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BIOCHEMICAL CHARACTERIZATION OF GLUTATHION-S-TRANSFERASE AND EFFECTS OF LEAD ACETATE IN *GAMMARUS PULEX*

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Glutathione transferases (GST s; EC.2.5.1.18) are a family of multifunctional proteins that catalyze the conjugation of GSH to the electrophilic centre of a large number of active molecules. A great number of drugs, halogenated hydrocarbons, epoxides and insectisides react with the sulphydryl group of glutathione. The reaction may be catalyzed by one or several glutathione trasferases, and it is therefore considered that a main function of these proteins is to aid detoxification of various xenobiotics.

In the reactions catalyzed by these transferases, the sulfur atom of glutathione (GSH) provides electrons for nucleic attack on or reduction of, the second, electrophilic substrate. A glutathione conjugate thus formed may be excreted as such or hydrolyzed to an S-(substituent)-cysteine derivative. The cysteine derivative can subsequently be N-acetylated to yield a mercapturic acid (mercaptate), which is a classical excretion product of xenobiotics. Alternatively, The cysteine moiety is cleaved at the C-S bond with elimination of pyruvate and NH_3 and conversion of the S-substituent to a corresponding mercaptan. By this transformation, the sulfur of the new metabolite is the only remainder of the GSH molecule. The thiol group may be blocked by glucuronosylation or methylation and the resulting thioglucuronide or methylthio group, have also been identified as excretion products. These new sulfur containing end products of xenobiotics are important major metabolites arising from conjugation with glutathione. Thus, the importance of GSH transferase activity is even greater than realized in the past.

In this study, optimum pH for glutathione-S-Transferase in Gammarus pulex, an invertebrata that is a member of the nourishment chain in aquatic environments and increasingly gains importance as a test organism in environmental toxicology, is determined, and the activities for the same enzyme are assayed in different substrate concentrations.We analyzed them by Michealis-Menten and Lineweaver-Burk plots. Furthermore the effect of lead acetate on enzyme activity is investigated. GST was exposed to lead acetate EC_{50} concentrations in order to get the changes of the GST activity after 4, 8, 16, 32 and 64 hours. As a result, in comparision with the control group, an important decrease in GST activity was observed.

The inhibition of the GST activity in aquatic macro-invertebrates is in general comparable with that in rat liver

Keywords:GST, lead acetate, *Gammarus pulex*

Introduction

Glutathione Transferases (GST s; EC.2.5.1.18) are a family of multifunctional proteins that catalyze the conjugation of GSH to the electrophilic centre of a large number of active molecules (Chasseaud, 1979; Jacoby and Habig, 1978; Mannervick and Danielson, 1988). GST s can probably also function as intracellular binding proteins by virtue of their ability to bind covalently to a wide number of xenobiotics such as biluribin, haem drugs, steroids, hormones and other substances including carcinogens and pesticides (Smith and Litwack, 1980). The glutathione transferases are normally present in large quantities, representing about 20% of the extractable protein of rat liver (Jacoby, 1978) but can be induced to greater than 20% (Arias et al., 1978). Most of the work has been carried out with enzymes from rat and human liver (Jakoby, 1978; Jacoby and Habig, 1980; Kamisaka et.al., 1975) but placenta as well as

sheep and mouse liver have also been sources for homogeneous preperations. The enzymes have been found in all mammalian tissue tested as well as in insects, protozoa, algae, fungi, and bacteria. Bacteria GST's however show structural and immunological properties that distinguish them from the GST's characterised from mammalian sources (Di Ilio et all.,1988 b; Piccolomini et al.,1989). GST activity has also been detected in a great number of invertebrate species and the tolerance of these organisms to the toxicity of foreign chemicals has often been related to their GST content (Stenersen et al., 1987; Dierckx, 1984; Clark, 1989). Non-vertebrate species are also of interest considering their potential use in environmental toxicology as test organisms.

The major outlines of the reactions catalyzed by these enzymes, whatever their source, are clear. The glutathione transferases may be considered as catalysts of all reactions in which glutathione, as the thiolate anion, can participate as a nucleophile, providing only that a compound with a sufficiently electrophilic group binds to the enzyme (Jacoby W.B., 1985).

In the present study, we report the biochemical characterization of GST and the effects of lead acetate in *Gammarus pulex*, an aquatic macro-invertebrate which plays an important role in the detritus chain.

Materials and Methods

Animals

Gammarus pulex were collected from the River Porsuk (Eskisehir), a high quality natural environment, and kept in tanks filled with running water, at a constant temperature, received artificial oxygenation.

Enzyme assay

Enzyme activity is determined spectrophotometrically at 340 nm by measuring the formation of the conjugate of glutathione (GSH) and 1-chloro-2-4-dinitrobezene (CDNB).

Reagents

Sodium phosphate buffer, 0.1 M, pH 6.5, containing 1mM EDTA

GSH, 20mM, in deionized water

CDNB, 20 mM, in 95% ethanol

To a 1 ml cuvette are added 850 µl of buffer, 50 µl of 20 mM GSH, and 50 µl of CDNB. The reaction is started by addition of 5 µl of enzyme. The increase in absorbance at 340 nm is monitored for three minutes.

Definition of Enzyme Unit: A unit of enzyme activity is defined as the amount of enzyme that catlyzes the formation of 1µmol of S-2, 4 dinitrophenyl glutathione per minute, using 1 mM concantration of GSH and CDNB.

Effect of pH on the GST Activity

Adding HCl, acidik sodium phospahate buffer samples, with 4, 5 values, and adding NaOH, bazic buffer samples with 7, 8, 9 values are obtained. These pH buffers with different values are used for the enzyme samples and their effects on activities were tested.

EC_{50} Value

Pb solutions were prepared by dissolving Pb acetate in distelled water. The animals were exposed for various time periods in a single toxicant concantration [(EC_{50}), (Kutlu M. ,1998)] to get the changes of the GST activity after 4, 8, 16, 32 and 64 hours.

Results and Discussion

In the present paper we report the characterization of GST, an enzymatic system that plays a key role in the biotransformation and metabolism of xenobiotics, from *Gammmarus pulex,* an aquatic macroinvertabrate which is

largely used as a test organism in ecotoxicological studies (Muirhead-Thomson, 1989). When assayed with 1-chloro 2,4-dinitrobenzene as substrate a relatively high activity value from whole animal cytosol was obtained. 1 U of enzyme activity is defined as the amount of enzyme that catalyzes the formation of 1 μm of product/min under the assay conditions. The results are expressed as specific activity, being the units of enzyme activity/mg of protein, as measured by the method of Lowry et.al. with bovine serum albumin as a standard.

GST activity was found in all aquatic macro-invertebrates investigated, as well as in other animal groups. The GST activity was relatively high (Bears et.al., 1981). In rat liver, which is a rich GST source, a specific GST activity of about 1000 is usually found (Dierickx, 1983 a-c). The GST activity was determined according to Habig et al (1974).

We also studied the kinetic properties. Kinetic properties were examined measuring the initial velocities of GST + CDNB concentrations varying from 5; 7.5; 10; 12.5; 15; 17.5; 20; 22,5 mm. They were analyzed by Michaelis-Menten (Fig. 1) and Lineweaver-Burk plots (Fig. 2).

Optimal conditions of substrate and cofactor concentration for cytosolic activity were determined. Alkaline pH activity data indicated that GST activity in *Gammarus pulex* tested was maximal pH range (6.5-8) and decreasing at higher values and non-detectable at pH:9.

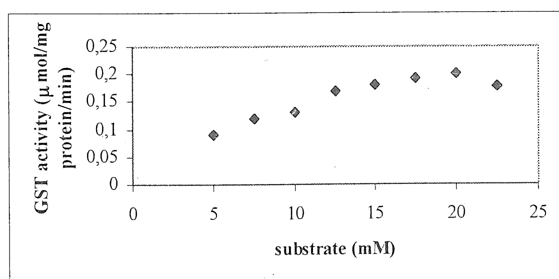

Fig. 1. Michealis-Menten Plot.

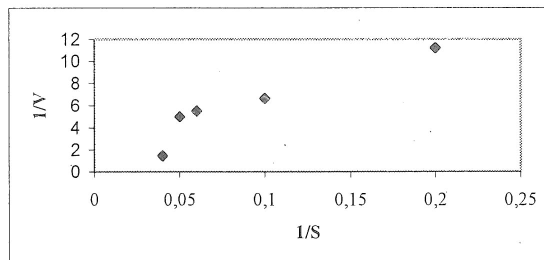

Fig. 2. Lineaweaver-Burk Plot.

In a serious of studies, we first observed that the treatment of lead acetate inhibit the activity of GST in *Gammarus pulex.*

To asses the relationship between cytosolic GST activity and the exposure time of lead acetate at EC_{50} , the activity was determined after 4, 8, 16, 32 and 64 hours of exposure.

In a serious of studies, we first observed that the treatment of lead acetate inhibits the activity of GST in *Gammarus pulex* (Fig.3). Figure 3 shows the activity of GST during the 64 hours of exposure at the toxicant concentration of EC_{50} . To asses the relationship between GST activity and the exposure time of Pb at EC_{50} , the activity was determined after 4, 8, 16, 32 and 64 hours of exposure.

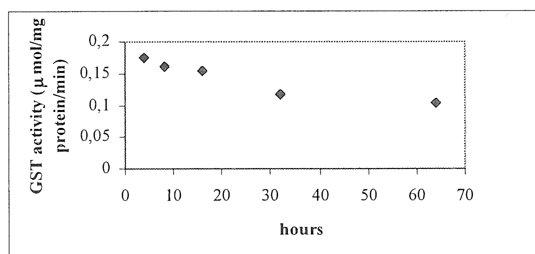

Fig. 3. Inhibition of lead acetate.

As a result; the activity of GST was inhibited by Pb acetate in *Gammarus pulex.* GST s can probably also function as intracellular binding proteins by virtue of their ability to bind covalently or non-covalently to a wide number of xenobiotics such us bilirubin, haem,

drugs, steroids hormones and other substances including carcinogens and pesticides (Smith and Litwack, 1980).

The catalytic function (Chasseaud, 1979) and the ligand complexing properties (Smith and Litwack, 1980) of GST are important for mammalians, since they detoxify a large number of chemicals. The multiplecity of GST isoenzymes is assumed to result from the need to conjugate numerous types of substances differing in the nature of their electrophilic centre and their molecular structures (Kettetrer et.al. 1988).

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