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NOTE

Reduced superoxide dismutase activity in *Palaemonetes argentinus* (Decapoda, Palemonidae) infected by *Probopyrus ringueleti* (Isopoda, Bopyridae)

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ABSTRACT: Cellular oxidative stress may promote damage or death in biological systems and may be caused by production of pro-oxidant molecules known as reactive oxygen species (ROS). The aim of this work was to analyze the activity of antioxidant enzymes (catalase [CAT], superoxide dismutase [SOD] and glutathione peroxidase [GPx]) in the shrimp *Palaemonetes argentinus* Nobili, 1901 infected by *Probopyrus ringueleti* (Verdi & Schuldt, 1987), a gill chamber parasite known for its capacity to cause host metabolic changes, including changes in oxygen consumption rates. Infested and non-infested shrimp were collected in the Patos Lagoon estuary (southern Brasil), where the prevalence of the parasite may be as high as 70%. No significant differences were observed for either CAT or GPx activities. However, SOD activity was significantly reduced in infected shrimp, suggesting that bopyrid isopod respiratory impairment resulted in reduced SOD enzyme activity.

KEY WORDS: *Palaemonetes argentinus* · *Probopyrus ringueleti* · Antioxidant enzymes · Parasitism

In the cellular metabolism of aerobic organisms some enzymatic and non-enzymatic reactions can produce oxyradicals, called reactive oxygen species (ROS) (Sies 1991). These are formed due to the incomplete reduction of oxygen, which may generate the superoxide anion ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2) or the hydroxyl radical ($^{\bullet}OH$), as well as other partially reduced molecules. $O_2^{-\bullet}$ may be produced in cells by auto-oxidation from small molecules such as flavins, catecholamines and hydroquinones, or upon 1-electron reduction of oxygen in reactions catalyzed by enzymes such as NADPH oxidase and xanthine oxidase, or from the mitochondrial respiratory chain (Freeman & Crapo 1982, Farber et al. 1990, Storey 1996). H_2O_2 is generated through the spontaneous dismutation of 2 molecules of $O_2^{-\bullet}$, or by the activity of superoxide dismutase (SOD). *In vivo* production of the $^{\bullet}OH$ radical is accelerated by the Haber-Weiss reaction catalyzed by metals (Davies 1994).

ROS are capable of damaging biological macromolecules such as DNA, carbohydrates, or proteins, thus compromising an organism. All cells possess enzymatic and non-enzymatic antioxidant defenses to inactivate these damaging molecules. According to Halliwell & Gutteridge (1989), an antioxidant is a substance that in low concentrations (i.e. low when compared to a substrate that may be oxidized) significantly inhibits or delays such oxidation. Most of the research carried out so far has characterized oxidative stress quantitatively by determination of antioxidant activity, and that activity has been used as a bioindicator of environmental pollution.

Oxidative stress is a harmful process characterized by cellular damage that occurs when the equilibrium between the rate of ROS production and ROS elimination by cellular antioxidant mechanisms is disrupted (Sies 1991). Since oxidative stress has been implicated in several pathologic conditions in mammals (e.g. mutagenesis, atherosclerosis, ischaemia-reperfusion, inflammation, etc.) (Davies 1994) and in molluscs and fish (Dio Giulio et al. 1989, Bainy et al. 1996), there is an interest in the antioxidant systems in crustaceans.

Various stress responses have been observed in crustaceans. These include black gill syndrome, molt retardation, and disoriented behavior as a consequence of aquatic pollution (Sindermann 1996). These effects can render animals more susceptible to parasitic infections that may affect the health of whole populations. Oxidative stress may also result when oxygen availability is low (Storey 1996), and in response to various chemical compounds (xenobiotics) (Videla et al. 1995, Bainy et al. 1996).

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To date, work on fish has focused on the effects of organic pollutants and has utilized antioxidant defense systems as biomarkers for polluted environments. Glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) activities have been reported in only a few invertebrates, chiefly molluscs (Bell & Smith 1994). Gamble et al. (1995) analyzed the GPx, CAT and SOD activities of *Mytilus edulis*, *Pecten maximus*, *Carcinus maenas* and *Asterias rubens*. Livingstone et al. (1995) tested the utilization of antioxidant systems from the digestive gland of *Mytilus galloprovinciallis* as biomarkers of polluted environments. Storey (1996) related increasing SOD, CAT and GPx activities in the land snail *Otala lactea* to a protective mechanism against oxidative stress during arousal. Antioxidant activity has also been reported in *Mercenaria mercenaria*, *Calyptogena magnifica* (Blum & Fridovich 1984), *Geukensia demissa*, *Rangia cuneata* (Wenning & Dio Giulio 1988) and *M. edulis* (Pipe 1992, Pellerin-Massicotte 1994). These reports cover organisms occurring in diverse habitats or submitted to various stressful conditions.

The aim of the present work was to analyze the activity of antioxidant enzymes of the shrimp *Palaemonetes argentinus* Nobili, 1901, infested by *Probopyrus ringueleti* Verdi & Schuldt, 1987, a gill chamber isopod parasite known for its capacity to promote host metabolic changes, including changes in oxygen consumption (Anderson 1975, Schuldt & Rodrigues-Capitulo 1987). As far as we know, this is the first attempt to determine the effects of parasitism on antioxidant enzyme status.

Material and methods. Shrimp were collected at Patos Lagoon estuary (southern Brazil), where the prevalence of *Probopyrus ringueleti* may be as high as 70% (Neves et al. unpubl.). Infested and non-infested shrimp were kept separated under laboratory conditions in 40 l aquaria for 15 d at 10 ppt salinity and 20°C. They were fed 3 times a week with ground beef *ad libitum*. The mean size and standard deviation of infested and non-infested shrimps were 2.95 ± 0.59 cm and 2.32 ± 0.68 cm, respectively.

Six pools of 4 either parasitized or unparasitized shrimps were homogenized in pH 7.6 buffer (Tris-HCl 20 mM, EDTA 1 mM, dithiothreitol 1 mM, sucrose 5 M, KCl 0.15 M) containing a protease inhibitor (PMSF 100 µM). The activities of the enzymes Cu,Zn-SOD, CAT, and selenium-dependent GPx were determined in whole animals (parasitized and unparasitized) following standard techniques (McCord & Fridovich 1969, Bleuter 1975, Sies et al. 1979 for the respective enzymes). Parasites were removed immediately before the determinations. SOD was defined as the amount of enzyme necessary to promote 50% inhibition of cytochrome *c* reduction min^{-1} at 25°C and pH 7.8. One unit of CAT was the quantity of the enzyme necessary to hydrolyze 1 µmol of H_2O_2 min^{-1} at 30°C and pH 8.0. Units of GPx were defined as the quantity of the enzyme necessary to oxidise 1 µmol of NADPH min^{-1} at 30°C and pH 7.0. Enzymatic activities were expressed in relation to protein concentration, which was determined as outlined by Layne (1957).

Shapiro-Wilks' and Levene's tests were used to verify the data normality and homogeneity of variances, respectively. Means were compared by Student's *t*-test for independent samples and were considered different when $p \leq 0.05$. All tests were run on 'Statistica for Windows' (v. 5.1b, StatSoft, Inc., 1996).

Results and discussion. Research on oxidative stress and antioxidant activity has mainly been developed for vertebrates, especially for species which present strategies to mitigate oxidative effects (Gil et al. 1987, Storey 1996) and those usually exposed to polluted environments (Videla et al. 1995, Bainy et al. 1996). In the present work, no significant differences ($p > 0.05$) were observed in either CAT or GPx activities. However, in infested shrimps SOD activity was significantly reduced ($p < 0.05$) (Fig. 1). As far as we know, this constitutes the first report of changes in antioxidant enzymes related to parasitic infestation. In this respect, it is relevant to mention the results of Nabih & El-Ansary (1993), who measured CAT and glutathione reductase activities in tissues of snails (*Biomphalaria glabrata* and *Bulinus truncatus*) susceptible to schisto-

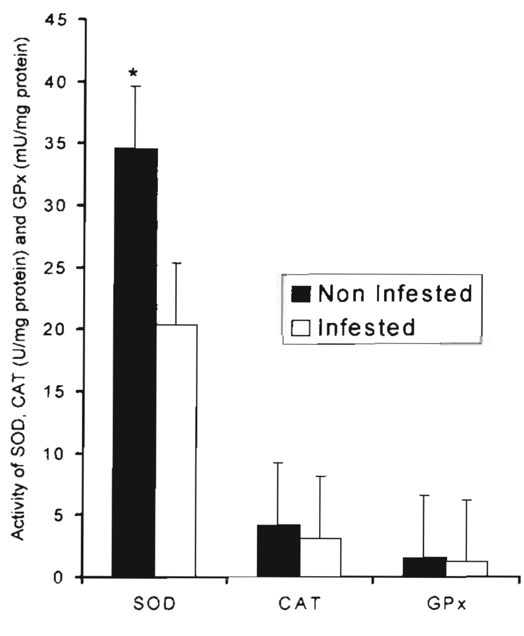

Fig. 1. *Palaemonetes argentinus*. Activity of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) of shrimp infested and uninfested by *Probopyrus ringueleti*. Vertical error bars = SE; *significantly different ($p < 0.05$)

some infection and compared them to those of the non-susceptible snail *Lymnaea truncatula*. *B. glabrata* and *B. truncatus* are aerobic while *L. truncatula* is a facultative aerobe. The enzymes of the aerobic species were more active. Also, Dykens (1984) observed significant CAT and SOD activity increases in the marine cnidarians *Anthopleura elegantissima* and *Cassiopeia xamachana* in response to reactive molecules produced by endosymbiotic algae.

Since SOD is directly related to dismutation of $O_2^{-\bullet}$ into H_2O_2, reduction of its activity (as observed in the present work) would suggest a lower capacity to avoid cytochrome *c* reduction by $O_2^{-\bullet}$. This, in turn, should decrease the capability to prevent cellular damage produced by ROS.

Shrimp parasitized by bopyrid isopods generally show a reduced respiratory rate (Anderson 1975, Schuldt & Rodrigues-Capítulo 1987). This may be a consequence of hydrodynamic changes produced by the presence of the parasite in branchial chambers and/or intense metabolic changes related to physiological alterations observed in parasitized hosts. The reduced respiratory rate may lead to lower levels of SOD and this, in turn, may make the shrimp more susceptible to damage by various marine pollutants.

Estuarine and marine environments have been used as major repositories of anthropogenic wastes for decades. These wastes have gradually accumulated and have had significant impact on sensitive habitat areas and aquatic communities (Kennish 1992). Pollutants typically associated with these wastes are polycyclic aromatic hydrocarbons (PAHs), chlorinated hydrocarbons (Walker & Livingstone 1992), heavy metals and nutrient elements (Kennish 1992). Chlorinated hydrocarbons and PAHs are lipophilic pollutants that are directly absorbed through organic membranes (Walker & Livingstone 1992).

At the Patos Lagoon estuary, nutrient enrichment is provided *in natura* by sewage discharge and by agricultural activities (Seeliger & Costa 1997). Although metal concentrations in the estuarine waters correspond to natural background levels (Seeliger & Knak 1982, Baumgarten & Niencheski 1990), the metal input, especially of suspended copper and lead, probably from industrial effluents, increases sporadically (Baumgarten 1987). The superficial coastal nearshore waters are also prone to pollution by hydrocarbon discharges from vessels during the washing of tanks (Seeliger & Costa 1997) or during charge and discharge operations in estuarine industries.

Considering that many of these pollutants are capable of absorption by aquatic organisms, and that they usually induce oxidative stress due to the production of oxyradicals during detoxification processes, it is possible that infested *Palaemonetes argentinus* with reduced SOD activities would be more susceptible to the effects of xenobiotic compounds.

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