Bull. Environ. Contam. Toxicol. (2004) 73:432–436

DOI: 10.1007/s00128-004-0447-4

Bulletin of Environmental Contamination and Toxicology

Biochemical Properties of Glutathione Peroxidase in *Gammarus pulex*

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Received: 31 August 2003/Accepted: 12 July 2004

Glutathione peroxidase (Glutathione: H_2O_2 Oxidoreductase, E.C. 1.11.9) catalyzes the reduction of various organic hydroperoxide with glutathione as hydrogen donor. It has been suggested that this enzyme functions in more times as a mechanism of protecting the cellular membrane system against peroxidative damage (Laurence et al. 1976). And the importance of selenium as an essential trace element is further concerned with this suggested function of the enzyme, It has a molecular weight of 84.000 kDa and 4 subunits per mol of the enzyme (Meister 1985).

Glutathione peroxidase (GSH-Px) catalyzes the oxidation of GSH to GSSG at the expence of $H_2O_2 : H_2O + 2GSH \longrightarrow GSSG + H_2O$.

According to selenium dependency, GSH - Px can be divided into two forms : Se - dependent and Se - independent GSH-Px (Fıskın 2000). Selenium dependent enzyme is active with both organic hydro peroxides and H_2O_2. The second type of GSH-Px consists of proteins that do not depend on selenium for catalysis and have negligible activity with H_2O_2 (Mannervik 1985).

Numerous chemical processes in aerobic cells lead to the production of peroxides by activated forms of oxygen. The peroxides may cause oxidative damage in biological tissues as well as decompose to generate free radicals and other reactive chemical species. (Vargas et al. 2001) The simplest hydroperoxide, H_2O_2 , can be detoxified by the selenium dependent glutathione peroxidase (Sawada et al. 1987).

Interest in aquatic toxicology results not only from the comparative point of view with possible benefits to the fishery profession but also from the human health stand point, since aquatic organisms may be sensitive first line indicators of environmental contamination which could have important puplic health significance. It has been the desire and need to establish baseline, information to define the aquatic model systems as completely as possible (Hendricks 1982).

Gammarus pulex is an invertebrata that is a member of the nourishment chain aquatic environments and increasingly gains importance as a test organism in environmental toxicology. In this study, we investigated the effects of lead acetate EC_{50} concentrations in the enzyme activity. Furthermore optimum pH for Se-dependent GSH-Px and Se-independent GSH-Px enzyme was determined.

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MATERIALS AND METHODS

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

One enzyme unit is defined as the amount of enzyme that transforms 1μmol of NADPH to NADP per minute at 37°C. The specific activity is expressed in units per milligram of protein (Cands et al. 1989).

Potassium Phosphate buffer (50mM pH= 7 EDTA 5 mM), Sodium azide (1mM), Reduced Glutathione (2mM), GSSG Reductase (1,2 unit/mL), NADPH (0.2mM) were used for the experiments. H_2O_2 (0.25mM) for Se- independent GSH-Px and Cumene hydroperoxyde, (1.5mM /mL etanol) for Se- dependent- Px, were prepared as the stock solutions.

Gammarus samples were homogenized with Potter Elvehjen homogenizer, in phosphate buffer and then centrifuged at 20.000 rpm for 15 minutes at 0°C. Supernatant was used for the enzyme assays. A mixture, containing NADPH, GSH, NaN_3, Glutathione reductase, and supernatant having the enzyme sample was preincubated for 10 min at 37 °C. The reaction was initiated by adding H_2O_2 for Se- dependent GSH- Px and Cumene Hydroperoxide for Se- independent GSH-Px. Absorbance changes were recorded at a wavelength of 340nm.

Pb solutions were prepared by dissolving Pb acetate in distelled water. The animals were exposed for various time periods in a single toxicant concentration [(EC_{50}), (Kutlu et al. 1998)] and *Gammarus pulex* glutathione peroxidase activity was measured after 4, 8, 16, 32, 64 and 96 hours.

RESULTS AND DISCUSSION

Glutathione peroxidase catalyzes the reduction of hydroperoxides with glutathione as the reductant. The simplest method is a spectrophotometric assay in which the reduction of GSSG is coupled to the oxidation of NADPH through Glutathione reductase (Cands et al. 1989).

Glutathione peroxidase activity has been demostrated in almost all mammalian tissues examined. In most animals the seleno protein is responsible for a substantial fraction of the activity (Flohe et al. 1984), but in the guinea pig (liver) the seleno protein is absent or present in a very small amount. The selenium-independent activity is in all case investigated in depth, ascribable to the glutathione transferases (Mannervik 1985). The ratio between selenium and selenium independent activities may vary not only between animal species but also from tissue to tissue in the same species (Tappel 1980).

In this study, we determined the effects of lead acetate on Glutathione peroxidase in Ga*mmarus pulex*. The animals were exposed to a single toxicant concentration

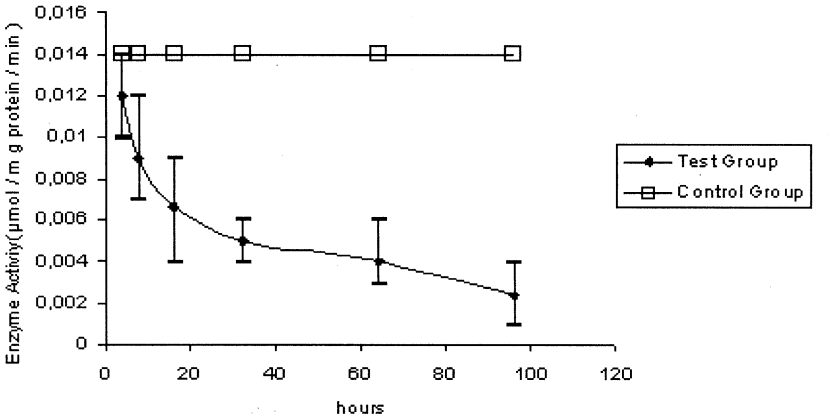

Figure 1. Se-dependent GSH-Px activity for *Gammarus pulex* at different hours (EC_{50} concentrations). Data are the means of at least three seperate determinations.

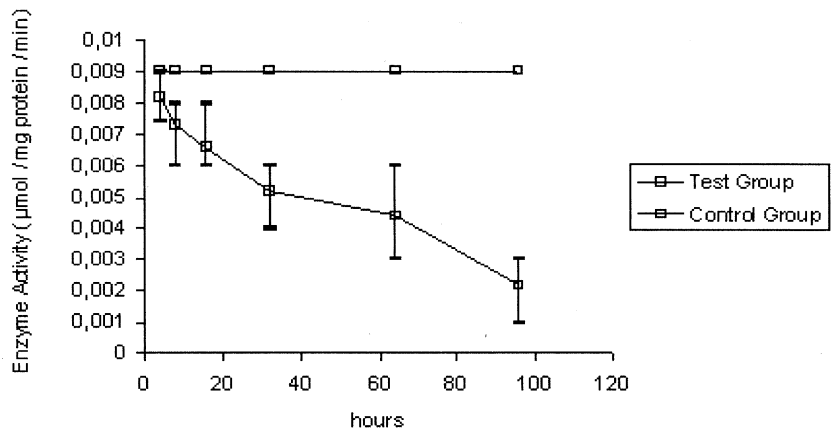

Figure 2. Se-independent GSH-Px activity for *Gammarus pulex* at different hours (EC_{50} concentrations). Data are the means of at least three seperate determinations.

(EC_{50}) for various time periods (4, 8, 16, 32, 64 and 96 hours) (Kutlu et al. 1998). Control groups of animals were subjected to the same procedures but exposed only to clean, condioned tap water. At the end of the exposure times, animals were homogenized mentioned above. After centrifugation, enzyme activity was measured. In a serious of studies, we first observed that the treatments of lead acetate slightly inhibited the activity of Glutathione peroxidase. Figure 1 shows the activity of Se-dependent glutathione peroxidase during the 96 hours of exposure at the toxicant concentration of EC_{50} and Figure 2 shows the activity of

Se- independent Glutathione peroxidase during the 96 hours of exposure at the toxicant concentration of EC_{50} .

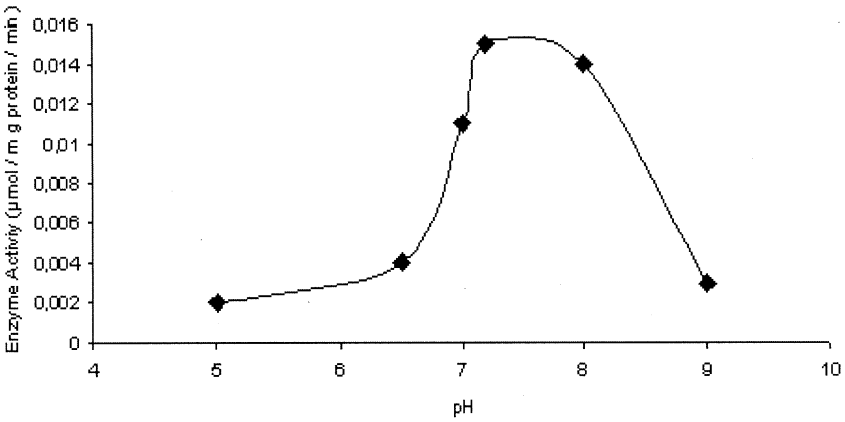

Figure 3. pH activity profile of *Gammarus pulex* Se-dependent GSH-Px in the presence of different buffer.

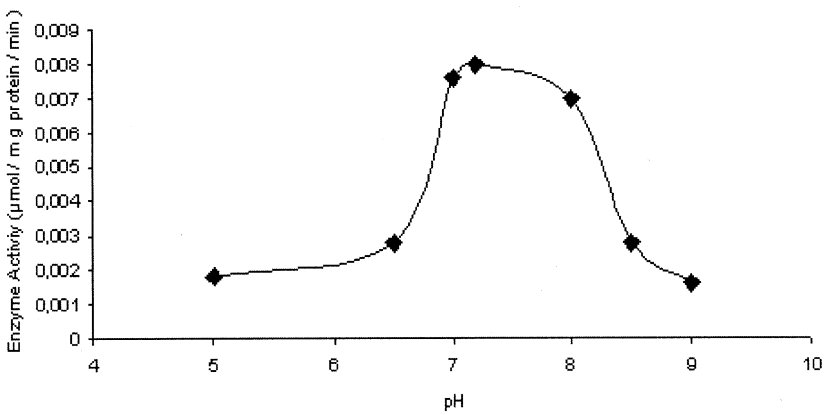

Figure 4. pH activity profile of *Gammarus pulex* Se-independent GSH-Px in the presence of different buffer.

As a result, activity of glutathione peroxidase was inhibited by lead acetate in *Gammarus pulex*. In a period of 96 hours of exposure, the activity became slightly low.

The effects of pH on Glutathione peroxidase activity was also investigated . For this purpose various buffer solutions (pH: 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9) were used as working buffers. Data regarding the effect of pH on Se-dependent Glutathione peroxidase activity in *Gammarus pulex* indicate that activity is maximal in the pH range 7-8.5 and decreasing at higher values (Fig 3) and Se-

independent Glutathione peroxidase activity is maximal in the pH range 7-8 and decreasing at higher values (Fig 4).

It can be concluded that an important decrease in the activities of Se-dependent GSH-Px and Se-independent GSH-Px, the antioxidant mechanism enzymes, in *Gammarus pulex*, exposed to lead acetate EC_{50} concentrations was observed. Optimum pH for Se-dependent GSH-Px and Se-independent GSH-Px enzyme was determined.

Acknowledgements This work was supported by grants from the Research Fund of Anadolu University, Eskişehir,Turkey and Presented at First International Conference on Pollution Eco-Chemistry and Ecological Processes, August 27-31, 2002, Shenyang, China.

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