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Shedding light on ovothiol biosynthesis in marine metazoans

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Ovothiol, isolated from marine invertebrate eggs, is considered one of the most powerful antioxidant with potential for drug development. However, its biological functions in marine organisms still represent a matter of debate. In sea urchins, the most accepted view is that ovothiol protects the eggs by the high oxidative burst at fertilization. In this work we address the role of ovothiol during sea urchin development to give new insights on ovothiol biosynthesis in metazoans. The gene involved in ovothiol biosynthesis *OvoA* was identified in *Paracentrotus lividus* genome (*PlOvoA*). *PlOvoA* embryo expression significantly increased at the pluteus stage and was up-regulated by metals at concentrations mimicking polluted sea-water and by cyclic toxic algal blooms, leading to ovothiol biosynthesis. *In silico* analyses of the *PlOvoA* upstream region revealed metal and stress responsive elements. Structural protein models highlighted conserved active site residues likely responsible for ovothiol biosynthesis. Phylogenetic analyses indicated that OvoA evolved in most marine metazoans and was lost in bony vertebrates during the transition from the aquatic to terrestrial environment. These results highlight the crucial role of OvoA in protecting embryos released in seawater from environmental cues, thus allowing the survival under different conditions.

Exposure to metals, toxins and more generally pollutants induces oxidative stress in marine organisms, which are able to respond with the induction of both enzymatic and non-enzymatic antioxidant defenses. These systems are necessary for sustaining marine life by maintaining a fine intracellular redox balance and minimizing undesirable cellular damage caused by reactive oxygen species (ROS). In addition to the well-known antioxidant enzymes, superoxide dismutase, catalase, and several peroxidases, organisms produce low molecular-weight non-enzymatic antioxidants, which also function as direct ROS scavengers[1]. Besides the ubiquitous tripeptide glutathione containing the reactive cysteine[2], some marine organisms produce ovothiol, the π-methyl-5-thiohistidine, first isolated from ovary, eggs and biological fluids of sea urchins and cephalopods[3–5]. Ovothiol exists in three forms (ovothiol A, B and C) differing in the degree of methylation at the aminoacidic side chain. In particular, ovothiol A is unmethylated, whereas ovothiol B and C are mono- or di-methylated, respectively. The sea urchin *Paracentrotus lividus* eggs contain high concentrations (millimolar) of ovothiol A, whereas *Strongylocentrotus purpuratus* eggs contain high concentrations of ovothiol C. Thanks to the aromaticity of the imidazole ring, ovothiol possesses a very acidic thiol group ($pKa = 1.4$), when compared to the other cellular thiols, such as glutathione, trypanothione or ergothioneine[6–8]. This unusual chemical feature confers ovothiol a thiolate anion form over a wide range of pH values[9] and makes it an efficient scavenger of radicals and peroxides[10,11] by providing also protection against peroxynitrite-induced damage[12]. These *in vitro* studies suggest that ovothiol is involved in the balancing of cellular redox homeostasis. It was previously hypothesized that, in sea urchin eggs, the reduced form of ovothiol is oxidized by hydrogen peroxide, produced during the oxidative burst at fertilization and is then regenerated by intracellular glutathione, thus providing a non-enzymatic glutathione peroxidase-like activity[13–15]. This system seems more efficient than catalase in destroying hydrogen peroxide at the concentrations produced during fertilization. The occurrence of ovothiol in other organisms and its involvement in different biological processes have also been described. In some pathogens, especially trypanosomes such as *Crithidia fasciculata* and *Leishmania donovani,* ovothiol has been suggested to be involved in the protection of the parasites from oxidative stress produced by macrophages during infection[16,17]. In the halotolerant green alga *Dunaliella salina* ovothiol has been proposed as a redox regulator in chloroplasts[18], whereas in the marine polychaete *Platynereis dumerilii* it has been suggested to act as a male pheromone during coupling[19]. In other marine organisms, ovothiol was identified in substructures of larger natural products, whose functions are still unknown. For example, ovothiol

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