Partial characterization of Glutamic Oxaloacetic Transaminase from the Clam *Ruditapes philippinarum* (Mollusca, Bivalvia)

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Molluscs are the principal subjects of marine pollution biomonitoring because of their widespread occurrence in coastal areas, their ability to accumulate various pollutants, and their tolerance to pollution. Biochemical indices of metabolism are subtle indicators of the physiological state of the organism. An important indice of the metabolism of molluscs under altered environmental conditions may be the activity levels of enzymes involved in adaptative readjustments (Goromosova & Tamozhnyaya, 1979). One of these enzymes could be the transaminase GOT (glutamic oxaloacetic transaminase, E.C.2.6.1.1), catalyzing the interconvertion of aspartate into 2-oxoglutarate, an intermediate of Krebs cycle. This enzyme, as well as the transaminase GPT (glutamic pyruvic transaminase, E.C.2.6.1.2), has been found in all the body tissues from all the molluscan species investigated (Bishop et al., 1983). Studies on kinetic characterization of the aminotransferase GOT showed a wide variability in the kinetic properties according to the species. This paper presents results of the kinetic characterization of GOT from *R. philippinarum*, as a first step of a further toxicological study in which this enzyme activity will be evaluated as a biochemical indicator of physiological stress induced by heavy metal exposure.

Material and methods.

Samples of *R. philippinarum* were obtained from a clam farm (Bay of Cádiz, Spain) in January and February 1990. Clams were depurated 24 hours in filtered sea water. Gills and digestive gland extracts were made by homogeneization in a mortar with glass powder in an adequate volume of the extraction buffer (80 mM Tris/HCl, pH=7.5, 0.25 M sucrose, 5 mM EDTA, 2 mM DTT and 1mM PMSF). The homogenate was filtered through a gauze, sonicated and centrifugated at 27000 g for 15 minutes. Then, the supernatant was filtered through a Sephadex G-25 column to remove endogenous salts, as well as some metabolites, and was used as the enzyme source for kinetic characterization assays. GOT activity was spectrophotometrically measured in the direction of oxaloacetate formation, following NADH oxidation at 340 nm in a coupled malate dehydrogenase reaction. The reaction mixture in a final volume of 2.5 ml contained 0.4 ml enzyme extract in adequate dilution, 0.6 U/ml de malate dehydrogenase, 1.2 U/ml de lactate dehydrogenase, 0.18 mM NADH, 80 mM phosphate buffer (pH=5.0-8.0) or 80 mM Tris/HCl buffer (pH=7.5-10), aspartate (0.1-100 mM) and 2-oxoglutarate 0.01-6 mM).

Results and discussion.

Figure 1 shows the effect of pH on GOT activity. GOT activity in Tris/HCl buffer is greater than in phosphate buffer, indicating a possible inhibitory effect of ion phosphate. The optimal pH for the glutamate oxaloacetate transaminase seemed to be at pH 8.0. This pH value is in agreement with those reported in other species of bivalves (7.7-8.5) by Bishop et al. (1983).

The apparent Km values for aspartate and 2-oxoglutarate at pH 8.0 in gills and digestive gland enzymes are listed in the following Table:

Organ	Km (asp)	Km(2-og)
Gills	0.73 mM	0.065 mM
Digestive gland	0.69 mM	0.076 mM

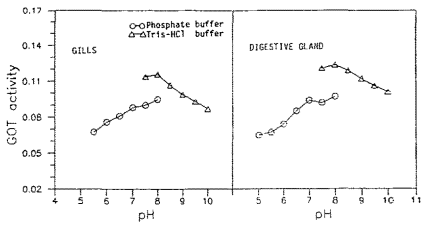

Fig.1. Effect of pH on GOT activity from gills and digestive gland.

These Km values are similar to those reported for the GOT from mussel gills (Paynter et al., 1984) and from squid axoplasm (Bishop et al., 1983). The double reciprocal plots of initial velocity versus substrate concentrations were clearly linear showing no co-operative effects in the enzyme (the Hill coefficient was aproximately equal to 1 in all the cases). The pH effect on the Km for aspartate and 2-oxoglutarate was also studied. No appreciable variation in the value of Km for 2-oxoglutarate was found, whereas a considerable increase of Km for aspartate was detected at pH 6 (Km = 1.36 mM). This strong reduction of the enzyme affinity to aspartate with a decreasing pH was also found in GOT mussel gills by Paynter et al. (1984). Figure 2 shows the effect of incubation temperature on GOT activity from gills and digestive gland. After a 5 minutes preincubation period, reaction was initiated with addition of 2-oxoglutarate, and NADH oxidation was measured for 2.5 minutes. Thermic denaturation of the enzyme occurs above 50°C. The activation energy, calculated from Arrhenius plot, was the same in gills and digestive gland (11.597 Kcal/mol).

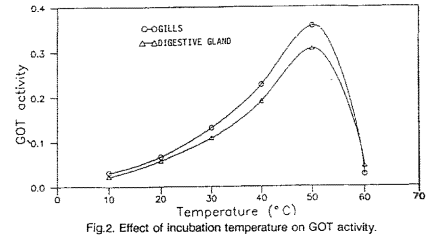

Fig.2. Effect of incubation temperature on GOT activity.

References.

BISHOP, S.H., ELLIS, L.L. AND BURCHAM, J.M.- 1983. Amino acid metabolism in molluscs. In: Hochachka, P.W. (ed.), *The Mollusca*, vol. 1, Academic Press, New York, pp. 243-327.

GOROMOSOVA, S.A. AND TAMOZHNYAYA, V.A.- 1979. Level of transaminase activity in tissues of mussel under normal conditions and in hypoxia. *Biologiya morya* (Kiev), No. 48, pp. 70-75.

PAYNTER, K.T., HOFFMANN, R.J., ELLIS, L.L AND BISHOP, S.H.- 1984. Partial characterization of the cytosolic and mitochondrial aspartate aminotransferase from ribbed mussel gill tissue. *J. Exp. Zool.*, 231, pp. 185-197.