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Anaerobiosis and metabolic plasticity of *Pinna nobilis*: Biochemical and ecological features

G. Laganà [a], D. Barreca [a, *], S. Giacobbe [b], E. Bellocco [a]

[a] Dipartimento di Scienze Chimiche, Università di Messina, Viale F. Stagno d'Alcontres 31, 98166 Messina, Italy
[b] Dipartimento di Scienze Ambientali e Biologiche, Università di Messina, Viale F. Stagno d'Alcontres 31, 98166 Messina, Italy

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ABSTRACT

Changes in the energetic metabolism were studied in the fan mussel *Pinna nobilis* L. exposed to environmental and anthropic stress. The high polymorphism of enzymes suggests an adaptation of the fan mussel to environmental variability peculiar of transitional waters with respect to the same species living in exposed coastal sea. The electrophoretic patterns showed a predominance of LDH-A4 and the presence of both mitochondrial and cytosolic MDH isozymes. Moreover, in all the analyzed tissues and organs, MDH activity was greater than the LDH one. Metabolic plasticity of the fan mussel is further highlighted by octopine dehydrogenase and superoxide dismutase electrophoretic patterns, showing the presence of many isoforms. These evidences are also confirmed by spectroscopic determinations of alanopine, tauropine, strombine and octopine dehydrogenase activity characterized by a specific trend due to environmental variability. Specific variations in anaerobic capacity of *P. nobilis* L. are discussed in relation to their distribution according to the marine-brackish gradient.

1. Introduction

Marine organisms modulate their metabolic pathways to counteract environmental factors such as temperature, salinity, oxygen or nutrient availability, maximizing benefits during favorable conditions and allowing survival during critical stages. For example, in many intertidal and infaunal invertebrates, the lack of a constant oxygen supply results in some spectacular tolerances (De Zwaan, 1977; Hochachka and Somero, 2002). These features are similar to some opportunistic species that can dominate a subtidal environment following a dystrophic crisis (Sato, 2006). Bivalves have evolved diverse and highly specialised strategies for surviving in hypoxic episodes including pathways that are efficient both in terms of ATP production, and in minimising H^+ and toxic end product accumulation (Hochachka and Somero, 2002). Under these circumstances, glycogen is metabolized to pyruvate and the cytosolic NADH/NAD^+ redox ratio is balanced by the reduction of pyruvate to lactate. Alternatively, NAD^+ can be recycled more efficiently by coupling an aminoacid to pyruvate, with formation of opines such as alanopine, tauropine, octopine, and strombine (Gäde, 1983; Gäde and Grieshaber, 1986). Stress factors induce alterations both in the amount of enzymes and in the production of isoforms possessing altered kinetic properties or post-translational modifications. In particular, since the estuarine and intertidal species are submitted naturally to multiple forms of daily stress, they display a wide range of metabolic adaptations, involving peculiar biochemical features (Storey and Storey, 1990;

* Corresponding author. Tel.: +39 090 6765187; fax: +39 090 6765186.
E-mail address: dbarreca@unime.it (D. Barreca).

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Hochachka, 1997; Hochachka and Somero, 2002; González-Wangüemerta et al., 2009). These adaptations of estuarine and intertidal bivalves to environmental variability have been poorly investigated in marine subtidal species, although they are frequently submitted to several types of stress causing short-term anaerobiosis (De Zwaan, 1977; Gäde and Grieshaber, 1986; Fields and Storey, 1987).

The Mediterranean endemic *Pinna nobilis* L., is one of the largest bivalves in the world. The "noble fan shell" lives partially buried in the soft bottom, with the broad posterior part of the shell that is elevated well above the sea floor. Fan shells are considered ecosystem engineers that remarkable increase habitat complexity, host epibiontic assemblages (Corriero and Pronzato, 1987; Cosentino and Giacobbe, 2008; Rabaoui et al., 2009) and locally influence oxygen and nutrient fluxes (Hewitt et al., 2006). Noble fan shell beds mostly occur from a maximum depth of −60 m in wave exposed areas up to −0.5 m in coastal lagoons (Zavodnik et al., 1991). In this respect, fan mussels might represent a profitable case-study to investigate metabolic adaptation of subtidal bivalves to environmental stress in oligotrophic shallow waters of sub-tropical areas (Butler et al., 1993). In this study, enzymatic markers and isozymes electrophoretic patterns of *P. nobilis* have been investigated in samples that are representative of a marine-brackish water gradient, to evaluate energetic metabolism and anaerobic adaptation in a long-lived moderately eurivalent species.

2. Methods

2.1. Sampling sites

The specimens of *P. nobilis* were collected in the Straits of Messina area (central Mediterranean) between May and June of 2011, from two nearby marine and brackish-water sites; the respective populations, that are not spatially separated, in agreement with Butler et al. (1993), might belong to the same metapopulation. The marine sites included a wave exposed sea-floor (St.1) and a more sheltered area (St.2), both at 7 m depth. The brackish water sites were located inside the channel that leads to the Faro lake (St.3) and in the inner part of the same lake (St.4), both at 1 m depth. In the Straits of Messina, the marine environment is characterized by tidal currents having a maximum speed of 3 m s^{-1}, with a period of 6 ¼ h. Such hydrological constraint leads to the upwelling of "Levantine Intermediate Water" that is colder, more salty and more nutrient-rich compared to the surface waters of Atlantic origin (Azzaro et al., 2007). This increase of primary production supports high density of benthic suspension feeders due to the water turbulences, oxygen availability and catabolytes clearing (Leonardi et al., 2009). The Faro lake is a meromictic basin, reaching a maximum depth of 29 m, characterized by anoxic and sulfidic waters, generally below 15 m in depth. Surface waters are mesotrophic, with a predominant heterotrophic biomass in the particulate matter and poorly oxygenated water can spread towards the surface in autumn. Exceptional seawater inflows cause strong alteration of the anoxic layer and temporary diseases on all aerobic organisms (Leonardi et al., 2009).

2.2. Sampling and sample treatment

Adult specimens having a comparable shell size were collected manually in each site and transported alive, within 10 min, into the laboratory by means of a thermic bag. Once sacrificed, they were measured according to García-March and Ferrer (1995) and their biomass was evaluated as shell-free wet weight. Soft tissues were weighted, dissected, and frozen at −20 °C until analysis. Adductor muscle, epatopancreas, gills and mantle were homogenized (1:4 w/v), using an Ultra Turrax tissue grinder, at 4 °C in 0.01 M potassium phosphate buffer (pH 7) containing 0.5 mM phenylmethyl sulfonyl fluoride (PMSF) and 1 mM β-mercaptoethanol. The homogenate was centrifuged for 15 min at 12 000 *rpm* at 4 °C. The supernatant was submitted to enzyme assays, protein determination and electrophoretic analysis.

2.3. Total protein and enzyme activities determinations

Total protein content was determined according to Bradford (1976). The dehydrogenase activities were analyzed at 25 ± 0.1 °C following the changes of absorbance at 340 nm. LDH and MDH activity was assayed according to Laganà et al. (2007). Alanopine (ADH), tauropine (TDH), strombine (SDH) and octopine (ODH) dehydrogenase activity was performed according to Baldwin et al. (1992). All the analyses were carried out in triplicate. One enzyme unit was defined as the amount catalyzing the production of 1.0 μM NAD^+ per min at 25 °C. In each experiment the values are the means ± standard deviation of three independent experiments ($n = 3$). Statistical comparisons of the results were performed by one-way ANOVA. Significant differences ($p < 0.05$) between the tested samples were analyzed using Turkey's test.

2.4. Isozymes determinations

Vertical electrophoresis was performed on 7.5% polyacrylamide gel according to Laganà et al. (2007). LDH staining solutions consisted of 60 mM lithium lactate, 0.336 mM nicotinamide adenine dinucleotide (NAD^+), 0.168 mM Nitro-blue tetrazolium (NBT), 0.056 mM phenazine methosulfate (PMS) in 0.1 M phosphate buffer (pH 7.0). MDH and ODH staining solution contained the same elements, but litium lactate was replaced with malate or octopine respectively. Staining of SOD was performed immediately after the achievement of electrophoresis using the nitrobluetetrazolium (NBT) photochemical method of Beauchamp and Fridovich (1971).