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Thermal behavior of myofibrillar proteins from the adductor muscles of scallops. A differential scanning calorimetric study (DSC)

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ABSTRACT

Muscles and different proteins obtained from scallops (*Chlamys tehuelchus* and *Zygochlamys patagonica*) were analyzed. In both species of scallop, the thermograms of striated and smooth muscles showed two endothermic transitions. The T_{max} values corresponding to striated and smooth muscles from *C. tehuelchus* were 53.2, 79.0ºC and 52.7, 78.0 ºC, respectively. The T_{max} corresponding to striated and smooth muscles of *Z. patagonica* were 55.0, 79.2ºC and 54.7, 78.7ºC, respectively. No significant differences were observed between the thermal transitions of myofibrils and actomyosin of both types of muscles from both species of scallop. Irrespective of the species, the thermal stability decreased when the pH of the muscles was increased. The increase in ionic strength greatly affected T_{max}, the ΔH total and the ΔH of the first transition. A significant decrease in ΔH total and ΔH corresponding to both transitions was observed, particularly in the striated muscles of *Zygochlamys patagonica*. These results indicate a major sensitivity of the adductor muscles of *Zygochlamys* to changes in the chemical environment.

Keywords: thermal stability, chemical environment, scallop.

INTRODUCTION

The study of the thermal behavior of myofibrillar proteins is of technological importance for determining and predicting the final quality of meat products because the functional and textural characteristics of meat depend mainly on their myofibrillar proteins. Differential scanning calorimetry (DSC) offers a direct method of studying the thermal transitions of muscle proteins in situ (Wright et al., 1977).

Invertebrate proteins have different thermal characteristics because they have an inherent myofibrillar protein called paramyosin (Paredi et al., 1994). Paramyosin constitutes the core of thick filaments in invertebrate muscles, where which is covered by a cortical layer of myosin (Cohen et al., 1971; Elfvin et al., 1976). Paramyosin considerably alters the characteristic texture of marine meat gel products

(Noguchi, 1979; Sano et al., 1986). Scallops have two kinds of adductors, the larger one which contains striated muscle and the a smaller one which contains smooth muscles. (Kondo and Morita, 1981). The smooth muscle of the Tehuelche scallop (*Chlamys tehuelchus*) contains more paramyosin than the striated muscle (Paredi, 1994). In addition, smooth muscles of the patagonian scallop contain more paramyosin than those of the tehuelche scallop (Paredi and Crupkin, 2000). Previous work suggested that different paramyosin-paramyosin or myosin-paramyosin interactions influence the thermal stability of myofibrillar proteins in marine invertebrates (Paredi et al., 1998). The purpose of this work was to use DSC to study the thermal behavior of myofibrillar proteins in striated and smooth muscles from two species of scallop in different chemical environments.

MATERIALS AND METHODS

Specimens of the tehuelche scallop (*Chlamys tehuelchus)* were collected at the Gulf of San Jose, Chubut, Argentina. Mature specimens with a length of 70mm, were selected. Patagonian scallops (*Zygochlamys patagónica)*were caught on the Argentine Continental Shelf in the Reclutas bed (39° 24' S; 55° 56' W*).* Mature specimens with a shell height of 55-65mm, were selected. Muscles were dissected and carefully freed of adhering pancreatic and liver tissues. All the procedures were carried out at 2-4°C.

Preparation of Myofibrillar Proteins

The procedure followed to obtain partially purified actomyosin was described by Paredi et al. (1990). Myofibrils was obtained according to Chantler and Szent-Gyorgyi (1980) .

The purity of proteins was assessed by SDS-PAGE 10% gels as reported by Laemmli (1970). The protein concentration was determined according to the Lowry method (Lowry et al., 1951).

Differential Scanning Calorimetry

DSC studies were performed in a Polymer Lab (PL-DSC, Reometric Scientific) according to previous work (Paredi et al., 1994). Temperature calibrations were made according to ASTM Norm E 474/80 using indium thermograms. The samples (20mg wet weight) were placed in the DSC hermetic pans, assuring good contact between the sample and the capsule bottom. Triplicate samples were analyzed. A hermetic pan with 18µl of distilled water was used as reference. After DSC analysis, capsules were punctured and the dry matter was determined by drying at 105°C overnight. All the samples were scanned at 10°C/min over the range of 10 to 100°C at a sensitivity of 0.5mV/cm. Total and partial denaturation enthalpies were estimated by measuring the area under the DSC transition curve.

pH and Ionic Strength Adjustment

Small pieces of muscle were dissected with a scalpel, treated with a 0.05M phosphate buffer solution, and stirred for 30 min at 0-4° C. The pH was adjusted to the desired value with 0.1N NaOH or 0.1N HCl. The ionic strengths were adjusted by the addition of NaCl at values between I=0.05 and 0.5.

Statistical Analysis

Analysis of variance was used and Duncan's New Multiple Range Test was applied to the data using a SYSTAT statistical analysis package (SYSTAT, 1994).

RESULTS AND DISCUSSION

DSC thermograms of striated whole muscle from patagonian scallops showed two endothermic transitions with T_{max} of 55.0 and 79.2° C (Figure 1a). In the thermograms of smooth whole muscle a similar profile was obtained and two endothermic transitions with T_{max} 54.7 and 78.7°C were observed (Data not shown). Similar DSC thermograms were obtained for striated and smooth muscles from the tehuelche scallop with T_{max} of 53.2, 79.0°C and 52.7, 78.0°C, respectively. To study the contribution of myofibrillar proteins to DSC transition of whole muscle, muscle depleted of sarcoplasmic protein fraction, myofibrils and actomyosin from smooth and striated muscles were also analyzed. Figure 1 shows that the endothermic transitions form muscle depleted of sarcoplasmic protein, myofibrils and actomyosin from striated muscle of Zygochlamys patagonica. Isolated and purified myofibrils and actomyosin from both types of muscle of both species of scallop showed thermograms similar to those of the respective whole muscles, with a slight displacement towards lower temperature. These results indicate that myosin and paramyosin contributed to the first transition and acting to the second transition. Similar results were reported for other invertebrates species (Paredi et al., 1994, 1996).

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 shown in Figure 1(b and c), when pH increased to 8.0 a displacement of the thermal transition to lower temperatures occurred. A significant decrease ($p<0.05$) in the T_{max} values for both transitions was found in striated muscle of the patagonian scallop at pH 8.0. Smooth muscle was affected in a similar manner by pH (data no shown). Striated muscles were more affected by the increase in pH than smooth ones. The DSC thermograms of two types of adductor muscle from the tehuelche scallop were similar to those of the patagonian scallop, but the smooth muscles were more affected by pH (data not shown). A significant decrease ($p<0.05$) in ΔH total and ΔHpeak I when the pH increased to 8.0 was observed in both types of muscles from the patagonian scallop (Table 1a). A significant decrease ($p<0.01$) was found at pH 8.0 in the ΔHpeak II of striated muscles from the patagonian scallop. These results agree with those obtained for other species of fish (Beas et al., 1990; Howell et al., 1991).

The denaturation entalphies (ΔH total and ΔHpeak I) from smooth muscles of the tehuelche scallop showed a significant decrease ($p < 0.05$) at pH 8.0 (Table 1b). A significant modification in the myosin-paramyosin zone of the thermograms with a decrease in the T_{max} values and endothermal areas was observed when the ionic strength was increased to 0.5 in both types of muscles from the patagonian scallop (Fig. 2c).

Table 1b: Denaturation enthalpies (ΔH total; ΔH peak I) corresponding to DSC thermograms of whole muscle of *Chlamys tehuechus* at different pH values.

PH	Smooth muscle		Striated muscle	
	ΔH total (J/g)	ΔH peakI (J/g)	ΔH total (J/g)	ΔH peakI (J/g)
5.5	20.09 ± 0.00	13.90 ± 0.00	19.00 ± 1.30	12.60 ± 0.90
7.0	18.42 ± 0.75	8.95 ± 0.50	18.29 ± 0.00	10.42 ± 0.00
8.0	11.01 ± 0.0*	5.98 ± 0.00*	17.12 ± 0.30	11.80 ± 0.30

Each value is the mean ± SD (n=4) *values significantly different from other values in the same column ($p<0.05$); # ($p<0.01$)

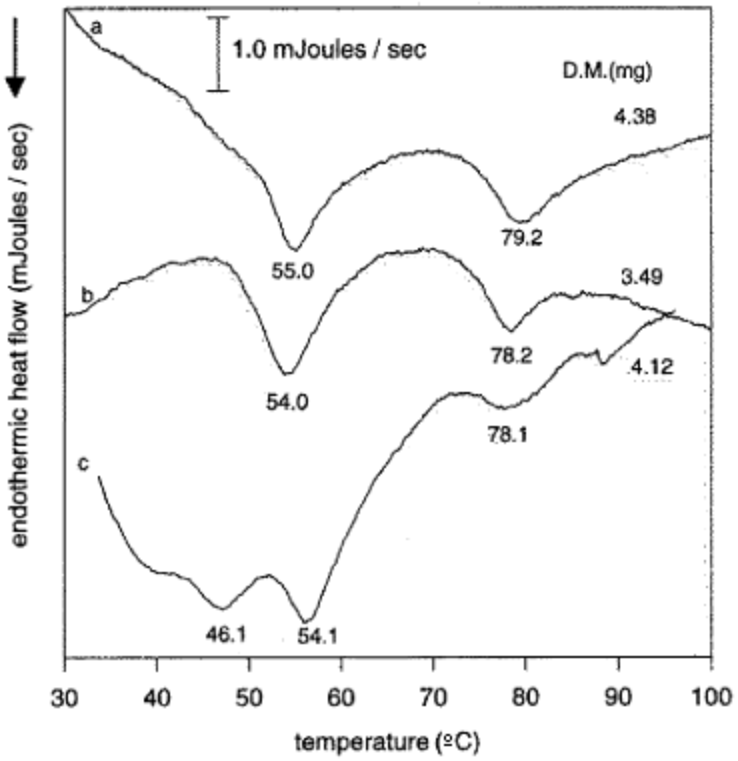

Figure 2:DSC thermograms of striated muscle of the patagonian scallop at different pH values (I=0.05). a) pH:7.0, b) pH :5.5, c) pH: 8.0 β=10°C/min. DM: dry matter

These results were similar for both species despite the fact that the striated muscles of the patagonian scallop were affected more than the smooth muscles. It has been postulated that at low ionic strength, molecules of myosin aggregate to form filaments which are stable than individual molecules present at high ionic strength (Samejima et al., 1983). Merrick and Johnson (1977) reported that solubility of paramyosin also increased at high ionic strength.

With respect to the second transition, actin was significantly affected by the increase in ionic strength and a displacement of the T_{max} value was observed with the increases in ionic strength in the patagonian scallop (Fig.2). A similar behavior was found for the tehuelche scallop. It had already been reported that KCl destabilizes the actin of bovine and fish striated muscles in this way (Stabursvik and Martens, 1980; Beas et al., 1990; Paredi et al., 1994).

As can be seen in Table 2 (a, b) irrespective of species of scallop a significant decrease ($p<0.05$) in the denaturation entalpies was observed in both types of muscle when the ionic strength increased to 0.5.

Table 2a: Denaturation enthalpies (ΔH total; ΔH peakI) corresponding to DSC thermograms of whole muscles of *Zygochlamys patagonica* at different ionic strength values.

Ionic strength	Smooth muscle			Striated muscle		
	ΔH total (J/g)	ΔHpeakI (J/g)	ΔHpeakII (J/g)	ΔH total (J/g)	ΔHpeakI (J/g)	ΔHpeakII (J/g)
0.05	17.80±0.87	11.21 ±1.0	6.60± 1.80	17.94 ± 0.80	13.17 ± 0.15	4.76 ± 0.16
0.25	13.51±0.64	9.50 ±0.33	4.02 ± 0.86*	8.92± 0.70*	8.14 ± 0.42*	2.18 ± 0.80*
0.50	7.41 ±0.03*	5.10 ±0.42*	2.31 ±0.45*	8.80 ±1.00	6.63 ± 0.03#	1.08 ± 0.80*

Each value is the mean±SD (n=4) * Values significantly different from other values in the same column (P<0.05), # (P < 0.01).

Table 2b: Denaturation enthalpies (ΔH total; ΔH peakI) corresponding to DSC thermograms of whole muscles of *Chlamys tehuelchus* at different ionic strength values.

Ionic Strength	Smooth muscle		Striated muscle	
	ΔH total (J/g)	ΔHpeakI (J/g)	ΔH total (J/g)	ΔHpeakI (J/g)
0.05	17.20 ± 0.00	8.80 ± 0.0	16.32 ± 0.70	7.45 ± 0.50
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*

Each value is the mean±SD (n=4) * Values significantly different from other values in the same column (P<0.05)

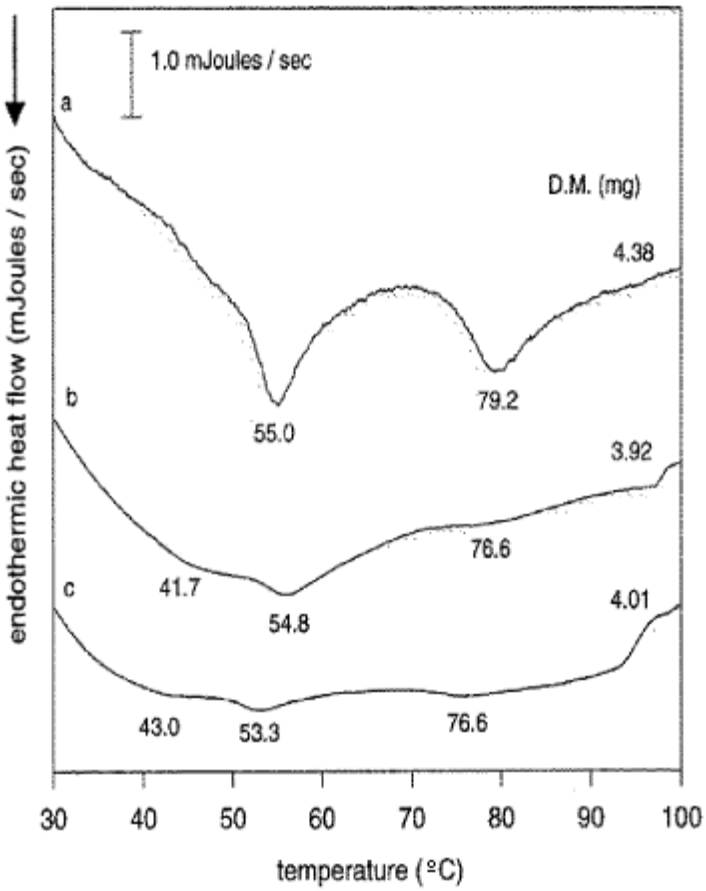

Figure 3: DSC thermograms of striated whole muscle of the patagonian scallop at different ionic strengths (I) (pH7.0). a) I=0.05, b) I=0.25, c)I= 0.5 β=10ºC/min, DM: dry matter.

CONCLUSIONS

The paramyosin content was not directly responsible for thermal stability in either species of scallop. In both smooth and striated muscle the zone corresponding to the first transition was affected more by the increase in pH and ionic strength. Modifications in the chemical environment affected the striated muscle of the patagonian scallop more than they affected the smooth muscle. Conversely, the smooth muscle was affected more than the striated muscle in the tehuelche scallop. *Zygochlamys patagonica* showed a major sensitivity to changes in the chemical environment.

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