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Induction of mixed-function oxygenase system and antioxidant enzymes in the coral *Montastraea faveolata* on acute exposure to benzo(*a*)pyrene

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Abstract

Components of the cytochrome P_{450} monooxygenase system (MFO) and antioxidant enzymes were investigated in the coral *Montastraea faveolata* exposed to the organic contaminant benzo(*a*)pyrene (B(*a*)P). For bioassays the corals were exposed to increasing concentrations of B(*a*)P (0.01 and 0.1 ppm) for 24 and 72 h, with water renewal every 24 h. Enzymatic activity of catalase (CAT), superoxide dismutase (SOD) and glutathione S-transferase (GST) were measured in host (polyp) and hosted (zooxanthellae) cells. NADPH cytochrome *c* reductase activity and contents of cytochrome P_{450} and P_{420} were only measured in the polyp. Antioxidant enzymes CAT and SOD in polyps and zooxanthellae and GST in polyps increased significantly at the highest concentration and maximum time of exposure. Cytochrome P_{420} was found in all colonies, and the cytochrome P_{450} content was greatest in the colonies from the highest concentrations of contaminant. NADPH cytochrome *c* reductase activity and the concentration of pigments did not vary between treatments. This is the first report of the induction of both detoxifying mechanisms, the MFO system and antioxidant enzymes on acute exposure to an organic contaminant in the reef-constructing coral species *M. faveolata*.
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1. Introduction

The mixed-function oxygenase (MFO) system, along with its main component the cytochrome P_{450} monooxygenase, is present in all living organisms and plays a fundamental role in the biotransformation of endogenous compounds (i.e., steroids, fatty acids, prostaglandins) and of a variety of exogenous compounds such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls and organochloride pesticides (Den Besten et al., 1990; Solé and Livingstone, 2005). The P_{450} cytochrome catalyzes the first step of the monooxigenation reaction in non-polar organic compounds by transferring electrons from the cytochrome P_{450} NADPH reductase enzyme complex in the microsomal membranes or vesicles (Wright and Welbourn, 2002). The subsequent reactions (corresponding to Phase 2) conjugate the products of the P_{450} system with hydrophilic endogenous molecules. The results of these conjugations are compounds more polar than the parental compounds, and thus, more easily excreted from the organisms (Lee, 1982).

The MFO system has been described in many marine invertebrates (Snyder, 2000) and its induction is currently used as an indicator of the exposure to contaminating substances (Fossi et al., 1998; Cajaraville et al., 2000). In molluscs (Gilewicz et al., 1984; Livingstone, 1988; Porte and Albagaiges, 1993), annelids (Lee, 1998; Galindo, 2004) and crustaceans (James and Boyle, 1998) the MFO system is induced during exposure to xenobiotic compounds such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). PAHs are highly persistent in marine sediments, accumulating in living tissues. MFO metabolism of PAHs leads to intermediate metabolites that strongly react with organic molecules, including proteins and nucleic acids (inducing carcinogenesis), leading to tissue damage and dysfunction (Wright and Welbourn, 2002). Some of these metabolites are reactive oxygen species (ROS) that could in turn induce the antioxidant defense mechanism(s) in these marine organisms (Regoli et al., 2002). The enzymes that constitute the MFO regulate the production of oxyradicals like

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the superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) (Winston and Di Giulio, 1991). This enzymatic response represents an adaptive mechanism that is induced by different environmental stressors (*e.g.* organic compounds, heavy metals, temperature, light), and it is less specific than those of the biotransformation enzymes. The study of both the MFO and the biotransformatin enzymes provides a better understanding of the toxic effect of contaminants and the response of the organisms (Livingstone, 1991). The cytochrome P_{450} contents, the activities of biotransformation enzymes (cytochrome *c* NADPH reductase, glutathione S-transferase) and those of the antioxidant enzymes (CAT, SOD and glutathione peroxidase) have all been correlated with increases of PAHs in tissues of various bivalve species, hence they have been regarded as biomarkers of PAHs exposure (Solé et al., 1994; Cheung et al., 2001; De Luca-Abbott et al., 2005).

In cnidarians, the MFO system has been shown to be present under natural conditions in six anemone species (Hefferman et al., 1996; Hefferman and Winston, 1998; Solé and Livingstone, 2005) and in the three scleractinian corals *Favia fragum, Siderastrea siderea* and *Montastraea faveolata* (Gassman and Kennedy, 1992; García et al., 2005). In contrast, Firman (1995) did not detect the MFO system in *M. faveolata* exposed for 90 days to the pesticide chlorane. In order to use the MFO system and the oxidative-stress enzymes as biomarkers of exposure to chemicals in ecosystems such as coral reefs, it becomes necessary to establish a relationship between the presence of contaminants in the surrounding waters and the biochemical responses of the organisms (Cajaraville et al., 1998). However, to date, studies on scleractinian corals have shown that some of their biochemical responses (oxidative-stress enzymes, antioxidant potential, thermal-stress proteins and cellular integrity components) are also influenced by changes in natural conditions such as light and temperature (Brown et al., 2002; Downs et al., 2005; Griffin and Bhagooli, 2004).

Coral reefs are highly productive ecosystems and they furnish economical benefits to nearby human populations (Hoegh-Guldberg, 1999). The continuous increase of polluting substances from man-made sources, the human developments along coastal areas, overfishing, and other global factors have changed water quality, which in turn is causing a generalized deterioration of reef ecosystems (Brown, 1997; Brown et al., 2002; McCulloch et al., 2003). In this study, we evaluated experimentally the induction of cytochrome P_{450}, the enzymatic activities of cytochrome *c* NADPH reductase, glutathione S-transferase, catalase and superoxide dismutase in *M. faveolata* exposed to benzo(*a*)pyrene at sublethal concentrations. Our results showed that induction of the biotransformation and of the antioxidant systems in this coral depended on both the concentration of the contaminant in water and the length of exposure.

2. Materials and methods

2.1. Chemicals

Benzo(*a*)pyrene, henceforth referred to as B(*a*)P, reduced β-nicotinamide adenine dinucleotide phosphate (NADPH), cytochrome *c*, glutathione (GSH), 1-chloro,2,4-dinitrobenzene, monobasic potassium phosphate, di-potassium hydrogen phosphate, phenylmethylsulfonyl fluoride (PMSF), dithiothreitol (DTT), hypoxanthine, xanthine oxidase and potassium cyanide were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethylene diaminetetraacetic acid (EDTA) was obtained from Research Organics, Inc. (Cleveland, USA). Iodoacetamine, pestatin A, leupeptin, aprotinin were obtained from Calbiochem EDM Biosciences, Inc. (Darmstadt, Germany). Sucrose, β-mercaptoethanol, and bovine serum albumin were obtained from Bio-Rad Corp. (San Diego, CA, USA). Hydrogen peroxide and dimethyl sulfoxide (DMSO) were obtained from Riedel de Haën AG (Seelze, Germany).

2.2. Animal collection and experimental conditions

Fragments of *M. faveolata* were collected at Cayo Paicla, Morrocoy National Park, Venezuela (10° 52′ N, 69° 16′ W) in February 2005. The mean surface area of the fragments was 9.93 ± 2.76 cm^2. Fragments were transported to the laboratory in closed 45-L plastic containers with aeration. Before they were placed separately into aerated closed 3.5-L glass aquaria, fragments were cleaned of associated biota such as sponges, polychaetes and algae. Seawater was renewed daily and filtered through fiberglass wool, activated carbon, a phytoplankton net and Whatman No. 1 filter paper. Acclimation period was 3 days and the bioassays were carried out for 24 and 72 h.

Three colonies were used for each dose level exposition. These levels were: acclimation control (C1), 24 h control (C2), 72 h control (C3), 24 h with DMSO (C4), 72 h with DMSO (C5), 24 h and 72 h with 0.01 ppm of B(*a*)P (T1 and T2, respectively), 24 h and 72 h with 0.1 ppm of B(*a*)P (T3 and T4, respectively).

The aquaria were kept aerated during the bioassays and they were covered with translucent plastic. Coral fragments were maintained under a regime of 12 h light: 12 h dark, with an irradiance of 10 μE/m^2 (Li-cor LI-250, underwater sensor UWQ6068). Water temperature (°C), salinity (‰), dissolved oxygen (mg/l), total dissolved solids (g/L) and pH were recorded daily (Hydrolab B, minisonde SURVEYOR 4[a]). During the bioassays the conditions were: 26.07 ± 0.81 °C, 37.48 ± 0.27‰, 5.25 ± 0.18 mgO_2/L, 35.99 ± 0.23 g/L of dissolved solids, and pH 8.21 ± 0.03. All coral fragments were frozen in liquid nitrogen at the end of experimental periods. Afterwards, the samples were stored at −80 °C.

2.3. Tissue preparation

2.3.1. Polyps

Coral tissues were removed from the coral skeleton with pressurized air, and immediately submerged and homogenized in two volumes of homogenization buffer on ice with an Ultra-Turrax blender. The homogenization buffer was 100 mM potassium phosphate at pH 7.6, containing 125 mM sucrose, 1 mM EDTA, 1 mM PMSF, 5 mM β-mercaptoethanol, 0.1 mM aprotinin, 1 μg/mL pepstatin A, and 1 μg/mL leupeptin. Homogenates were centrifuged for 5 min at 2000 ×*g* at 4 °C, to

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