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Role of some organic substrates in Cadmium uptake by a Marine Bacterium

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INTRODUCTION

It has been shown for a long time, that microorganisms are able to take up metals and particularly cadmium from their medium (Tornabene and Edwards, 1972; Gauthier and Flatau, 1980). The amount of cadmium absorbed seems to be an intrinsic characteristic of the strain rather than that of the species (Tynecka et al., 1981 a, b) and cadmium sensitive strains generally take up more cadmium than resistant ones (Gauthier et al., 1986). For a given strain however, the cellular content of cadmium also depends on extrinsic physicochemical parameters like temperature, pH (Titus and Pfister, 1982), salinity or the presence of organic matter (Flatau et al., 1986).

Saccharose (SAC), glucose (GLU), sodium succinate (SUC), gluconate (GLC), acetate (ACE), glycerophosphate (GLY), pyruvate (PYR), organic substrates eventually used as carbon sources or involved in the tricarboxylic cycle were therefore investigated to determine their possible role in the fixation of cadmium by a marine pseudomonad. As sensitivity to metals of microorganisms greatly depends on their nutritional state (Brynhildsen et al., 1988), this study was carried out on freshly harvested cells (fresh cells) and starved cells.

RESULTS AND DISCUSSION

Although the presence of glucose stimulated respiratory activity in fresh cells, it induced a decrease of 27 % in the cellular amount of cadmium. In starved cells however, glucose stimulated respiratory activity and induced an increase of 27 % in the cellular amount of cadmium. This could suggest the presence of an efflux mechanism which would be activated in non limited cells only, as supposed by Brynhildsen et al., (1988). On the other hand, the addition of energy and carbon (as glucose) may have energized the transport of cadmium in starved cells as it was supposed for the transport of zinc in *Escherichia coli* (Bucheder and Broda, 1974).

The other substrates had a more attenuated effect on Cd uptake by fresh cells. A trend in the inhibition of Cd uptake was supposed but could not be confirmed because of the too slight variations of the results relative to their variance.

On the other hand, a significative stimulation of Cd uptake in starved cells by the tested substrates was observed (Fig. 1), which was not correlated to the stimulation of respiratory activity.

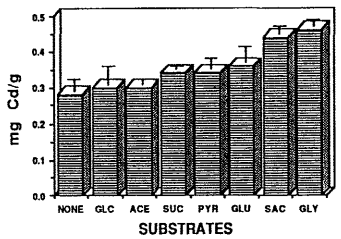

Fig. 1 : *Stimulation of cadmium uptake by starved cells in the presence of gluconate (GLC), acetate (ACE), succinate (SUC), pyruvate (PYR), glucose (GLU), saccharose (SAC) and glycerophosphate (GLY), (final concentration, 2g/l).*

In conclusion, the uptake of cadmium greatly depends on the nutritional state of cells at least for the tested strain. In starved cells, the supply of energy and carbon stimulated Cd uptake, probably because this latter was probably an energy-dependend mechanism (Flatau et al., 1989). On the other hand the absorption of cadmium could be limited by efflux mechanisms activated in non limited organisms.

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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

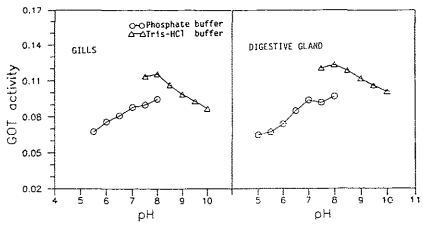

Fig.1. Effect of pH on GOT activity from gills and digestive gland.

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).

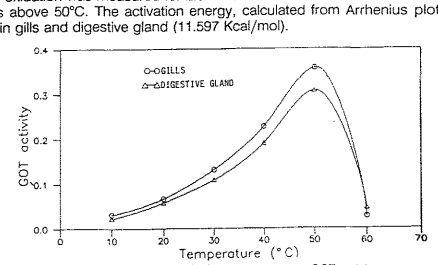

Fig.2. Effect of incubation temperature on GOT activity.

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