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Short communication

Sulfatase activity in the oyster *Crassostrea virginica*: Its potential interference with sulfotransferase determination

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Abstract

Two sulfatase isoforms, a soluble one with an optimum pH of 5.0, and a microsomal one with an optimum pH of 7.6, were observed in digestive gland, gonads, mantle and gills of the oyster *C. virginica*. The highest sulfatase activity was recorded in the digestive gland cytosol and is likely to interfere with the in vitro determination of sulfotransferase activity. Indeed, the sulfatase inhibitor Na_2SO_3 led to an increase of measured sulfotransferase activity ($31 \pm 9\%$), suggesting that those sulfatases might be partially responsible for the low sulfotransferase activities found in *C. virginica*.

Keywords: Sulfatase; Sulfotransferase; Mollusk; Metabolism

Sulfation modulates the biological activity of many xenobiotics and endogenous compounds including steroid hormones. The sulfation pathway is reversible and comprises two enzyme systems: the sulfotransferases, which catalyse the sulfation reaction and are located in the cytoplasm (Kauffman, 2004), and the sulfatases, which catalyse the hydrolysis of sulfate esters and are located in the lysosomes, the nucleus, the mitochondria or the endoplasmic reticulum (Coughtrie et al., 1998; Zhu et al., 1998). The determination of sulfotransferase activity in hepatopancreas/digestive gland cytosol of marine mollusk and crustacean species has indicated low or undetectable activities (Schell and James, 1989; Li and James, 1993; Janer et al., 2005) and has lead to the hypothesis that inhibitors of sulfotransferase or high levels of sulfatases present in hepatopancreas/digestive gland interfere with the determination of sulfotransferase activity in vitro. Indeed, hepatopancreas cytosol of the lobster *Panulirus argus* inhibited sulfotransferase activity present in the sheepshead minnow (*Cyprinodon variegates*) (Schell and James, 1989), and digestive gland cytosol of *Mytilus galloprovincialis* inhibited sulfotransferase activity in red mullet (*Mullus barbatus*) liver cytosol (G. Janer and C. Porte, unpublished data). Several sulfatase enzymes and genes have been reported in mollusks (Spaulding

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and Morse, 1991; Wittstock et al., 2000), and are possibly involved in the catabolism of sulfated polysaccharides in their herbivorous diet. However, there is still little information about the enzyme kinetics and subcellular distribution of sulfatases. Thus, this work was designed to determine sulfatase in the cytosolic and microsomal fractions of the oyster *Crassostrea virginica*, and to explore its possible interference with the measurement of sulfotransferase activities in vitro.

Digestive gland, gonads, gills and mantle of Eastern oysters (*C. virginica*, 4 years old) were dissected, frozen in liquid nitrogen, and kept at −80 °C until used. Subcellular fractions (cytosol, microsomes) were prepared as described previously (Morcillo et al., 1998), with the addition of a second ultracentrifugation step to wash the microsomes. The protein content was determined with the BCA^{TM} Protein Assay kit (Pierce Chemical Co.).

Sulfatase activity was measured as in Zhu et al. (1998) using estrone-sulfate (E_1SO_4) as substrate. The assay tubes contained 20 mM Tris–HCl buffer pH 7.6 (microsomes) or 20 mM sodium acetate buffer pH 5.0 (cytosol), 4 mM $MgCl_2$, 30 μM E_1SO_4 (0.2 μCi; 50 Ci/mmol NEN Life Science Products Inc., Boston, MA), and 30–45 μg of microsomal/cytosolic protein, in a final volume of 150 μL. After 20 min incubation at 30 °C the reaction was stopped by adding 200 μL of ice-cold water, and 3 mL of toluene. The tubes were vortexed, centrifuged (2500 × *g* for 15 min), and 400 μL of the supernatant was counted for [^{3}H] in a β-counter. Sulfatase activity was calculated by the difference of radioactivity in the aqueous phase in the system incubated at 30 °C compared to the same system kept in ice. Blanks without proteins were included in each assay to validate the extraction efficiency.

Sulfatase activity towards E_1SO_4 was linear with up to at least 0.3 mg protein/mL and with time for at least 30 min. Activities also increased linearly with temperatures ranging between 22 and 37 °C. Dependence on pH was investigated and different curves were obtained for the cytosolic and microsomal forms (Fig. 1). The cytosolic activity was optimum at pH 5.0, decreased sharply at pH 6.0 and a very low activity was found at pH 7.0 (6% of that at pH 5.0). In contrast, two optimal pH peaks were observed at pH 5.0 and 7.6 using microsomes as a source of sulfatase. The peak at pH 5.0 might be due to the presence of some residual cytosol in the microsomal fraction, despite of the fact that microsomes were carefully washed. The apparent Km and Vmax were 62 ± 14 μM and 2,263 ± 204 pmol/min/mg protein (n = 4) for sulfatase activity in the cytosolic fraction (pH 5.0), and 130 ± 21 μM and 958 ± 53 pmol/min/mg protein (n = 4) in the microsomal fraction (pH 7.6). These activities are in the range of those reported in rats towards the same substrate (Zhu et al., 1998), and the pH optimum found in the cytosol and microsomal fractions are similar to those reported for soluble and membrane bound sulfatases, respectively, in numerous species (Hanson et al., 2004). The identification of sulfatase activity in the microsomal fraction indicates the presence of a sulfatase isoform located in the endoplasmatic reticulum, as previously reported for vertebrate species. These results contrast with existing data for invertebrate species that indicate the existence of only soluble isoforms (Hanson et al., 2004). Certainly, the highest sulfatase activity was observed in the cytosolic

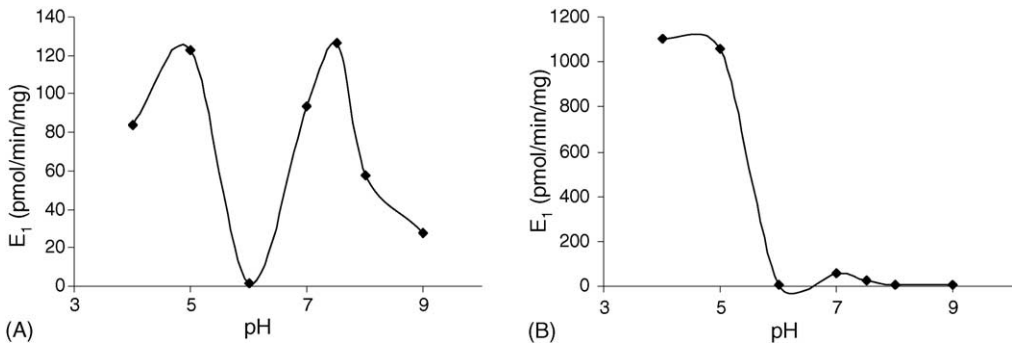

Fig. 1. pH dependence of (A) microsomal and (B) cytosolic E1-SO_4 sulfatase activity. Values are mean of duplicates.