# Thermal Stability of Myofibrillar Proteins from Smooth and Striated Muscles of Scallop (*Chlamys tehuelchus*): A Differential Scanning Calorimetric Study

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Denaturation of proteins from striated and smooth muscles of scallop (*Chlamys tehuelchus*) was studied with DSC by monitoring maximum temperatures of transitions and denaturation enthalpies. DSC thermograms of both striated and smooth muscles free of connective tissue showed two transitions, ($T_{max}$ of 53.2 and 79 °C) and ($T_{max}$ of 52.7 and 78 °C), respectively. These results indicate that the different paramyosin content of the muscles did not influence the thermal stability of their proteins. The DSC thermograms of myofibrils and actomyosin were similar to those corresponding to respective whole muscles. Myosin from striated muscles showed a cooperative single peak with $T_{max}$ of 48.8 °C. Similar $T_{max}$ values were observed in DSC thermograms of myosin from smooth muscle. As pH and ionic strength increased, the thermal stability of whole muscle decreased. Smooth muscles were more affected than striated muscles. The pH increment significantly affected denaturation enthalpies ($\Delta H_{total}$ and $\Delta H_{peak\ I}$) of whole smooth muscles. Denaturation enthalpies ($\Delta H_{total}$ and $\Delta H_{peak\ I}$) significantly decreased ($p < 0.05$) when ionic strength increased to 0.5 in both type of muscles.

**Keywords:** *Scallop adductor muscle; thermal stability myofibrillar proteins; paramyosin; differential scanning calorimetry*

## INTRODUCTION

The study of the thermal behavior of myofibrillar proteins is of technological importance to determine and predict the final quality of meat products because functional and textural characteristics of meat depend mainly on their myofibrillar proteins. Differential scanning calorimetry (DSC) offers a direct method to study the thermal transitions of muscle proteins in situ (Wright et al., 1977). The thermal denaturation of myofibrillar proteins was studied in several fish species by DSC (Hastings et al., 1985; Poulter et al., 1985; Park and Lanier, 1989; Beas et al., 1990; Howell et al., 1991; Hsu et al., 1993; Ogawa et al., 1993; Davies et al., 1994). Compared with the myofibrillar proteins stability of several mammalian, avian, and fish species there are few DSC reports on myofibrillar proteins of marine invertebrate species (Akahane et al., 1985; Hastings et al., 1985; Paredi et al., 1994, 1995, 1996). Paramyosin is a myofibrillar protein characteristic in marine invertebrate (Cohen et al., 1971). It is the core of thick filaments in the muscle of invertebrate where it is covered by a cortical layer of myosin (Cohen et al., 1971; Elfvin et al., 1976). It has been reported that the paramyosin considerably alters the characteristic texture of the marine meat gel products (Noguchi, 1979; Sano et al., 1986). Recently, it has also been suggested that either paramyosin content or different myosin−paramyosin interactions could influence the thermal stability of myofibrillar proteins in marine invertebrates (Paredi et al., 1994). Paramyosin content in the myofibrillar proteins of these organisms varies with species and muscle types (Horie et al., 1975; Winkelman, 1976; Levine et al., 1976). It was reported that the smooth muscle of scallops (*Chlamys tehuelchus*) contains more paramyosin than the striated one (Paredi, 1994). The purpose of this work was to study the possible influence of paramyosin content on thermal behavior of myofibrillar proteins, by DSC determinations of both smooth and striated muscles of scallops (*Chlamys tehuelchus*). In addition, the influence of chemical environment on the thermal stability of the myofibrillar proteins was also investigated.

## MATERIALS AND METHODS

Specimens of *Chlamys tehuelchus* (D'orbigny) were collected from June 1995 through October 1996 from San Jose Gulf, Chubut, Argentina. Mature specimens, 70 mm in length, were selected. The maturity of gonads was determined by macroscopic observation, and the histology was determined by the procedure described by Lasta and Calvo (1978). 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. The striated and smooth muscles were separated. All procedures were

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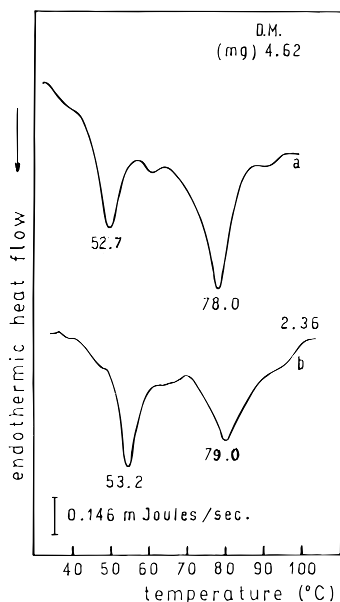


**Figure 1.** DSC thermograms of whole muscle from scallop: (a) smooth and (b) striated (pH 6.8). Heating rate = 10 °C/min. DM, dry matter.

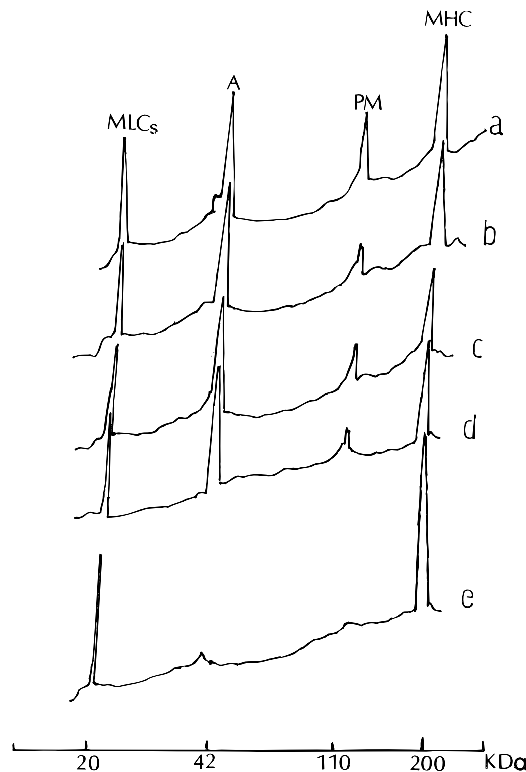


**Figure 2.** Densitometric patterns of SDS−PAGE (10%) gels: (a) myofibrils of smooth muscles, (b) myofibrils of striated muscles, (c) actomyosin of smooth muscles, (d) actomyosin of striated muscles, (e) myosin of striated muscles. MHC, myosin heavy chain (200 kDa); PM, paramyosin (110 kDa); A, actin (42 kDa); MLCs, myosin light chains (20 kDa).

carried out at 0−4 °C, and the tissues were immediately used for protein isolation.

**Preparation of Myofibrillar Proteins.** Myofibrils were obtained according to the method described by Chantler and Szent-Gyorgyi (1980). The procedure followed to obtain partially purified actomyosin was decribed previously (Paredi et al., 1990). Myosin was obtained from adductor muscles by ammonium sulfate fractionation according to the method of Focant and Huriaux (1976), with slight modifications as previously described (Paredi, 1994). The range of saturation of ammonium sulfate used was 40−65% in the presence of 0.5 M NaCl and 5 mM phosphate buffer (pH 7.0) (5 mM ATP and 20 mM $MgSO_4$). The final suspension of myosin was precipited with 5 mM phosphate buffer (pH 6.5).

**Protein Determination.** Protein concentration for myofibrils, actomyosin, and myosin was determined by the Lowry method, with bovine serum albumin (BSA) as the standard (Lowry et al., 1951).

**Criterion of Purity for Protein Preparations.** The purity of myofibrils, actomyosin, and myosin 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. A linear response was obtained with 30 μg of protein. 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 (DSC) 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 using indium thermograms. The samples (17−20 mg wet weight) were placed in DSC hermetic pans, ensuring a good contact between the sample and the capsule bottom. Quadruplicate samples were analyzed. As reference, a hermetic capsule with 18−19 μL of distilled water was used. After DSC analysis, the capsules were punctured, and the dry matter weight was determined by drying at 105 °C overnight. All the samples were scanned at 10 °C/min over the range of 10−100 °C, at a sensitivity of 0.5 mV/cm. Total denaturation enthalpies($\Delta H_{total}$) and denaturation entalphies of first transition ($\Delta H_{peak\ I}$) 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 the Sigma Scan Package (The Scientific Measurement Program 3.90, 1992).

**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 $I$ values between 0.05 and 0.5.

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

## RESULTS AND DISCUSSION

**Thermal Denaturation of Both Striated and Smooth Whole Adductor Muscle Proteins.** DSC thermograms of striated whole muscles showed two endothermic transitions with $T_{max}$ of 53.2 and 79.0 °C (Figure 1). In thermograms of smooth whole muscle, two endothermic transitions with $T_{max}$ of 52.7 and 78.0 °C were present (Figure 1). No significant differences ($p < 0.05$) were found between the $T_{max}$ of both types of muscles.

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.

Myofibrils, actomyosin, and myosin endothermic transitions were also analyzed to investigate the contribution of myofibrillar proteins to DSC transitions of both smooth and striated whole muscles. The purity of myofibrils, actomyosin, and myosin was previously checked by 10% SDS−PAGE. Densitometric analysis

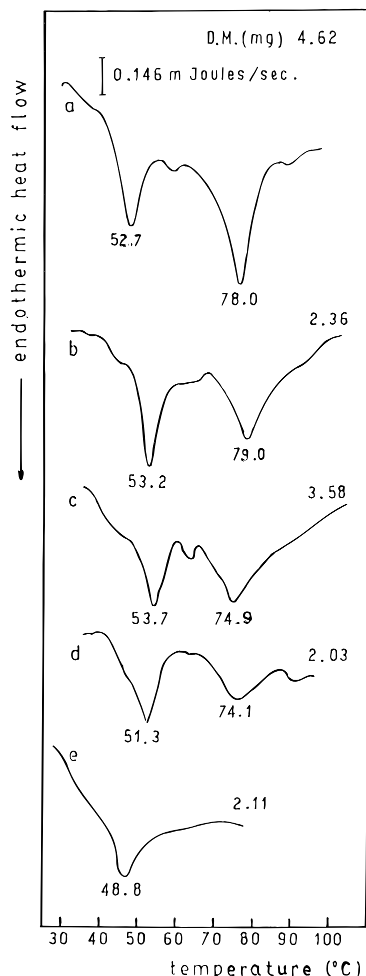


**Figure 3.** DSC thermograms of scallop: (a) whole smooth muscle, (b) striated muscle, (c) myofibrils of striated muscle, (d) actomyosin of striated muscle, (e) myosin of striated muscle; $I = 0.05$ and pH 6.8. Heating rate = 10 °C/min. DM, dry matter.

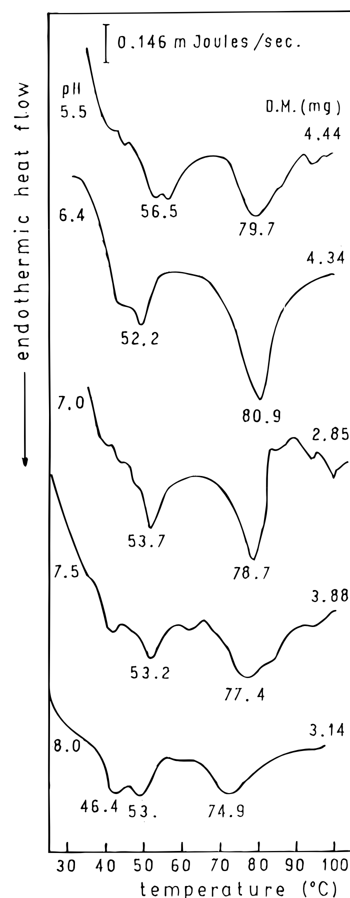


**Figure 4.** DSC thermograms of whole smooth muscle of scallop at different pH values ($I = 0.05$). Heating rate = 10 °C/min. DM, dry matter.

profiles of 10% SDS−PAGE gels of myofibrils, actomyosin, and myosin can be observed in Figure 2. The characteristic polypeptidic bands corresponding to myofibrils and to each major myofibrillar protein were present in the gels. As can be seen in Figure 2, the most important difference observed in the densitometric analysis of proteins was that both myofibrils and actomyosin of smooth muscles have a higher paramyosin content than those of striated muscles. These results were similar to those previously reported (Paredi, 1994).

Myofibrils and actomyosin isolated and purified from striated muscle showed thermograms similar to those of the respective whole muscles, with a displacement toward lower temperatures (Figure 3). The DSC thermograms corresponding to myofibrils and actomyosin from smooth muscles were similar to those of the striated one (data not shown). DSC thermograms of purified myosin from striated muscles showed a cooperative single peak with $T_{max}$ of 48.8 °C (Figure 3); similar profiles were observed in myosin from smooth muscles (data not shown).

Paredi et al. (1994) reported two major endothermic transitions in whole adductor muscles from the bivalve mollusc *Aulacomya*. In that work, the first transition was related to myosin and paramyosin denaturation, and the second was related to actin denaturation. In this way, the first transition in DSC thermograms of both myofibrils and actomyosin from scallop would be related to myosin and paramyosin denaturation, and the second transition would be related to actin denaturation. In addition, the results would also indicate a higher thermal stability for the native muscle than for the isolated myofibrillar proteins. These results agree with those reported by Wright et al. (1977), Xiong et al. (1987), and Davies et al. (1988).

**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, as the pH increased above 7 there was a displacement of thermal transitions to lower temperatures in both smooth and striated muscles. Smooth muscles were much more modified by pH increment than striated ones (Figures 4 and 5). 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 in both types of muscles. These results agree with those obtained in other fish species (Beas et al., 1990; Howell et al., 1991; Paredi et al., 1994). A significant decrease ($p < 0.05$) in $\Delta H_{total}$ and $\Delta H_{peak\ I}$ at pH 7.5 and pH 8.0 was observed in smooth muscle (Table 1). No significant differences were observed in $\Delta H_{total}$ and $\Delta H_{peak\ I}$ in striated muscles for increasing pH values (Table 1).

The effect of ionic strength on the thermal stability of the protein in smooth and striated whole muscles is

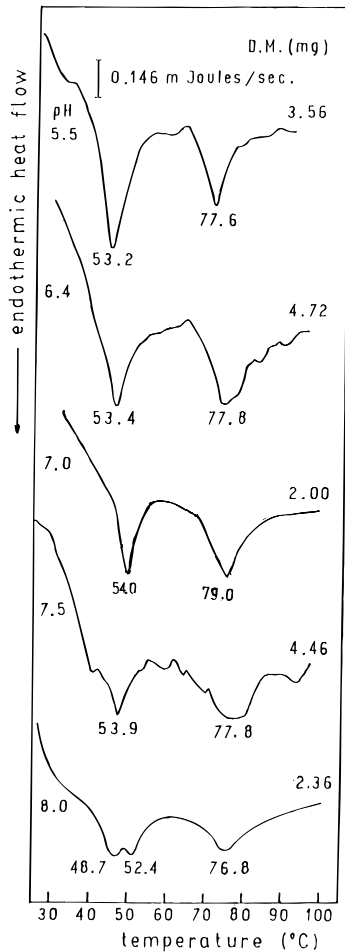


**Figure 5.** DSC thermograms of striated whole muscle of scallop at different pH values ($I = 0.05$). Heating rate = 10 °C/min. DM, dry matter.

**Table 1. Denaturation Enthalpies ($\Delta H_{total}$ and $\Delta H_{peak\ I}$) Corresponding to DSC Thermograms of Whole Muscle at Different pH Values**[a]

| | smooth muscle | | striated muscle | |
|---|---|---|---|---|
| pH | $\Delta H_{total}$ (J/g) | $\Delta H_{peak\ I}$ (J/g) | $\Delta H_{total}$ (J/g) | $\Delta H_{peak\ I}$ (J/g) |
| 5.5 | 20.09 ± 0.0 | 13.90 ± 0.0 | 19.00 ± 1.30 | 12.60 ± 0.90 |
| 6.4 | 19.51 ± 1.33 | 11.30 ± 0.40 | 17.20 ± 1.30 | 13.00 ± 0.90 |
| 7.0 | 18.42 ± 0.75 | 8.95 ± 0.50 | 18.29 ± 0.00 | 10.42 ± 0.00 |
| 7.5 | 14.23 ± 0.15* | 7.62 ± 0.15* | 16.60 ± 0.00 | 10.55 ± 0.00 |
| 8.0 | 11.01 ± 0.0* | 5.98 ± 0.0* | 17.12 ± 0.30 | 11.80 ± 0.30 |

[a] Each value is represented as mean ± SD ($n = 4$). An asterisk (*) indicates that the values are significantly different from others in the same column ($p < 0.05$).

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 in both smooth and striated muscles. These results agree with those observed in other fish species (Howell et al., 1991; Beas et al., 1991) and marine invertebrates (Paredi et al., 1994). It has been postulated that, at low ionic strength, molecules of myosin aggregate to form filaments with greater stability than the individual molecules that exist at high ionic strength (Samejima et al., 1983). Merrick and Johnson (1977) reported that solubility of paramyosin also increased at high ionic strength.

The $T_{max}$ of the actin area shows a trend to decrease when ionic strength increased to 0.5 in DSC thermo-

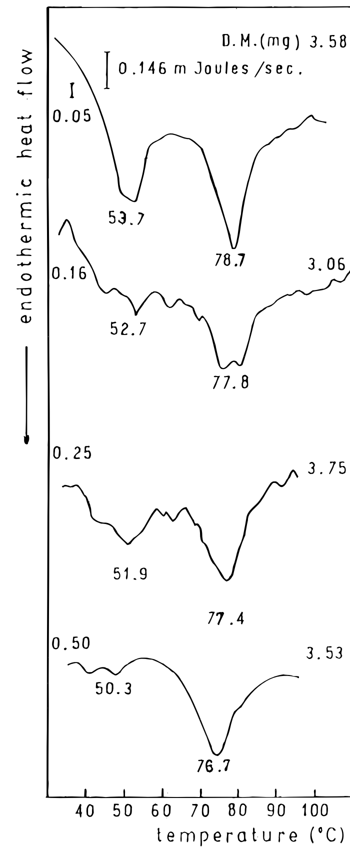


**Figure 6.** DSC thermograms of whole smooth muscle of scallop at different ionic strength values (pH 7.0). Heating rate = 10 °C/min. DM, dry matter

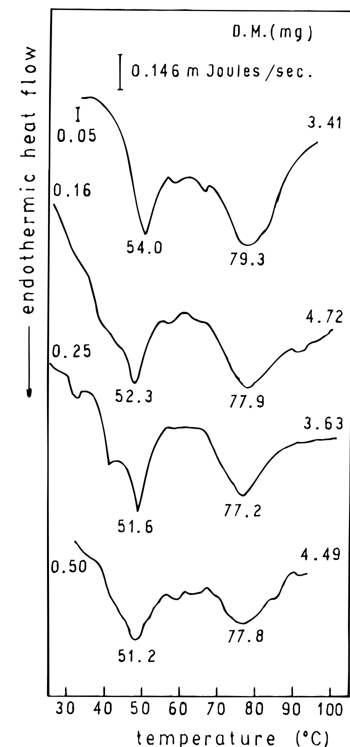


**Figure 7.** DSC thermograms of whole striated muscle of scallop at different ionic strength values (pH 7.0). Heating rate = 10 °C/min. DM, dry matter.

grams of smooth muscle. A significative decrease ($p < 0.05$) of the $T_{max}$ corresponding to the actin area was observed in striated muscles (Figure 7). In agreement

**Table 2. Denaturation Enthalpies ($\Delta H_{total}$ and $\Delta H_{peak\ I}$) Corresponding to DSC Thermograms of Whole Muscles at Different Ionic Strength Values[a]**

| | smooth muscle | | striated muscle | |
|---|---|---|---|---|
| ionic strength | $\Delta H_{total}$ (J/g) | $\Delta H_{peak\ I}$ (J/g) | $\Delta H_{total}$ (J/g) | $\Delta H_{peak\ I}$ (J/g) |
| 0.05 | 17.20 ± 0.0 | 8.80 ± 0.0 | 16.32 ± 0.70 | 7.45 ± 0.50 |
| 0.16 | 18.30 ± 0.16 | 8.30 ± 0.20 | 12.60 ± 1.30 | 8.62 ± 0.90 |
| 0.25 | 19.50 ± 0.25 | 7.90 ± 0.20 | 14.65 ± 1.00 | 8.54 ± 0.90 |
| 0.50 | 7.95 ± 0.03* | 0.71 ± 0.06* | 10.13 ± 0.90* | 4.39 ± 0.90* |

[a] Each value is represented as mean ± SD ($n = 4$). An asterisk (*) indicates that the values are significantly different from others in the same column ($p < 0.05$).

with these results, it had been reported that KCl destabilizes actin of bovine and fish striated muscles (Stabursvik and Martens, 1980; Beas et al., 1990; Howell et al., 1991).

As can be seen in Table 2, a significant decrease ($p < 0.05$) of the denaturation enthalpies ($\Delta H_{total}$ and $\Delta H_{peak\ I}$) was observed in both types of muscles, when the ionic strength increased to 0.5. Similar results were obtained with other fish species (Howell et al., 1991; Paredi et al., 1994).

In conclusion, both whole smooth and striated muscles of scallops showed two endothermic transitions during thermal denaturation. The $T_{max}$ of the first transition was similar in both smooth and striated muscles. Paramyosin content was higher in smooth muscle than in striated ones. These results indicate that the paramyosin content has no significant influence in the thermal stability of muscle proteins. When pH and ionic strength were increased, the thermal stability of whole muscles decreased. Smooth muscles were more affected than striated muscles. Ionic strength appeared to have a greater effect on denaturation enthalpies than pH. Different myosin−paramyosin interactions would be related with the thermal behavior observed in both types of muscles due to the effects of the chemical environment.

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