Defenses against oxidative stress in the Antarctic scallop *Adamussium colbecki* and effects of acute exposure to metals

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Abstract

Since a general pathway of toxicity induced by pollutants is the enhancement of reactive oxygen species, biochemical responses associated with variations in the antioxidant cellular system have been often proposed as biomarkers of pollutant-mediated toxicity associated with oxidative stress. Antarctic organisms live in an extreme environment characterized by low water temperature, high level of dissolved oxygen, presence of ice and strong seasonal changes in light intensity and availability of food, conditions which could influence both the formation of reactive oxygen species and the mechanisms for their removal. In this respect and considering the utility of this as a key species for monitoring marine Antarctic environment it was of interest to investigate the antioxidant defense system of the scallop *Adamussium colbecki*.

The parameters examined in the digestive gland of the scallop were the concentration of glutathione and the activity of several glutathione dependent and antioxidant enzymes (glyoxalase I and II, glutathione S-transferases, glutathione peroxidases, glutathione reductase, catalase, superoxide dismutase). Very high levels of catalase suggest a possible adaptation to Antarctic extreme conditions, while the high activities of glutathione S-transferases are more probably related to the feeding behavior of Pectinids. Enzymes from *Adamussium colbecki* generally appeared to be active at low temperatures but, with a few exceptions, their activities increased with rising temperature. Exposure of *A. colbecki* to sublethal concentrations of Cu or Hg resulted in a significant reduction in the levels of total glutathione and in the activity of catalase and glutathione S-transferases. Antioxidant responses of *A. colbecki* could represent a useful tool in assessing the biological impact of environmental pollutants in the Antarctic ecosystems.

Introduction

Marine bivalves are commonly used in temperate waters as suitable indicators of environmental pollution (Phillips, 1980). These organisms can concentrate contaminants within the tissues, so providing a time-integrated measurement of their bioavailability. Moreover, in environmental disturbance assessment, the integration of chemical data with biological responses (the so called biomarkers) is strongly recommended in order to assess effects of pollutants on the organisms (Bayne et al., 1988).

A similar approach is urgently also needed for Antarctic ecosystems, where the increase of human activities makes necessary the development of such integrated biomonitoring programs. In fact, an effective biological monitoring would ‘allow the assessment of the impacts of ongoing activities and facilitate the early detection of the possible unforeseen impacts’ (Protocol on Environmental Protection to the Antarctic Treaty, 1991).

Biomarkers can be investigated at different levels of biological organization and, at the biochemical one, many studies are based on the enhancement of reactive oxygen species as a general pathway of toxicity induced by pollutants and associated with oxidative stress (Winston, 1991). In this respect, antioxidant cellular responses to pollutants are well documented for several temperate molluscs (Winston & Di Giulio, 1991), while data are still limited for Antarctic inver-
tebrates. Also considering the extreme Antarctic environmental conditions (higher oxygen solubility at low water temperature and seasonality in light intensity and food availability) it was of interest to make a preliminary characterization of the cellular antioxidant system in the scallop *Adamussium colbecki* which is considered a key species for biomonitoring pollution in Antarctic ecosystems (Berkman & Nigro, 1992).

The possible biochemical adaptation to cold seawater has been considered by evaluating the enzymatic responses at different temperatures.

The utility of antioxidant responses as biomarkers of oxidative stress induced by pollutants has also been examined in scallops exposed to metals under different laboratory conditions.

**Materials and methods**

**Sampling and laboratory exposures**

Specimens of *Adamussium colbecki* were collected by Scuba diving in Terra Nova Bay (Ross Sea) near the Italian Antarctic Base. For preliminary biochemical characterization, digestive glands were dissected from 20 specimens, grouped in 5 pools and maintained in liquid nitrogen till processing for analyses.

Exposures to metals (Cu and Hg at 20 and 5 µg l\(^{-1}\) respectively) were carried out at the Italian Antarctic Base during the Austral summer 1995–96. Scallops were acclimatized without sediments for some days in running filtered seawater and they were not fed during the experiments. Seawater (1 l ind\(^{-1}\)) was changed and redosed daily and the temperature maintained at 0 ± 1 °C. At each sampling time 30 specimens (from control, Cu and Hg exposed groups) were collected and digestive glands dissected with the same procedures previously described.

**Biochemical analyses**

Sample preparation was carried out at 4 °C and detailed procedures have been described elsewhere (Regoli et al., 1997). Total glutathione was assayed by the enzymatic method of Akerboom & Sies (1981). Glyoxalase I (EC 4.4.1.5) was measured by the increase in absorbance due to the formation of S-D-lactoylglutathione (LSG) with GSH formed from S-D-lactoylglutathione (LSG). Glutathione S-transferase (GST) (EC 2.5.1.18) activities were determined, according to Habig & Jakoby (1981), using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. Glutathione reductase (EC 1.6.4.2.) was assayed following the decrease in absorbance at 340 nm due to the oxidation of NADPH in the presence of GSSG (Ramos-Martinez et al., 1983). Glutathione peroxidase (GPx) activities were measured in a coupled enzyme system where the formed GSSG is converted to its reduced form by glutathione reductase (Lawrence & Burk, 1976); H\(_2\)O\(_2\) or cumene hydroperoxide were used as substrates (respectively for the Se-dependent, EC 1.11.1.9, and the sum of Se-dependent and Se-independent, EC 2.5.1.18, activities). The rate of blank reaction was subtracted from the total rate. Catalase (EC 1.11.1.6) activity was measured, according to Greenwald (1985), by the decrease in absorbance at 240 nm due to H\(_2\)O\(_2\) consumption. Superoxide dismutase (SOD) (EC 1.15.1.1) activity was determined by its ability to inhibit the reduction of cytochrome c by O\(_2\)^− generated by the xanthine oxidase/hypoxanthine system (McCord & Fridovich, 1969). One unit of SOD has been calculated as the amount of enzyme inhibiting by 50% the reduction of cytochrome c. Protein concentration was determined according to Lowry et al. (1951) by using Bovine Serum Albumin (BSA) as standard.

**Results**

Table 1 reports the levels of glutathione and of antioxidant enzymes measured at different temperatures between 0 ° and 34 °C, in the digestive gland of *Adamussium colbecki*. Enzymatic activities generally increased at higher temperatures but with some considerable differences among the enzymes. In this respect, increasing activities of glyoxalase I and glutathione S-transferase were observed up to 19 °C, while similar or even reduced values were found at higher temperatures. A similar trend was exhibited by glutathione peroxidases (especially with cumene hydroperoxide) with maximum enzymatic activity at 15 °C. On the other hand, the increase of glyoxalase II and glutathione reductase was more constant up to 32 °C, while no clear effect of temperature was evident for catalase activity. Superoxide dismutase was measured only at 10 °C, since the blank reaction was greatly affected by temperature, making difficult the interpretation of differences.