Thermal Denaturation of *Aulacomya ater ater* (Molina) Myofibrillar Proteins: A Differential Scanning Calorimetric Study

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Denaturation of the proteins from the adductor muscle of *Aulacomya ater ater* (Molina) was studied with DSC by monitoring maximum temperatures of transitions and denaturation enthalpies. Whole muscle free of connective tissue showed two transitions (T_{max} 50.5 and 72.5 °C) and ΔH of 2.5 cal/g. Sarcoplasmic proteins contributed to both denaturation peaks. The DSC thermograms of actomyosin were similar to those of whole muscle. Two endothermic peaks (36 and 50.5 °C) were observed in DSC thermograms of myosin. Paramyosin contributed to myosin transitions. With increasing pH and ionic strength, the thermal stability of whole muscle decreased. The total denaturation enthalpy significantly decreased with an increase of the ionic strength.

INTRODUCTION

The study of the thermal behavior of myofibrillar proteins is of technological importance in determining and predicting the final quality of meat products because functional and textural characteristics of meat depend mainly on their myofibrillar proteins.

The classical methods of measuring protein denaturation provide only a partial view of the phenomenom, and frequently the extraction and purification processes influence the native state of the proteins. Differential scanning calorimetry (DSC) offers a direct method to study the thermal transitions of muscle proteins *in situ* (Wright et al., 1977).

Stabursvik and Martens (1980) and Xiong et al. (1987) used DSC to examine the effect of pH and salts on the thermal stability of myofibrillar proteins in mammalian and avian muscle. Differences in the heat stability of myofibrillar proteins of fish and mammalian species have been reported by Martens and Vold (1976). Thermal denaturation of myofibrillar proteins was studied using DSC in several fish species (Hastings et al., 1985; Poulter et al., 1985; Davies et al., 1988; Beas et al., 1990; Howell et al., 1991). As compared with the myofibrillar thermostability of several mammalian, avian, and fish species, there are only a few DSC studies that included myofibrillar proteins of marine invertebrate species (Hastings et al., 1985; Akahane et al., 1985). As a result, the contributions of paramyosin and other proteins of invertebrate muscles to thermal transitions have not yet been conclusively studied.

Paramyosin forms the cores of the thick filaments in the muscle of invertebrates, where it is covered by a cortical layer of myosin (Cohen et al., 1971; Elfvin et al., 1976). The content of paramyosin in the myofibrillar proteins of invertebrates varies with species and muscle types (Horie et al., 1975; Winkelman, 1976; Levine et al., 1976). In addition, it has also been reported that the paramyosin considerably alters the characteristic texture of the marine meat gel products (Noguchi, 1979; Sano et al., 1986).

The purpose of this work was to study the thermal behavior of myofibrillar proteins of *Aulacomya* adductor muscles by monitoring both peak temperature maxima (T_{max}) and transition enthalpies by DSC. The effects of pH and ionic strength upon thermal stability were also studied.

MATERIALS AND METHODS

Specimens of *Aulacomya ater ater* (Molina) were collected from May 1991 through October 1992 from San José Gulf, Chubut, Argentina. Mature specimens, 60–70 mm in length, were selected. The maturity of gonads was determined by macroscopic observation and the histology of the mantle by the procedure described by Vinuesa and Tortorelli (1980). After the shells were cleaned, 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. All of the procedure was done at 0–4 °C, and the tissues were immediately used for isolation of protein.

Preparation of Myofibrillar Proteins. The procedure followed to obtain partially purified actomyosin was described previously (Paredi et al., 1990). Myosin was obtained from adductor muscles by the modified method of Focant and Huriaux (1976). Paramyosin was obtained from adductor muscles by the ethanol precipitation method (Stafford and Yphantis, 1972). EDTA (10 mM) and DTT (0.5 mM) were present throughout the preparation to minimize the proteolytic degradation of paramyosin.

Exudative Sarcoplasmic Fraction. The exudative sarcoplasmic fraction was obtained by centrifugation of whole muscle at 2500*g* for 20 min at 4 °C.

Protein Determination. Protein concentration for actomyosin, myosin, paramyosin, and the exudative sarcoplasmic fraction was determined according to the Lowry method, with bovine serum albumin added as standard (Lowry et al., 1951).

Criterion of Purity for Protein Preparations. The purity of actomyosin, myosin, and paramyosin was assessed by SDS–PAGE in 10% gels using a Shandon vertical gel apparatus, as reported by Portzio and Pearson (1977). The protein loaded on the gel was varied to check linearity of heavy myosin, actin, paramyosin, and light myosin chains. With 30 μg of protein a linear response was obtained. Quantitative composition of each protein 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 accessory (Kyoto, Japan).

Differential Scanning Calorimetry. Differential scanning calorimetric studies were performed in a DuPont 910 system attached to a Hewlett-Packard 7046 B recorder. The temperature calibrations were performed according to ASTM Norm E 474/80

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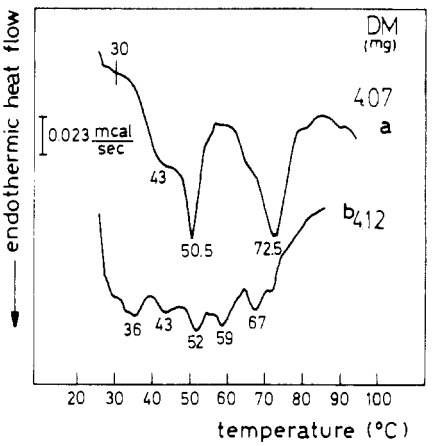

Figure 1. DSC thermograms of *Aulacomya* (a) whole muscle and (b) exudative sarcoplasmic fraction. Heating rate was 10 °C/min. DM, dry matter.

using indium thermograms. The samples (13–20 mg wet weight) were placed in DSC hermetic pans, assuring a good contact between the sample and the capsule bottom. As reference, a hermetic capsule with 17–18 μL of distilled water was used. After DSC analysis, the capsules were punctured and the dry matter weight determined by drying at 105 °C overnight. All of the samples were scanned at 10 °C/min over the range 10–100 °C, at a sensitivity of 0.5 mV/cm. Total denaturation enthalpies (ΔH) were estimated by measuring the area under the DSC transition curve (a baseline was constructed as a straight line from the start to the end of the endotherm). The endotherm areas were measured with a Morphomat 306 Zeiss image analyzer (Carl Zeiss, Ober Cochem, Germany).

pH and Ionic Strength Adjustment. Small pieces of muscle were dissected with a scalpel, treated with a solution of 0.1 M phosphate buffer, and stirred for 30 min at 4 °C. The pH was adjusted to the desired value with 0.1 N NaOH or 0.1 N HCl. The ionic strengths (I) were adjusted by the addition of NaCl at values between I = 0.005 and 0.5.

Statistical Analysis. Analysis of variance was applied to the data using the statistical analysis package SYSTAT (Wilkinson, 1990).

RESULTS AND DISCUSSION

Thermal Denaturation of *Aulacomya* Whole Adductor Muscle Proteins. DSC thermograms of whole muscle have the characteristic profile shown in Figure 1. Because of their complexity, it is convenient to describe the transitions in terms of their T_{max} (Wright et al., 1977). The DSC thermograms of whole muscle of fresh *Aulacomya* show two endothermic transitions, with T_{max} values of 50.5 ± 0.5 and 72.5 ± 0.5 °C , and a shoulder at about 43 °C (Figure 1). The denaturation enthalpy of whole muscle was 2.50 ± 0.05 cal/g. Because of the small amount of connective tissues in bivalve molluscs muscles (Soudan, 1965) and fish muscles (Brown, 1986), the endothermic transitions can be assigned to denaturation of myofibrillar protein and sarcoplasmic proteins. The DSC profiles of the exudative sarcoplasmic fraction of whole muscle showed transitions at 36, 43, 52, 59, and 67 °C (ΔH = 1.7 cal/g) (Figure 1).

The DSC thermograms of whole mammaliam muscle showed three transitions with T_{max} values of 57–60, 62–67, and 74–80 °C at a heating rate of 10 °C/min (Wright et al., 1977; Wagner and Añon, 1986; Xiong et al., 1987). The T_{max} values of *Aulacomya* muscle transitions were lower than those of mammaliam muscles. Similar profiles were reported with other fish species (Martens and Vold, 1976; Akahane et al., 1985; Hastings et al., 1985; Poulter et al., 1985; Beas et al., 1990; Howel et al., 1991). Connell (1961), Hasnain et al. (1979), Hastings et al. (1985), and Davies et al. (1988) attributed higher heat lability to fish proteins than to mammalian muscle proteins. Akahane et al. (1985) observed large variations in heat stability among the myofibrillar proteins isolated from rabbit, carp, and scallop.

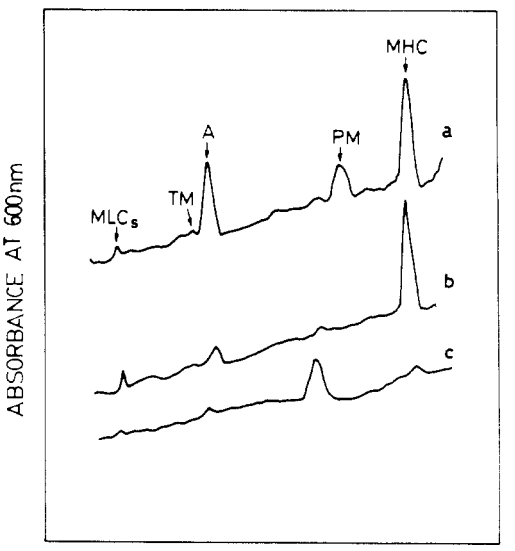

Figure 2. Densitometric patterns of SDS–PAGE (10%) gels: (a) actomyosin; (b) myosin; (c) paramyosin. MHC, myosin heavy chain (200 kDa); PM, paramyosin [(a) 110 kDa; (c) 106 kDa]; A, actin (42 kDa); TM, tropomyosin (36 kDa); MLCs, myosin light chains (17 kDa).

To investigate the contribution of actomyosin, myosin, and paramyosin to the thermal transitions of whole muscle (shown in Figure 1), these myofibrillar proteins were isolated from *Aulacomya* muscle. Densitometric analysis profiles of SDS–PAGE 10% gels of actomyosin, myosin, and paramyosin can be observed in Figure 2. The purities of these proteins were estimated at about 87, 95, and 90%, respectively. DSC thermograms of actomyosin show a similar profile to that of whole muscle with T_{max} at 42.5, 48, and 68 °C (Figure 3). There was a displacement of the thermal transitions to temperatures lower than those of whole muscle. The DSC thermogram of myosin shows two endothermic transitions at 36 and 50.5 °C and a shoulder at 29 °C. Wright et al. (1977) concluded that the existence of up to three myosin transitions implies structural changes in three discrete regions of the molecule. The three regions of the molecule differing in thermal stability could be related to the unfolding of the hinge region and head and tail sections (Samejima et al., 1983; Wright and Wilding, 1984).

The DSC profile of paramyosin shows thermal transitions at 34, 42, 51, and 66 °C. Halsey and Harrington (1973) identified two melting transitions at 44 and 64 °C in paramyosin, corresponding to the lower of two transitions with the trypsin-sensitive or hinge region related to the catch mechanism of molluscan muscle. Taking into account that the myosin/paramyosin ratio in *Aulacomya* adductor muscle is about 4:1 (Paredi et al., 1990), the thermogram of whole muscle could be reconstructed on the basis of thermograms corresponding to actomyosin, paramyosin, and sarcoplasmic proteins. These results would indicate that myosin and paramyosin contribute mainly to the first transition and that actin is responsible for most of the second transition in whole muscle. In addition, the results would also indicate a higher thermal stability for the native muscle than for the isolated myofibrillar proteins. These results are in agreement with those reported by Wright et al. (1977), Xiong et al. (1987), and Davies et al. (1988).

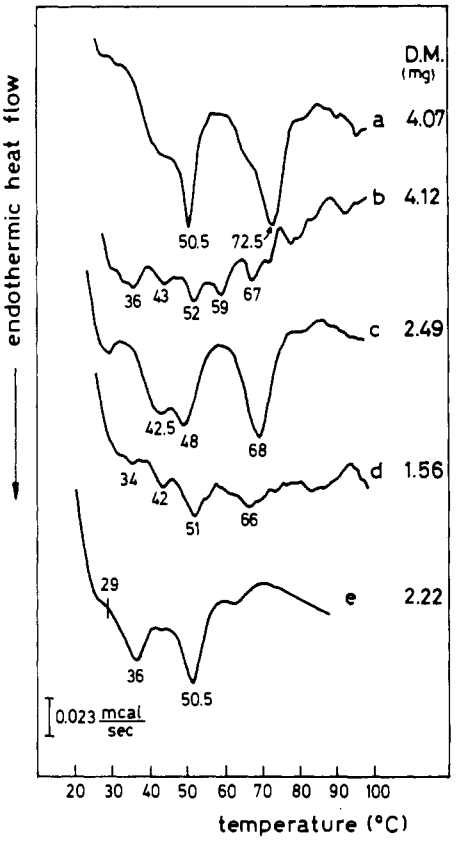

Figure 3. DSC thermograms of *Aulacomya*: (a) whole muscle; (b) exudative sarcoplasmic fraction; (c) actomyosin; (d) paramyosin; (e) myosin. Heating rate was 10 °C/min. DM, dry matter.

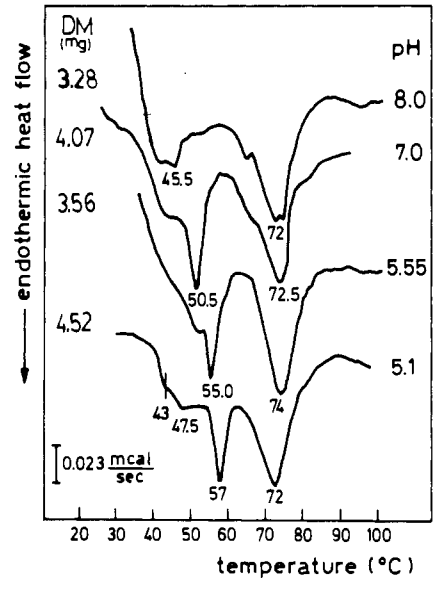

Figure 4. DSC thermograms of whole muscle of *Aulacomya* at different pH values ($I = 0.05$). Heating rate was 10 °C/min. DM, dry matter.

T_{max} values corresponding to whole muscles of different species are shown in Table 1. It had been reported that the principal difference between the DSC thermograms corresponding to muscles of different species was the values obtained for the different thermal transitions (Stabursvick and Martens, 1980). Among fish species, thermal stability of myosin increases in species adapted to higher environmental temperatures (Hastings et al., 1985; Poulter et al., 1985; Howell et al., 1991). Also, the thermal stability of the isolated native myosin in tropical fish was reported to be higher than that of cold-water fish (Davies et al., 1988). As can be seen in Table 1, similar values of T_{max} were obtained with *Aulacomya* living at an environmental temperature of about 10 ° C and with tropical species with habitat temperatures in the range 23–27 °C. The fact that *Aulacomya*, living at temperatures of 10 °C, presented a T_{max} of the first transition higher that those of cold-water fish and similar to those of tropical fish could be due to an inherent higher stability of myosin from *Aulacomya* and, at least partially, to the presence of paramyosin that forms the core of the thick filaments covered by cortical layers of myosin in the invertebrate muscles (Waterson et al., 1974). Structural myosin–paramyosin interactions could be responsible for the high T_{max} value of the first transition in the DSC thermogram of whole muscle of invertebrates. In agreement with this hypothesis, the first peak in the DSC thermogram of whole muscle of squid from cold-water habitat is similar to that of whole muscle of *Aulacomya* (Hasting et al., 1985).

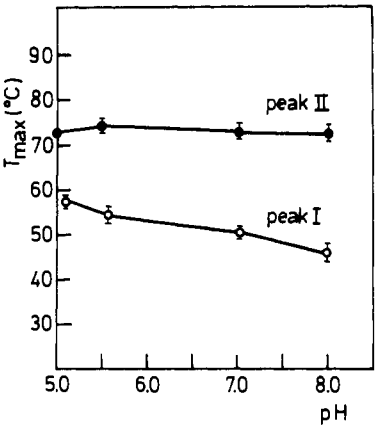

Figure 5. T_{max} of peaks I and II corresponding to the DSC thermograms of whole muscle at different pH values. Heating rate was 10 °C/min. Each value represents a mean of three determinations ± SD.

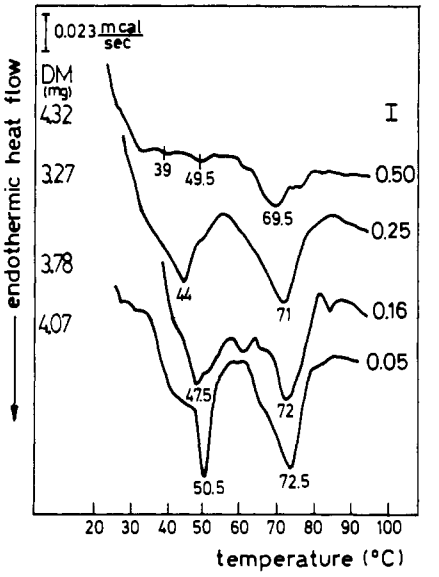

Figure 6. DSC thermograms of whole muscle of *Aulacomya* at different ionic strength values (pH 7.0). Heating rate was 10 °C/min. DM, dry matter.

Effect of pH and Ionic Strength on the Thermal Behavior of Myofibrillar Proteins. The characteristics associated with the chemical environment such as pH and ionic strength could modify both the thermal stability and the conformational structure of the proteins (Wright and Wilding, 1984). As can be seen in Figures 4 and 5, when pH increased, a displacement of thermal transitions to

Table 1. Peak Temperatures of Transition (T_{max}) in Whole Muscle for Different Species

species	habitat temp (°C)	T_{max} (°C)			
rabbit	a	60.0		80.0	Wright et al. (1977)
bovine (M. semimembranosus)	a	58.8	66.0	80.2	Wagner and Añon (1986)
cod (*Gadus morhua*)	2–8	42.0		76.0	Hastings et al. (1985)
squid (*Loligo farbesi*)	2–6	50.0		77.0	Hastings et al. (1985)
hake (*M. hubbsi*)	8–10	46.5		75.3	Beas et al. (1990)
A. ater ater (Molina)	8–12	50.5		72.5	present work
sea bream (*Gymnocranius rivulatus*)	23–27	51.7		72.6	Howell et al. (1991)

[a] Warm-blooded mammals.

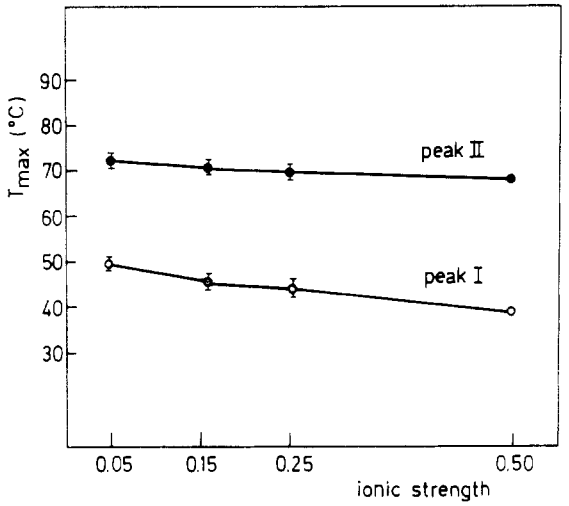

Figure 7. T_{max} of peaks I and II corresponding to the DSC thermograms of whole muscle at different ionic strength values. Heating rate was 10 °C/min. Each value represents a mean of three determinations ± SD.

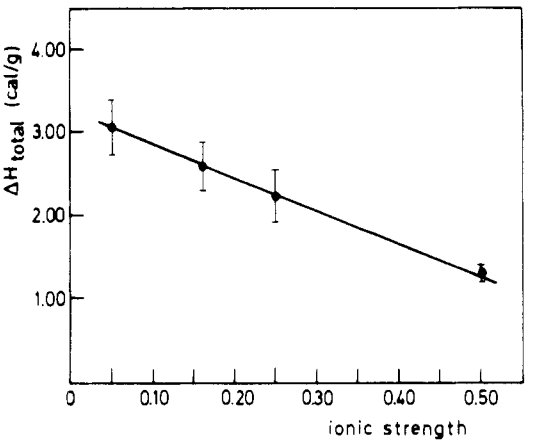

Figure 8. Denaturation enthalpy corresponding to DSC thermograms of whole muscle at different ionic strength values. Each value represents a mean of three determinations ± SD.

lower temperatures occurred. The decrease in the thermal stability was accompanied by significant modifications ($p < 0.05$) in the myosin–paramyosin area of the thermogram at pH 8. Conversely, no major changes were observed in the actin area. These results are in agreement with those obtained in other fish species (Beas et al., 1990; Howell et al., 1991). Total denaturation enthalpy did not show a defined trend for increasing pH values (data not shown).

The effect of ionic strength on the thermal stability of the protein in whole muscle is shown in Figures 6 and 7. Great modifications in the myosin–paramyosin zone of the thermogram with a displacement of T_{max} to lower values and a decrease in endothermal areas was observed when the ionic strength increased to 0.5. This response of proteins to ionic strength is in agreement with that observed in other fish species (Howell et al., 1991; Beas et al., 1991). It has been postulated that at low ionic strength molecules of myosin aggregate to form filaments with greater stability than the individual molecules which exist at high ionic strength (Samejima et al., 1983). Merrick and Johnson (1977) reported that solubility of paramyosin increased at high ionic strength. In addition, a significant decrease ($p < 0.05$) of the T_{max} corresponding to the actin peak and a slight decrease of its area were observed. In agreement with these results, KCl destabilizes actin in bovine muscle and the degree of destabilization depends on KCl concentration (Stabursvik and Martens, 1980). Moreover, the destabilization of hake actin by KCl was also reported (Beas et al., 1990; Howell et al., 1991).

As can be seen in Figure 8, a significant decrease ($p < 0.05$) of the denaturation enthalpy with an increase in ionic strength was observed. Similar results were obtained with myofibrils of several fish species (Howell et al., 1991) and surimi treated with 3% NaCl (Beas et al., 1991).

In conclusion, whole muscle of *Aulacomya* showed two endothermic transitions during thermal denaturation. The T_{max} values of the first transition were similar to those of tropical fish species. As pH and ionic strength were increased the thermal stability of whole muscle decreased. Ionic strength appeared to have a greater effect on denaturation enthalpies than pH.

The knowledge of thermal stability of the muscle proteins of *Aulacomya* provides information as to how the muscle will behave during freezing and under frozen storage. Moreover, it is important to optimize processes such as curing (acid conditions) or the production of gelled marine meat products (high salt concentrations affecting the gelation of myofibrillar proteins) by selecting the proper pH and ionic strength levels.

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