UNRAVELING THE MERCAPTURIC ACID PATHWAY IN BIVALVES: ABSORPTION, METABOLISM AND EXCRETION OF CDNB IN PACIFIC OYSTERS, CRASSOSTREA GIGAS

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The mercapturic acid pathway (MAP) is a major phase II detoxification route, comprising the conjugation of electrophilic substances to glutathione (GSH), a reaction catalyzed by glutathione S-transferase (GST). In mammals, GSH-conjugates are transported out of cells, followed by the removal of Glu/Gly GSH-constituent amino acids by γ-glutamyltransferase and dipeptidases ectopeptidases. The resulting cysteine S-conjugates are reabsorbed and, finally, N-acetylation of the product generates a mercapturic acid. This pathway is poorly investigated in alternative biological models, such as bivalves, which are key organisms for aquatic ecosystems, important resources for aquaculture activities and broadly used in environmental studies. We used 1-chloro-2,4-dinitrobenzene (CDNB) as a model compound to study the MAP in Pacific oysters, Crassostrea gigas. Animals were exposed to 10 μM CDNB and MAP metabolites were followed over 24h in the seawater, gills, digestive gland and hemolymph. CDNB presented a rapid decay in seawater (half-life ~2 h), with MAP metabolites peaking as fast as 15 min (GSH conjugate), 1 h (Cys conjugate), and 4 h (mercapturic acid). Biokinetic modeling of the MAP pointed to a fast uptake and metabolism of CDNB, consistent with gills being a key organ for absorption, initial biotransformation, and, probably, excretion of metabolites. Data indicated that hemolymph participated as a milieu for metabolite transport, and suggested that digestive gland plays a secondary role in the bivalve MAP. Furthermore, biochemical and molecular analysis revealed a robust upregulation of phase II biotransformation in gills (GST transcription (4h) and activity (24h)). This is the first validation of a functional and interorgan MAP pathway in bivalves, indicating hemolymph-mediated cooperation among tissues, and highlighting a major role for gills in the metabolism of waterborne electrophilic substances. Experimental and modeled data are fully resonant with a classical mechanism for phase II xenobiotic metabolism in bivalves, indicating that water is a sink for MAP metabolites.

Keywords: mercapturic acid pathway, bivalves, glutathione

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