# ARTICLES

# Postmortem Changes in Adductor Muscles from *Aulacomya ater ater* (Molina) Stored at 2–4 °C. A Differential Scanning Calorimetric Study

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Postmortem thermal behavior of *Aulacomya* striated adductor muscles stored at 2–4 °C was investigated by differential scanning calorimetry (DSC). The exothermal peak present in prerigor muscles from different species was not evident in DSC thermograms of either fresh or cold-stored adductor muscles. ATP content and pH of the muscles fell sharply in the first 8 h of storage. At this time the greatest increases in both total denaturation enthalpy and denaturation enthalpy related with the first transition were observed. The addition of exogenous ATP to whole muscle after 18 h of storage (low endogenous ATP level) produced a decrease in total enthalpy as a result of a decrease in the peak I enthalpy. These results support the idea that the exothermal peak is not evident in DSC thermograms because it coincides with the first endothermal transition.

**Keywords:** *Adductor muscle; thermal stability; cold storage; differential scanning calorimetry*

## INTRODUCTION

The denaturation of myofibrillar proteins and the decrease in water holding capacity of the meat of *Aulacomya* adductor muscles stored at 2–4 °C were reported in a previous work (Paredi et al., 1990). A decrease of about 44% in both $Mg^{2+}$-ATPase activity and reduced viscosity of actomyosin after the first day of storage were also reported. These changes were attributed to a conformational change of the protein by intrinsic viscosity and surface hydrophobicity determinations (Paredi et al., 1992).

Differential scanning calorimetry (DSC) is a direct method to study the thermal transitions of muscle proteins *in situ* (Wright et al., 1977). The myofibrillar thermostability of several mammalian, avian, and fish species has been studied by DSC (Stabursvik and Martens, 1980; Xiong et al., 1987; Hastings et al., 1985). However, only a few DSC studies included myofibrillar proteins of marine invertebrate species (Hastings et al., 1985; Akahane et al., 1985; Paredi et al., 1994); there are no DSC studies on cold-stored marine invertebrate muscles.

The purpose of this work was to study the thermal behavior of myofibrillar proteins of *Aulacomya* adductor muscles stored at 2–4 °C by DSC.

## MATERIALS AND METHODS

Specimens of *Aulacomya ater ater* (Molina) were collected from April 1992 through May 1993 from the San Jose 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 was examined according to the procedure of Vinuesa and Tortorelli (1980). After the shells were cleaned, striated muscles were dissected and the adhering pancreatic and liver tissues were carefully removed. Adductor muscles were stored at 2–4 °C for up to 48 h.

**Differential Scanning Calorimetry.** Differential scanning calorimetry studies were performed using a DuPont 910 system with a Hewlett-Packard 7046 B recorder. Temperatures were calibrated according to ASTM Norm E 474/80 using indium thermograms. Unstored (zero time) and stored samples of muscle (13–20 mg of wet weight) were placed in DSC hermetic pans, with a good contact between the sample and the capsule bottom assured. In addition, muscles at 18 h postmortem were run alone and with 8 μL of 20 mM ATP, which were mixed inmediately before heating. As a 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 was determined by drying at 105 °C overnight. All of 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 enthalpies of first ($\Delta H_{peak\ I}$) and second ($\Delta H_{peak\ II}$) transitions were estimated by measuring the area under the DSC transition curve (a base line was constructed as a straight line from the start to the end of the endotherm). The total areas of the thermograms were divided into partial areas corresponding to each transition. The endotherm areas were measured with a Sigma Scan package (The Scientific Measurement Program 3.90, 1992).

**ATP Content.** A portion of pooled, cut, and mixed muscles was homogenized with 10% trichloroacetic acid (TCA). The

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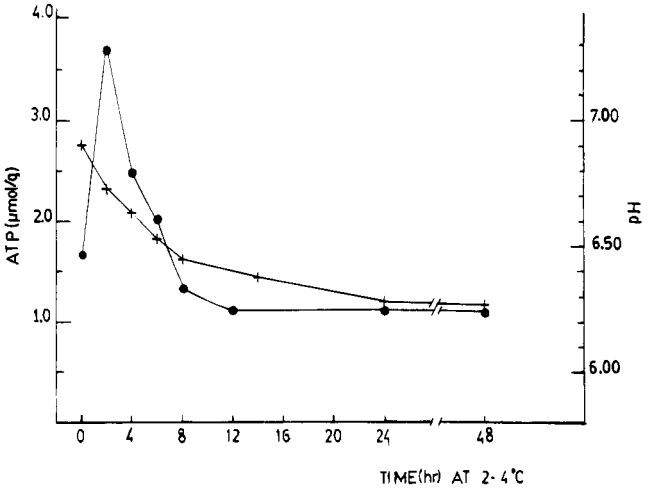


**Figure 1.** Changes in ATP content (●) and pH (+) in *Aulacomya* adductor muscles stored at 2–4 °C.

homogenate was centrifuged at 700*g*. The residue was extracted again with 5% TCA. The supernatants were combined and neutralized to pH 6.8 with 20% KOH solution under cooling with ice. The neutralized extract was centrifuged, and the precipitate was washed with 5% TCA neutralized with KOH. Aliquots of supernatants were taken to measure ATP by the luciferin–luciferase assay as described by Romano and Laborde (1978), adapted for bivalve adductor muscles. A PICO-ATP luminometer (Jovin and Ivon Ste, France) was employed for light peak measurement.

**pH Determination.** About 5 g of the adductor striated muscles was cut into small pieces and homogenized with 25 mL of distilled water. The pH of the homogenate was measured with an Orion digital pH-meter.

**Preparation of Myofibrillar protein.** The procedure followed to obtain partially purified actomyosin was described previously (Paredi et al., 1990).

Aggregation of protein was monitored by measuring OD at 600 nm using a Beckman DU8 spectrophotometer.

**Statistical Analysis.** Analysis of variance (ANOVA) and least significant difference (lsd) were applied to the data using analysis package SYSTAT (Wilkinson, 1990).

## RESULTS AND DISCUSSION

The ATP concentration and the pH of *Aulacomya* muscle for different storage times are shown in Figure 1. ATP content increased for a short time after death. This agreed with the results of Hiltz and Dyer (1971), who reported that the nucleotide was regenerated by the degradation of arginine phosphate prior to the destruction of ATP in marine invertebrate species. A maximum of ATP concentration occurred 2 h after death, then the concentration dropped sharply in the following 8 h of storage, and remained unchanged thereafter. These results agreed with those reported for *Aulacomya* in poor biological condition (low glycogen, lipids, protein, and energy content) (De Vido de Mattio et al., 1992).

The pH fell sharply in the first 8 h of postmortem storage and only slightly from 8 to 48 h of storage (Figure 1). The decrease in the pH of invertebrate muscle is mainly due to the production of octopine as a result of the reaction between pyruvate and arginine (Hiltz and Dyer, 1971). The behavior of ATP content and pH in *Aulacomya* muscle would indicate that the onset of rigor takes place at about 8 h after death.

The DSC thermograms of whole *Aulacomya* adductor muscle at 0 and 2 h of postmortem storage are shown in Figure 2. In a previous work (Paredi et al., 1994)

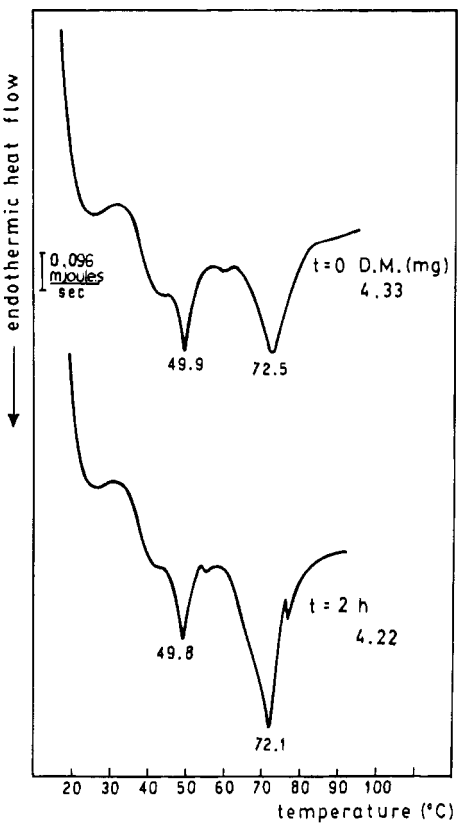


**Figure 2.** DSC thermograms of *Aulacomya* whole muscle after 0 and 2 h of postmortem storage at 2–4 °C. Heating rate = 10 °C/min; DM, dry matter.

two endothermic transitions with peak temperature maxima ($T_{max}$) values of 50.5 ± 0.5 and 72.5 ± 0.5 °C and a shoulder at about 43 °C had been found in the DSC thermograms of fresh *Aulacomya* muscle free of connective tissue. It was suggested that myosin and paramyosin contributed to the first transition and that actin was responsible for most of the second transition in whole muscle.

The DSC thermograms of muscle in prerigor from different species showed an exothermal peak with a $T_{max}$ at 50–55 °C (Martens and Vold, 1976; Wright et al., 1977; Park and Lanier, 1988). This peak was not evident in DSC thermograms of fresh *Aulacomya* whole adductor muscles (Paredi et al., 1994) or in the thermograms of *Aulacomya* muscle stored at 2–4 °C (Figure 2).

Wright et al. (1977) and Park and Lanier (1988) reported that the importance of the exothermal peak decreased with development of rigor and that it finally disappeared in postrigor muscle. It is widely accepted that the disappearance of the exothermal peak begins with the onset of rigor and corresponds to a drop both in ATP concentration and in pH.

The absence of an exothermic peak in prerigor *Aulacomya* muscle could be due to the proximity between the $T_{max}$ for the first endothermal transition (50 °C for fresh *Aulacomya* adductor muscle) and the temperature of the exothermal peak.

This would produce a decrease in the area of the first endothermal transition and result in the nonexistance of the exothermal peak. To examine this premise, the $\Delta H_{total}$ and the $\Delta H_{peak\ I}$ of whole muscle after 18 h of storage at 2–4 °C (low ATP levels), with and without exogenous ATP, were determined.

The changes in total enthalpy ($\Delta H_{total}$) and in peak I enthalpy ($\Delta H_{peak\ I}$) for whole muscle after 18 h of storage

**Table 1. Effect of ATP Addition to Muscle after 18 h of Postmortem Storage on the $\Delta H_{total}$ and $\Delta H_{peak\ I}$[a]**

| muscle | $\Delta H_{total}$ (J/g) | $\Delta H_{peak\ I}$ (J/g) |
|---|---|---|
| without ATP | 11.38 ± 0.42 | 6.70 ± 0.21 |
| with ATP | 8.99 ± 0.33 | 4.77 ± 0.20 |

[a] Each value represents a mean of four determinations ± SD.

**Table 2. Denaturation Enthalpies and Transition Temperatures Corresponding to DSC Thermograms of Whole Muscle at Different Periods of Storage at 2–4 °C[a]**

| time (h) at 2–4 °C | $\Delta H_{total}$ (J/g) | $\Delta H_{peak\ I}$ (J/g) | $T_{max}$ (°C) peak I | $T_{max}$ (°C) peak II |
|---|---|---|---|---|
| 0 | 9.83 ± 1.13 | 4.98 ± 0.67 | 50.00 ± 0.90[c] | 72.50 ± 0.84 |
| 2 | 11.30 ± 0.88 | 5.69 ± 0.92 | 49.80 ± 0.60[d] | 72.10 ± 0.37 |
| 4 | 10.33 ± 1.63 | 5.11 ± 1.25 | 48.80 ± 1.70 | 71.20 ± 1.50 |
| 6 | 11.93 ± 1.42 | 6.53 ± 1.21 | 51.50 ± 0.70 | 73.00 ± 0.62 |
| 8 | 15.49 ± 1.42[b] | 9.79 ± 1.62[b] | 52.00 ± 0.80[c,d] | 72.70 ± 0.63 |
| 10 | 11.06 ± 1.25 | 5.53 ± 0.63 | 51.25 ± 0.25 | 71.50 ± 0.00 |
| 14 | 11.22 ± 1.38 | 6.02 ± 0.04 | 51.40 ± 1.70 | 72.00 ± 0.30 |
| 24 | 11.35 ± 0.50 | 6.28 ± 0.08 | 50.00 ± 0.00 | 72.00 ± 0.00 |
| 48 | 10.21 ± 0.29 | 5.69 ± 0.21 | 51.50 ± 0.50 | 70.75 ± 0.25 |

[a] Each value represents a mean of four to six determinations ± SD. [b] Values significantly different from others in the same column ($P < 0.05$). [c,d] Values in the same column having the same letter are significantly different ($P < 0.05$).

at 2–4 °C, with and without exogenous ATP, are presented in Table 1. The addition of ATP produced a decrease in the total enthalpy as a result of a decrease in the peak I enthalpy. These results support the idea that the exothermic peak is not evident in DSC thermograms because it overlaps with the first endothermic transition.

The variations of $\Delta H_{total}$, $\Delta H_{peak\ I}$, and $T_{max}$ during the cold storage of adductor *Aulacomya* muscles are shown in Table 2. Low values of $\Delta H_{total}$ and $\Delta H_{peak\ I}$ obtained at zero time could be related to high contents of both phosphagens and ATP in the muscles. A significant increment ($P < 0.05$) in both $\Delta H_{total}$ and $\Delta H_{peak\ I}$ occurs 8 h after death, corresponding to decreases in both ATP concentration and pH (Figure 2). The values of enthalpy fell in the first 10 h of storage, and no major changes were observed thereafter. $\Delta H_{peak\ II}$ remained around 5.2 ± 0.4 J/g during cold storage. In addition, the $T_{max}$ value of peak I corresponding to 8 h of storage was significantly different ($P < 0.05$) with respect to those at 0 and 2 h (Table 2). No major changes were observed in $T_{max}$ values of peak II during postmortem storage (Table 2).

The increment observed in denaturation enthalpy after 8 h of storage could be related to the onset of rigor mortis and the formation of the actomyosin complex. It has been reported that changes in both thick and thin filaments may occur only after the proteins have lost ATP, which acts as an allosteric agent in the myofibillar structure (Honikel et al., 1981).

The enthalpy of thermal transitions of the protein is the result of endothermic and exothermic contributions. Endothermic contributions are due to the breakup of hydrogen bonds, and exothermic contributions are due to the aggregation and the breakup of hydrophobic interactions of the protein (Myers, 1990). The decrease in $\Delta H$ after 8 h of postmortem storage may be due to aggregation, breakup of the internal hydrophobic interactions, or a decrease of the endothermic contribution from the breakup of hydrogen bonds. The optical density of actomyosin obtained from adductor muscles at different storage times did not change. This would indicate that no aggregation took place in the first 24 h of storage. On the other hand, an increase in protein surface hydrophobicity of actomyosin from *Aulacomya* adductor muscle stored for up to 48 h was reported (Paredi et al., 1992). This result suggests that a weakening of hydrophobic interactions would occur earlier during postmortem storage. Taking into account the increment in hydrophobicity in denatured actomyosin and the decrease in hydrophobic interactions of the protein, an increment in $\Delta H$ could be expected. However, these changes would be not evident by DSC analysis because the contributions by a decrease in hydrophobic interactions could be less than those due to the rupture of hydrogen bonds.

In conclusion, no evidence of an exothermic peak was observed in the DSC thermograms of whole *Aulacomya* muscle during early postmortem storage. This is an important difference with respect to the DSC thermograms of both fish and mammalian prerigor muscles. In addition, the results of this work would suggest that the onset of rigor mortis in *Aulacomya* adductor muscle, could be determined by measurements of total $\Delta H$, which correlate with the $\Delta H$ of the first transition.

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