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Antioxidant systems and lipid peroxidation in *Bathymodiolus azoricus* from Mid-Atlantic Ridge hydrothermal vent fields

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Abstract

Enzymatic defenses involved in protection from oxygen radical damage were determined in gills and mantle of *Bathymodiolus azoricus* collected from three contrasting Mid-Atlantic Ridge (MAR) hydrothermal vent fields (Menez-Gwen, Lucky Strike and Rainbow). The activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidases (GPx) (total and Se-dependent), and levels of total oxyradical scavenging capacity (TOSC), metallothioneins (MT) and lipid peroxidation (LPO) were determined in *B. azoricus* tissues and the impact of metal concentrations on these antioxidant systems and lipid peroxidation assessed. SOD, CAT, TOSC, MTs and LPO levels were higher in *B. azoricus* gills while glutathione peroxidases (total and Se-dependent) were higher in the mantle, and with the exception of CAT, were of the same order of magnitude as in other molluscs. TOSC levels from Menez-Gwen indicate that the vent environment at this site is less stressful and the formation of ROS in mussels is effectively counteracted by the antioxidant defense system. TOSC depletion indicates an elevated ROS production in molluscs at the other two vent sites. Cytosolic SOD, GPx and LPO were more relevant at Lucky Strike (Bairro Alto) where levels of essential (Cu and Zn) and toxic metals (Cd and Ag) were highest in the organisms. CAT activity and LPO were predominant at the Rainbow vent site, where an excess of Fe in mussel tissues and in vent fluids (the highest of all three vent sites) may have contributed to increased LPO. Therefore, three distinct pathways for antioxidant enzyme systems and LPO based on environmental metal speciation of MAR vent fields are proposed for *Bathymodiolus* gills. At Menez-Gwen, TOSC towards peroxyl and hydroxyl radicals and peroxynitrite are predominant, while at Lucky Strike cytosolic SOD activity and GPx are the main antioxidant mechanisms. Finally at Rainbow, catalase and lipid peroxidation are dominant, suggesting that resistance of mussels to metal toxicity at these vent fields decreases in the sequence Menez-Gwen > Lucky Strike and Rainbow.

Keywords: Antioxidant systems; Lipid peroxidation; Hydrothermal vent field; *Bathymodiolus azoricus*; TOSC; Metallothioneins

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1. Introduction

Since their discovery in 1977 (Corliss et al., 1979), populations of invertebrates at hydrothermal vents, particularly the Mytillidae, have attracted attention due to their capacity to live in one of the most extreme environments on earth. Hydrothermal vent environments are characterised by high temperature and pressure, low pH, enriched in toxic sulphide species (0.9 mM near the vents, (Blum and Fridovich, 1984)), radionuclides and naturally high concentrations of bioavailable metals that would be toxic or even lethal to non-vent marine species (Desbruyères et al., 2001; Geret et al., 1998). To live in this extreme environment, bivalve species such as the Mytilid *Bathymodiolus azoricus*, the dominant species of Mid-Atlantic Ridge (MAR) vents, derive a substantial portion of their food from free living chemoautotrophic sulphur-oxidizing bacteria and symbiotic methanotrophic bacteria present in mussel gills, that use vent substances to produce organic compounds and energy (Fiala-Medioni and Felbeck, 1990; Raulfs et al., 2004).

Metals like Fe, Zn, Cu and Mn essential for these bivalve species become toxic if present in excess. Others, like Cd, Ag, Ba and Sr, are toxic and do not seem to be related to the normal metabolism of these species (Rousse et al., 1998). Bioaccumulation of metals (Fe, Zn, Mn, Cd, Ag, Ba and Sr) in tissues of *Bathymodiolus* sp., from MAR, occurs mainly in the gills (the main interface between the organism and the hydrothermal source and where symbiotic activity is more important), in the digestive gland, and to a less extent in the mantle (Rousse et al., 1998). Furthermore, metal accumulation in *Bathymodiolus* is site specific and depends on the chemical composition of hydrothermal fluid. The survival of these vent molluscs depends on their accumulation, excretion or detoxification capacity (Chassard-Bouchaud et al., 1986). By binding to specific ligands, metals (Zn, Cu, Cd and Ag) induce the synthesis of heat-stable low molecular weight cytosolic metallothioneins (MTs) (Rousse et al., 1998). MTs are also involved in metal detoxification processes that occur in thiotrophic and methanotrophic symbiotic bacteria in *Bathymodiolus* gills. However, the role of symbiotic bacteria in metal detoxification in this species is still not well understood (Rousse et al., 1998). Although MT appears not to have a determinant role in metal detoxification in this species (Rousse et al., 1998; Fiala-Médioni et al., 2000), MTs could protect cells from oxidative stress by acting as oxyradical scavengers and through metal binding/release dynamic mechanisms (Langston et al., 1998; Viarengo et al., 2000).

Hydrogen sulphide is a potent inhibitor of antioxidant enzymes and reacts spontaneously with oxygen to generate toxic oxygen (Fridovich, 1998) and sulphide species, which in turn are capable of inflicting DNA damage (Pruski and Dixon, 2003). These reactive oxygen species (ROS) include superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and the highly reactive hydroxyl radical ($OH^{\bullet}$), peroxyl radicals ($ROO^{\bullet}$), alkoxyl radicals ($RO^{\bullet}$) and peroxynitrite (HOONO). All these oxidant species have significant damaging potential to biological targets, but different reactivity and formation pathways (Regoli and Winston, 1999; Winston et al., 1998) and therefore total oxyradical scavenging capacity (TOSC) reflects the susceptibility to oxidative stress with a greater predictive value, since the overall impairment in neutralizing ROS reactivity will anticipate alterations at other levels (Regoli, 2000).

The pro-oxidant/antioxidant balance and detoxification of ROS are crucial for cellular homeostasis (Winston and Di Giulio, 1991; Lemaire and Livingstone, 1993; Livingstone, 2001). Prominent among these antioxidants are the enzymes superoxide dismutase (SOD, EC 1.15.1.1—converts $O_2^{\bullet -}$ to H_2O_2), catalase (CAT; EC 1.11.1.6—converts H_2O_2 to water) present in peroxisomes and glutathione peroxidase (GPx; EC 1.11.1.9—detoxifies H_2O_2) and organic hydroperoxides produced by lipid peroxidation present in mitochondria and in the cytosol (Di Giulio et al., 1995; Halliwell and Gutteridge, 1999). Antioxidant enzymes are induced by various environmental pro-oxidant conditions: exposure to various types of compounds, temperature (Buchner et al., 1996; Abele et al., 1998) and hypoxia/hyperoxia (Abele-Oeschger et al., 1994). Metal ions can also stimulate lipid peroxidation by oxidizing poly-unsaturated fatty acids and other damages (Cavaletto et al., 2002).

The potential for the formation of toxic oxygen radical species during metabolic processes in vent mussels is unknown, and knowledge of the enzymatic activity against oxygen toxicity in the hydrothermal vent animals is sparse. The only information reported deals with enzymatic activity (SOD, CAT and GPx) in tissues