1980).

Preparation of Samples. After cleaning the shells, striated muscles were dissected. Muscles were carefully freed from adhering pancreatic and liver tissues and rinsed with 5 mM phosphate buffer (pH 7.0) containing 40 mM NaCl and 0.1 mM phenylmethanesulfonyl fluoride.

Storage of Samples. A batch of adductor muscles was immersed for 10 min in 10% Na polyphosphates (5.3% Na tripolyphosphate and 4.7% Na pyrophosphate) solution prior to freezing in a plate freezer at −30 °C for 2 hours (treated samples). Frozen muscles were stored up to 6 weeks at −30 °C. A second control batch of adductor muscles was frozen and stored without the polyphosphates bath (control samples). Four subsamples from these two batches, each consisting of eight adductor muscles, were withdrawn for analysis at zero time after the freezing and after 1, 2, 3, 4, and 6 weeks of storage. Unfrozen adductor muscles with and without polyphosphates bath were also analyzed.

Actomyosin Preparation. Actomyosin was obtained from adductor muscles by the modified method of Focant and Huriaux (1976), as described in a previous work (Paredi et al., 1990). 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-

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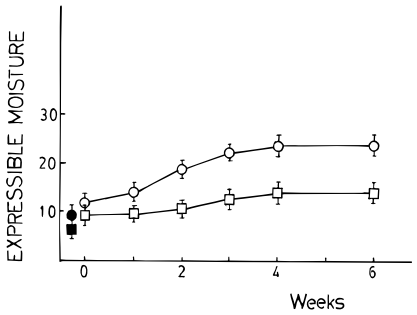

Figure 1. Changes in expressible moisture in frozen stored muscles: (○) control, (□) 10% polyphosphates (5.3% Na tripolyphosphate + 4.7% Na pyrophosphate), and (●, ■) unfrozen samples. Results are expressed as means (12 determinations). Bars represent confidence limits ($p < 0.05$). Percent of expressible moisture is g of expressible moisture/100 g of muscle. There were significant differences ($p < 0.01$) between mean values of unfrozen samples and different storage periods in control and treated samples. No significant differences were found among means of weeks 3, 4, and 6 of storage. There were significant differences ($p < 0.01$) between control and treated samples throughout the storage period.

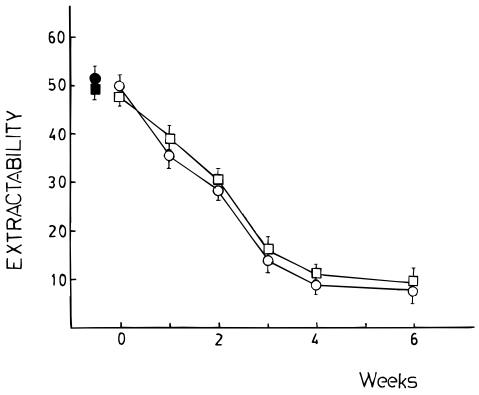

Figure 2. Changes in extractability of actomyosin of adductor muscles during storage at −30 °C. Results are expressed as means (12 determinations). Bars represent confidence limits ($p < 0.05$). Extractability of actomyosin is described as mg of actomyosin/g of muscle. For other details, see legend of Figure 1. There were no significant differences between mean values of unfrozen and zero time of frozen storage samples. Significant differences ($p < 0.01$) were found for mean values among different storage periods except between weeks 4 and 6. There were no significant differences between control and treated samples throughout the storage period.

PAGE 10%. Extractability of actomyosin was expressed as mg of actomyosin/g of muscle.

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

Protein Determinations. The protein concentration of actomyosin was determined by the Lowry method with bovine serum albumin as a standard (Lowry et al., 1951).

Mg^{2+}-ATPase Activity. Mg^{2+}-ATPase activity was measured in 0.15 mg mL^{-1} actomyosin in a solution of 20 mM Tris-maleate (pH 6.8), 30 mM KCl, 2 mM $MgCl_2$, 1.6 mM ATP, and 0.1 mM $CaCl_2$. The reaction was stopped after 1 min at 30 °C with TCA at 10% (final concentration) (Paredi et al., 1990). Phosphorus was determined by the Chen methods (Chen et al., 1956).

SDS−Polyacrylamide Gel Eletrophoresis (SDS−PAGE). SDS−PAGE of actomyosin was performed in 10% gels using a Shandon Southern vertical gel apparatus (U.K.), as previously reported (Portzio and Pearson, 1977). Quantitative actomyosin composition was determined by the scanning of gels at 600 nm with a Shimadzu dual-wavelength chromatogram scanner Model CS 910 equipped with a gel-scanning accesory (Kyoto, Japan). The protein load on the gel was varied to check linearity of myosin heavy, actin, and myosin light chains. With 30 μg of actomyosin, a linear response was obtained. Myosin/actin and myosin/paramyosin ratios were calculated by dividing myosin heavy plus myosin light chain areas by actin and paramyosin, respectively.

Expressible Moisture. The expressible moisture was measured in frozen and unfrozen muscles by the method of Wierbicki et al. (1957) with the modification introduced by Ciarlo et al. (1985). Frozen samples were previously thawed at 10 °C.

Statistical Analysis. Analysis of variance was applied to the data along with Duncan's new multiple range test and the Student's *t*-test using the statistical analysis package SYSTAT (Wilkinson, 1990).

RESULTS AND DISCUSSION

The changes in expressible moisture from *Aulacomya* adductor muscles during freezing and frozen storage, for both treated and control samples, are presented in Figure 1. Samples that were not frozen produced 6% of expressible moisture if they had been immersed in a polyphosphates bath and 8% if they had not. Freezing produced significative ($p < 0.01$) increases in the amounts of expressible moisture in both treated and control muscles. For control samples the expressible moisture reached a maximun after 2 weeks of storage (Figure 1). In samples that had been immersed in polyphosphates solution, the expressible moisture was significantly less ($p < 0.01$) than in untreated samples at all stages of storage. Immersion in polyphosphates solutions also reduced the drip in frozen stored scallops (Hardy and Smith, 1970) and in *Aulacomya* adductor muscles stored at 2−4 °C (Paredi et al., 1992).

This change in the water-holding capacity (WHC) is a very sensitivity indicator of changes in the charges and structure of myofibrillar proteins (Hamm, 1960, l975). The decrease in the WHC of *Aulacomya* adductor muscle stored at 2−4 °C was also related to changes in the biochemical and physicochemical properties of myofibrillar proteins by Paredi et al. (1990, 1992). These reports would suggest that the increases in expressible moisture of frozen stored *Aulacomya* muscles could also be related to changes in the properties of myofibrillar proteins.

Freezing did not affect the actomyosin extractability of both treated and control adductor muscles (Figure 2). However, the actomyosin extractability was significantly reduced ($p < 0.01$) during frozen storage. It was reduced by about 33% after 2 weeks of storage and by about 75% after 4 weeks of storage (Figure 2). Decreases in actomyosin extractability in frozen fish has been reported by Dyer (1951), Connell (1968), Sikorski et al. (1976), Matsumoto (1979) and Jiang and Lee (1985). Since no significant differences were observed between polyphosphates-treated and untreated samples, the actomyosin extractability would not be affected by this treatment.

The reduced viscosity of actomyosin from treated and control samples during freezing and frozen storage is shown in Figure 3. A 15% decrease ($p < 0.05$) in viscosity after freezing was observed. During frozen storage the viscosity showed a significant ($p < 0.01$) decrease that reached 60% after 4 weeks. Thereafter the viscosity stabilized. No significant differences were observed in the viscosity of actomyosin from treated and untreated muscles.

The Ca^{2+}-dependent Mg^{2+}-ATPase activity of actomyosin for treated and control samples is shown in Figure 4. In treated samples the activity fell ($p < 0.05$) by 10% during freezing, while in control samples the

Table 1. Relative Percentages of Myosin, Paramyosin, and Actin and Myosin/Actin and Myosin/Paramyosin Ratios in Actomyosin from Frozen Stored Muscles

		relative percentage (%)[a]			ratio[a]	
weeks at −30 °C	sample	M	PM	A	M/A	M/PM
unfrozen	control	50.90 ± 3.00[b]	13.70 ± 0.23[b]	36.00 ± 2.80[b]	1.44 ± 0.13[b]	3.90 ± 0.24[b]
	treated	50.20 ± 2.00[b]	15.50 ± 2.00[b]	34.00 ± 1.50[b]	1.50 ± 0.10[b]	3.50 ± 0.90[b]
4	control	26.00 ± 3.00[c]	11.00 ± 3.50[c]	61.00 ± 1.90[c]	0.42 ± 0.08[c]	2.62 ± 1.00[c]
	treated	28.20 ± 0.45[c]	8.00 ± 2.00[c]	62.50 ± 1.40[c]	0.43 ± 0.02[c]	2.80 ± 0.40[c]

[a] Mean ± confidence limits ($n = 6$, $p < 0.05$); M, myosin; PM, paramyosin, A, actin. [b,c] Values in the same columns with different superscripts are significantly different ($p < 0.01$).

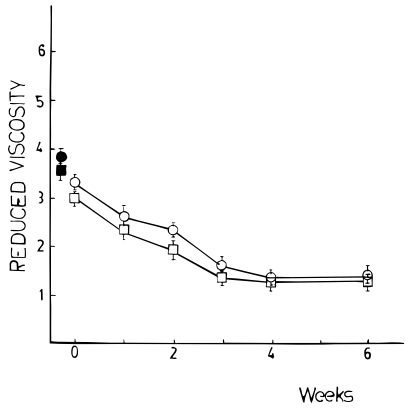

Figure 3. Reduced viscosity of actomyosin from frozen stored muscles at −30 °C. Results are expressed as means of 12 determinations. Bars represent confidence limits ($p < 0.05$). For other details see legend of Figure 1. There were significant differences ($p < 0.05$) between mean values of unfrozen and zero time of frozen storage samples. Significant differences ($p < 0.01$) were found for mean values among different storage periods, except among weeks 3, 4, and 6. There were no significant differences between control and treated samples throughout the storage period.

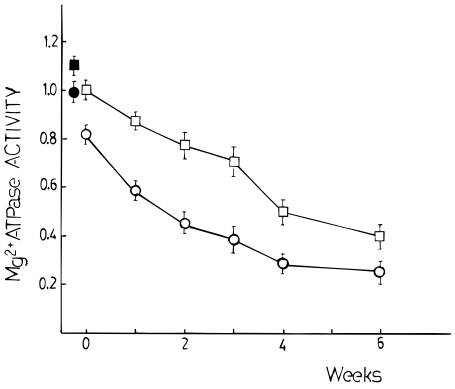

Figure 4. Changes in Mg^{2+}-ATPase activity of actomyosin from frozen stored muscles. Mg^{2+}-ATPase activities are μmol of inorganic phosphate released within 1 min at 30 °C/mg of protein. Results are expressed as means of 12 determinations. Bars represent confidence limits ($p < 0.05$). For others details, see legend of Figure 1. There were significant differences between mean values of unfrozen and frozen samples for control ($p < 0.01$) and treated ($p < 0.05$) samples. Significant differences ($p < 0.01$) were found for mean values among different storage periods, except between weeks 4 and 6. There were significant differences ($p < 0.01$) between control and treated samples throughout the storage period.

activity fell ($p < 0.01$) by 20%. Although the patterns of decreases in Mg^{2+}-ATPase activity for both treated and control samples during frozen storage are similar, the activity of treated samples was significantly higher ($p < 0.01$) than that of control samples throughout the storage period. Similar effects of the polyphosphates on the Mg^{2+}-ATPase activity of actomyosin from *Aulacomya* adductor muscles stored at 2−4 °C have been previously reported (Paredi et al., 1992).

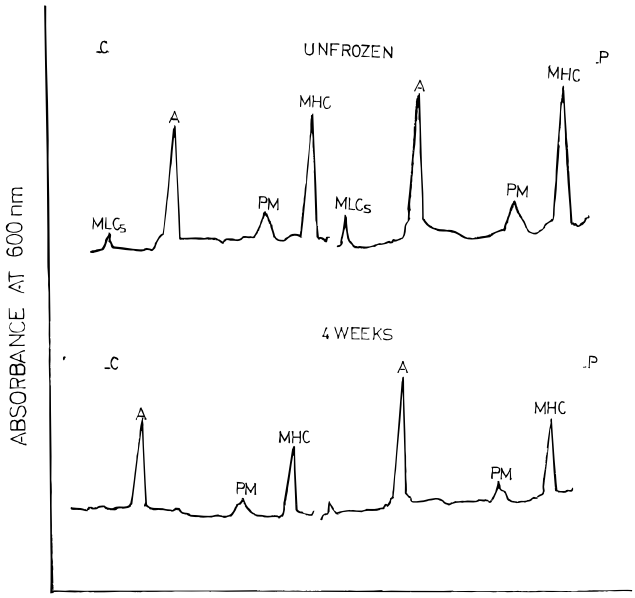

Figure 5. Densitometric analysis profiles of SDS−PAGE gels of actomyosin from control (C) and polyphosphates-treated (P) adductor muscles frozen stored at −30 °C: MHC, myosin heavy chain (200 kDa); PM, paramyosin (110 kDa); A, actin (42 kDa); MLCs, myosin light chains (17 kDa).

Increased protein functionality by phosphates is mediated through changes in hydrophobic protein interactions (Trout and Schmidt, 1987). Exposed hydrophobic groups of native myosin are located almost exclusively in the head region (Boredjo, 1983). It is generally accepted that the head of the myosin heavy chain has the ability to hydrolize ATP and to release energy for muscle contractions (Suzuki, 1981). Therefore the protective action of polyphosphates on the Mg^{2+}-ATPase activity of actomyosin in frozen stored muscles could be due to interactions with hydrophobic groups at the head of the myosin. A protective effect of inorganic polyphosphates on Ca^{2+}-ATPase in carp myofibrils was reported by Yagi et al., (1985).

The composition of actomyosin obtained by densitometric analysis of gels after SDS−PAGE (10%) is shown in Table 1. Relative percentages of myosin, paramyosin, and actin in actomyosin of unfrozen control adductor muscles at zero time were about 51, 14, and 36%, respectively. These results are similar to those reported in a previous work (Paredi et al., 1990). The composition of actomyosin did not change with immersion in a polyphosphates solution (Table 1). The relative composition of actomyosin in both treated and control samples was not greatly affected by freezing (data not shown). However during frozen storage, the relative percentages of myosin and paramyosin significantly decreased ($p < 0.01$) in treated and untreated samples during the first 4 weeks of storage (Table 1; Figure 5).

Correspondingly the relative percentage of actin increased. The myosin/actin ratio significantly ($p < 0.01$) decreased about 70%, and the myosin/paramyosin ratio significantly ($p < 0.01$) decreased about 30 and 20% in control and treated samples, respectively. At present the nature of the changes that lead to decreases in the myosin and paramyosin in the actomyosin of frozen stored adductor muscles is not known. Our results would indicate that myosin and paramyosin are less stable than actin in frozen stored muscles. Paramyosin forms the core of the thick filaments in the muscle of invertebrates, where it is covered by a cortical layer of myosin (Cohen et al., 1971; Szent-Gyorgyi et al., 1971; Elfvin et al., 1976). Therefore our results would indicate that thick filaments are more affected than thin filaments during frozen storage of adductor muscles. The stability of actin during frozen storage of fish was reported (Connell, 1960; Sikorski et al., 1976; Shenouda, 1980).

Polyphosphates delayed the decrease in enzymatic activity and provided some protection for the myosin light chains without affecting either the extractability or the viscosity of actomyosin from frozen stored muscles. Therefore the effect of polyphosphates on actomyosin denaturation could be attributed to interactions between the polyphosphates and the head of the myosin.

CONCLUSIONS

The expressible moisture in *Aulacomya* adductor muscles increased during freezing and frozen storage. These changes were accompanied by actomyosin denaturation. The myosin and paramyosin of the actomyosin complex were most affected. Immersion in polyphosphates solution was effective in reducing water loss in stored muscle. In addition polyphosphates did not affect the extractability and the viscosity of actomyosin and delayed the decrease in Mg^{2+}-ATPase activity.

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Received for review February 20, 1996. Accepted August 5, 1996.[⊗] This investigation was supported by grants from Consejo Nacional de Investigaciones Cientificas y Tecnicas (CONICET).

JF960113V

[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1996.