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Thermal Denaturation of Myofibrillar Proteins of Striated and Smooth Adductor Muscles of Scallop (*Zygochlamys patagonica*). A Differential Scanning Calorimetric Study

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Denaturation of proteins from striated and smooth muscles of scallop (*Zygochlamys patagonica*) was studied with differential scanning calorimetry (DSC) by monitoring maximum temperatures of transition and denaturation enthalpies. DSC thermograms of both striated and smooth whole muscles showed two transitions: Tmax 55.0, 79.2 °C; and Tmax 54.7, 78.7 °C, respectively. The DSC thermograms of myofibrils and actomyosin were similar to those corresponding to their respective whole muscles. As pH and ionic strength increased, the thermal stability of whole muscles decreased. The pH increase (5.0−8.0) significantly ($p < 0.01$) decreased the denaturation enthalpies (ΔH total, ΔH peakI, and ΔH peakII) of whole striated muscles. A significant decrease ($p < 0.05$) in the ΔH total and the ΔH peakI was also observed in DSC thermograms of smooth muscles at pH 8.0. Denaturation enthalpies (ΔH total and ΔH peakI) significantly decreased ($p < 0.01$) when the ionic strength increased from 0.05 to 0.5 in both types of muscles. Striated muscles were more affected than smooth muscles by changes in the chemical environment.

KEYWORDS: Thermal denaturation; myofibrillar proteins; Patagonian scallop

INTRODUCTION

Study of the thermal behavior of myofibrillar proteins is of technological importance with regard to determining and predicting the final quality of meat products. Differential scanning calorimetry (DSC) offers a direct method to study the thermal transitions of muscle proteins in situ (*1*). The thermal denaturation of myofibrillar proteins was studied in several fish species by DSC (*2*−*9*).

DSC studies on myofibrillar proteins of marine invertebrate species were also reported (*2*, *10*−*13*). Invertebrate myofibrillar proteins have different thermal characteristics because they have an inherent protein called paramyosin (*11*). Paramyosin constitutes the core of thick filaments in invertebrate muscles where it is covered by a cortical layer of myosin (*14*, *15*). In addition, it has been reported that paramyosin considerably alters the characteristic texture of marine meat gel products (*16*, *17*). Recently, it has also been suggested that either the paramyosin content or the different myosin−paramyosin interactions would be related, at least partially, with the thermal behavior observed in myofibrillar proteins of marine invertebrates due to changes in the chemical environment (*18*). Paredi and Crupkin (*19*) reported that the smooth muscles of the Patagonian scallop have a greater content of paramyosin than striated muscles. In addition, the paramyosin content in the smooth muscles of the Patagonian scallop is higher than that in the corresponding muscles of *Chlamys tehuelchus* (*19*, *20*). The purpose of this work was to study by DSC the thermal behavior of myofibrillar proteins in striated and smooth muscles from the Patagonian scallop. The influence of the chemical environment on the thermal stability of myofibrillar proteins was also investigated.

MATERIALS AND METHODS

Samples. Specimens of *Zygochlamys patagonica* (King Broderip 1832) were caught in the Argentine Continental Shelf in the Reclutas bed (39°24′S; 55° 56′W) from March through October 2000. Mature specimens, 55−65 mm shell height, were selected. After the shells were cleaned, the adductor muscles were dissected. Muscles were carefully freed from adhering pancreatic and liver tissues, and rinsed with a 5 mM phosphate buffer (pH 7.0) containing 40 mM NaCl and 0.l mM phenylmethylsulfonyl fluoride. The striated and smooth muscles were separated. All the procedure was done at 0−4 °C and the tissues were immediately used for isolation of proteins.

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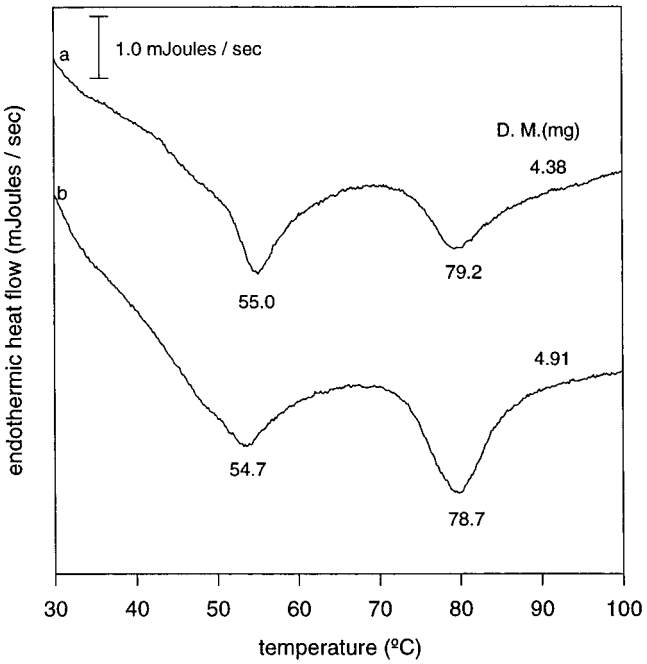

Figure 1. DSC thermograms of whole muscle from scallop: (a) striated; (b) smooth. Heating rate, 10 °C/min; DM, dry matter.

Preparation of Myofibrillar Proteins. Myofibrils were obtained according to the method described by Chantler and Szent-Gyorgyi (*21*). The procedure followed to obtain partially purified actomyosin was previously described (*22*).

Protein Determination. Protein concentration for myofibrils and actomyosin was determined by the Lowry method, with bovine serum albumin added as standard (*23*).

Criterion of Purity for Protein Preparations. The purity of myofibrils and actomyosin was assessed by SDS−PAGE in l0% gels using a Sigma Microslab gel apparatus, as reported by Laemmli (*24*). 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. The quantitative composition of each protein was determined by the scanning of gels at 600 nm with a Shimadzu dual-wavelength chromatogram scanner model CS 9l0 equipped with a gel scanning accessory (Kyoto, Japan).

Differential Scanning Calorimetry. Differential scanning calorimetric (DSC) studies were performed in a Polymer Lab (PL-DSC, Rheometric Scientific). Temperature calibrations were performed according to ASTM Norm E 474/8O using indium thermograms. 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. A hermetic capsule with 19−20 μL of distilled water was used as reference. 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 to 100 °C at a sensitivity of 0.5 mV/cm. Total and partial denaturation enthalpies (ΔH total, ΔH peakI, and ΔH peakII) were estimated by measuring the area below the DSC transition curve (a baseline was constructed as a straight line from the start to the end of the endotherm).

PH and Ionic Strength Adjustment. Small pieces of muscle were dissected with a scalpel, treated with a solution of 0.1 M phosphate buffer pH 7.0 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. Ionic strengths (I) were adjusted by the addition of NaCl at I values between 0.05 and 0.5.

Statistical Analysis. Analysis of variance and the Duncan's new multiple range test were performed, using the statistical analysis package Statistica/MAC (*25*).

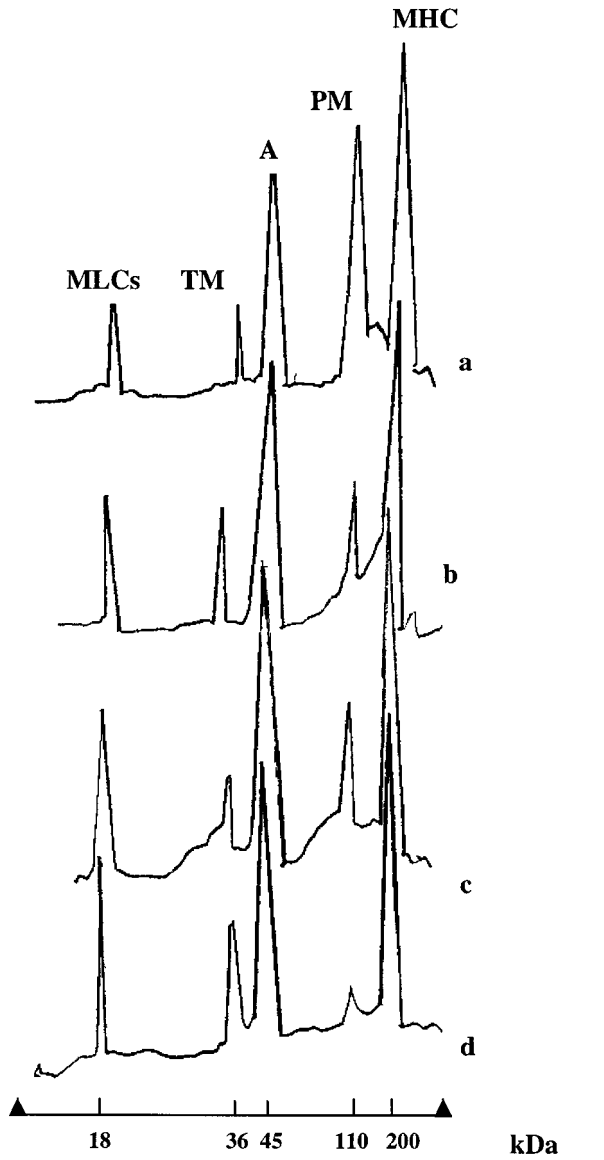

Figure 2. Densitometric patterns of SDS–PAGE (10%) gels. (a) Myofibrils of smooth muscles; (b) myofibrils of striated muscles; (c) actomyosin of smooth muscles; (d) actomyosin of striated muscles. MHC, myosin heavy chain (200 KDa); PM, paramyosin (110 KDa); A, actin (45 KDa); TM, tropomyosin (36 KDa); MLCs, myosin light chains (18 KDa).

RESULTS AND DISCUSSION

Thermal Behavior of Both Striated and Smooth Whole Adductor Muscle Proteins. DSC thermograms of striated and smooth whole muscles showed two endothermic transitions with Tmax of 55.0, 79.2, and 54.7, 78.7 °C, respectively (**Figure 1**). No significant differences ($P < 0.05$) were found between the Tmax of both types of muscle.

Because of the small amount of connective tissues in bivalve mollusc muscles (*26*) and fish muscles (*27*), the endothermic transitions can be assigned to denaturation of myofibrillar and sarcoplasmic proteins.

Whole muscle, myofibrils, and actomyosin were analyzed by DSC to investigate the contribution of myofibrillar proteins to DSC transitions of both smooth and striated whole muscles. The purities of myofibrils and actomyosin were previously checked by SDS−PAGE 10%. Densitometric analysis profiles of SDS−PAGE l0% gels of myofibrils and actomyosin from both smooth and striated muscles can be observed in **Figure 2**.

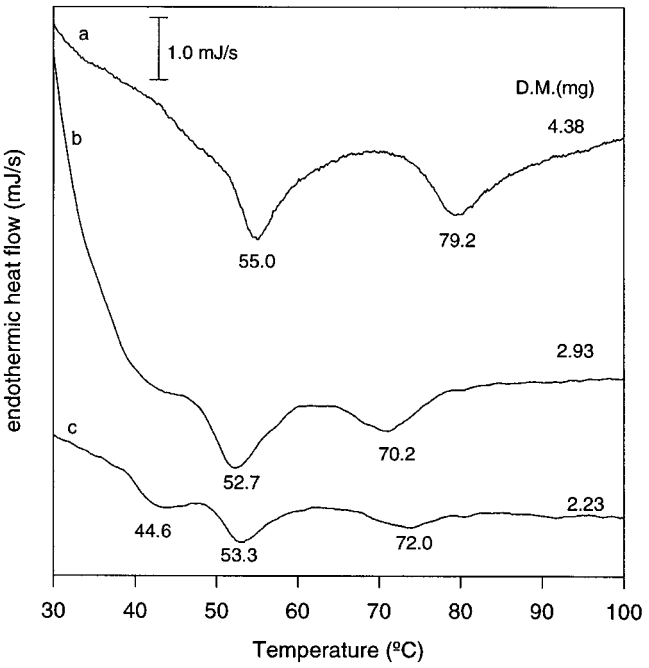

Figure 3. DSC thermograms of scallop: (a) whole striated muscle, (b) myofibrils of striated muscle, (c) actomyosin of striated muscle. Heating rate, 10 °C/min; DM, dry matter.

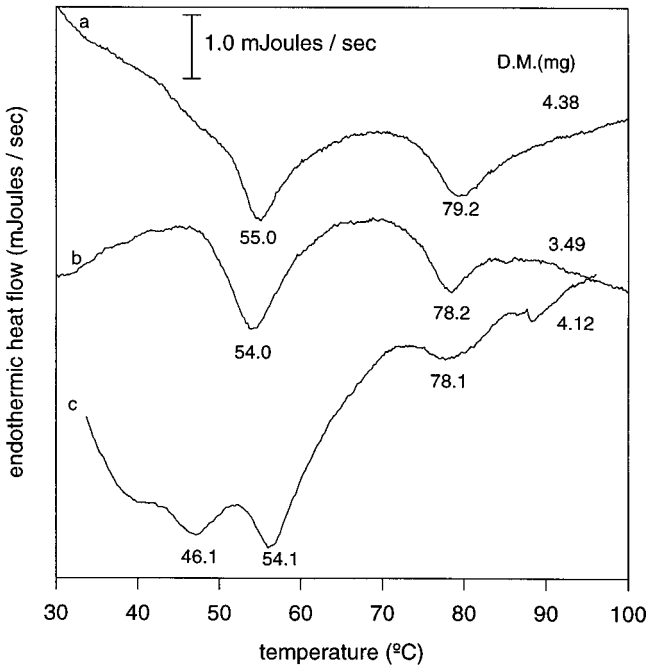

Figure 4. DSC thermograms of whole striated muscle of scallop at different pH values ($I = 0.05$): (a) pH 7.0; (b) pH 5.5; (c) pH 8.0. Heating rate, 10 °C/min; DM, dry matter.

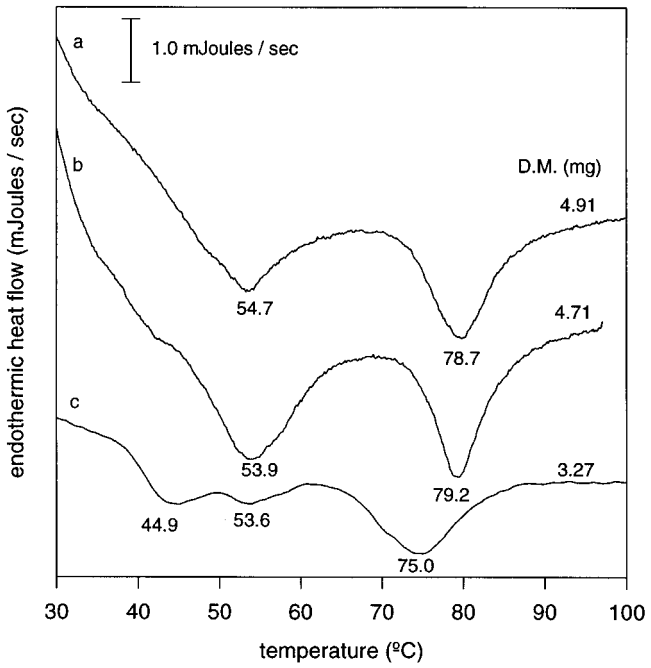

Figure 5. DSC thermograms of smooth whole muscle of scallop at different pH values ($I = 0.05$): (a) pH 7.0; (b) pH 5.5; (c) pH (8.0). Heating rate, 10 °C/min; DM, dry matter.

The characteristic polypeptidic bands corresponding to myofibrils and to each major myofibrillar protein were present in the gels. As **Figure 2** also shows, the most important difference observed in the gels was that both myofibrils and actomyosin of smooth muscles have higher paramyosin contents than those of striated muscles.

Myofibrils and actomyosin isolated and purified from striated muscles showed thermograms similar to those of their respective whole muscles, with a displacement toward lower temperatures (**Figure 3**). Similar DSC thermograms were obtained with myofibrils and actomyosin from smooth muscles (data not shown). Paredi et al. (*11*) reported two major endothermic transitions in whole muscles from the bivalve mollusc *Aulacomya*. In that work the first transition was related to myosin and paramyosin denaturation, and the second transition was related to actin denaturation. In this way, the results of DSC termograms of both myofibrils and actomyosin from Patagonian scallop indicate that myosin and paramyosin contributed to the first transition and actin contributed to the second transition. Similar results were reported for other invertebrate species (*13*). In addition, the results would also indicate that the native muscle has higher thermal stability than the isolated myofibrillar proteins. These results agree with those reported by other authors (*1*, *28*, *29*). In addition, the results shown in **Figures 1**, **2**, and **3** would indicate that the paramyosin content does not have a direct influence on the thermal stability of proteins.

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 (*30*). As it is shown in **Figures 4** and **5**, when the pH increased to 8.0 a displacement of the thermal transition to lower temperatures occurred in both striated and smooth muscles. The decrease in 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 (*5*, *7*, *11*). A significant decrease ($p < 0.01$) in the ΔH total, the ΔH peakI, and the ΔH peakII when the pH increased to 8.0 was observed in striated muscles (**Table 1**). A significant decrease ($p < 0.05$) was also found at pH 8.0 in the ΔH total and the ΔH peakI of smooth muscles. These results agree with those obtained in other fish species (*5*, *7*, *18*). The results of the denaturation enthalpies would indicate that striated muscles are more susceptible to increased pH (8.0) than are the smooth muscles.

The effect of ionic strength on the thermal stability of the protein in striated and smooth whole muscles is shown in **Figures 6** and **7**, respectively. Great modifications in the

Table 1. Denaturation Enthalpies (ΔH Total; ΔH Peak I; ΔH Peak II) Correponding to DSC Thermograms of Whole Muscle of *Zygochlamys patagonica* at Different pH Values[a]

	smooth muscle			striated muscle		
pH	ΔH total (J/g)	ΔH peakI (J/g)	ΔH peakII (J/g)	ΔH total (J/g)	ΔH peakI (J/g)	ΔH peakII (J/g)
5.5	17.70 ± 0.00	9.95 ± 0.00	7.63 ± 0.00	18.01 ± 0.10	12.40 ± 0.00	5.68 ± 0.00
7.0	17.10 ± 1.36	9.80 ± 0.55	7.40 ± 0.85	17.50 ± 0.40	12.10 ± 1.00	5.02 ± 0.38
8.0	12.78 ± 0.78*	6.59 ± 0.70*	6.19 ± 0.76	8.23 ± 1.00#	6.50 ± 0.23#	1.74 ± 1.0#

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

Table 2. Denaturation Enthalpies (ΔH Total; ΔH Peak I) Corresponding to DSC Thermograms of Whole Muscles of *Zygochlamys patagonica* at Different Ionic Strength Values[a]

	smooth muscle			striated muscle		
ionic strength	ΔH total (J/g)	ΔH peak I (J/g)	ΔH peak II (J/g)	ΔH total (J/g)	ΔH peak I (J/g)	ΔH peak II (J/g)
0.05	17.80 ± 0.87	11.21 ± 1.00	6.60 ± 1.80	17.94 ± 0.80	13.17 ± 0.15	4.76 ± 0.20
0.25	13.51 ± 0.64*	9.50 ± 0.33	4.33 ± 0.90	10.92 ± 0.60*	8.14 ± 0.40*	2.18 ± 1.10
0.50	7.41 ± 0.03#	5.10 ± 0.42#	2.31 ± 0.50	8.40 ± 0.70#	6.50 ± 0.03#	1.08 ± 0.90

[a] Each value is represented as mean ± SD ($n = 4$). Values significantly different in the same column *($p < 0.05$), # ($p < 0.01$).

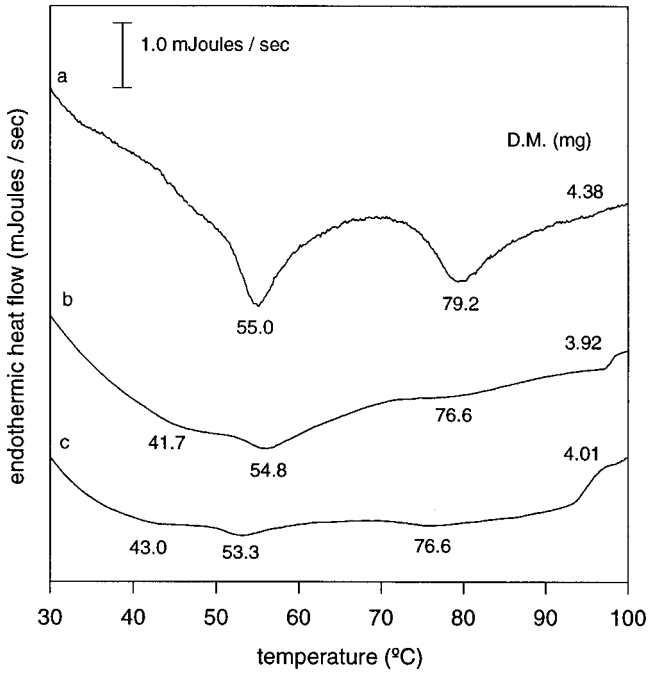

Figure 6. DSC thermograms of whole striated muscle of scallop at different ionic strength values. (a) I= 0.05; (b) I= 0.25 (c) I=0.5. (pH 7.0). Heating rate = 10 °C/min. DM= dry matter.

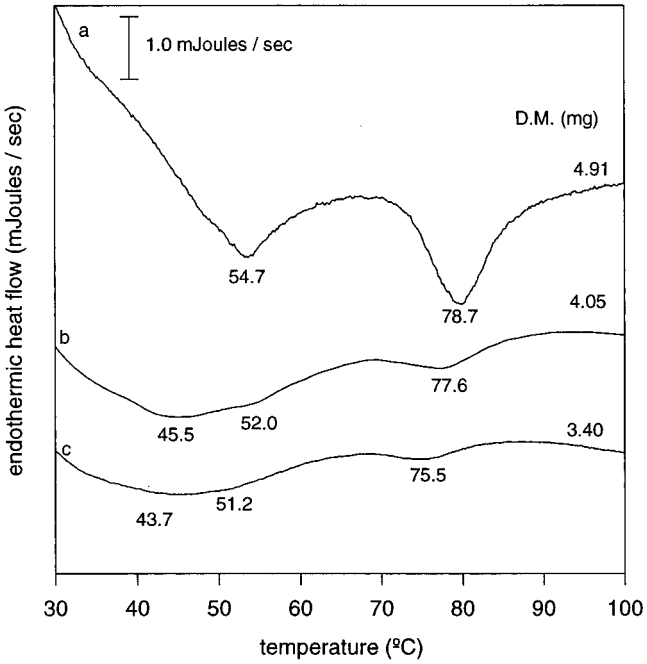

Figure 7. DSC thermograms of whole smooth muscle of scallop at different ionic strength values: (a) $I = 0.05$; (b) $I = 0.25$; (c) $I = 0.5$ (pH 7.0). Heating rate, 10 °C /min; DM, dry matter.

myosin−paramyosin zone of the thermogram with a displacement of Tmax 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 (*6*, *7*) and marine invertebrates (*11*). It has been postulated that at low ionic strengths, molecules of myosin aggregate to form filaments with greater stability than the individual molecules that exist at high ionic strengths (*31*). Merrick and Johnson (*32*) reported that solubility of paramyosin also increased at high ionic strength.

The Tmax of the actin area shows a significant decrease ($p < 0.05$) when ionic strength increased to 0.5, in DSC thermograms of both types of muscles (**Figures 6** and **7**). In agreement with these results it had been reported that KCl destabilizes the actin of bovine and fish muscles (*5*, *7*, *33*).

As it can be seen in **Table 2** a significant decrease ($p < 0.01$) of the denaturation enthalpies (ΔH total and ΔH peakI) was observed in striated muscles when the ionic strength increased to 0.5. The ΔH total and the ΔH peakI of DSC thermograms of smooth muscles also showed a significant decrease ($p < 0.01$) at an ionic strength of 0.5 (**Table 2**). Similar results were obtained with other fish species (*7*, *11*). No significant difference ($p > 0.05$) was observed in the ΔH peakII in both types of muscle.

CONCLUSIONS

The paramyosin content does not have a direct influence on the thermal stability of myofibrillar proteins. Striated muscles were more susceptible to changes in the chemical environment than the smooth muscles were. Ionic strength appeared to have a greater effect on denaturation enthalpies than pH did.

Knowledge of the thermal stability of muscle proteins of Patagonian scallop provides information about how the muscles will behave during freezing and under frozen storage. Moreover,

it is important to optimize processes such as curing (acid condition) or production of gelled marine meat products (high salt concentrations affecting the gelation of myofibrillar protein) by selecting the proper pH and ionic strength levels.

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