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EFFECTS OF LEAD AND SOME IONS ON THE ACTIVITY OF GLUTATHIONE REDUCTASE IN *GAMMARUS PULEX*

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Gammarus pulex, a member of the Amphipoda, has been commonly used as a test organism in environmental toxicology in recent years. Glutathione reductase is an enzyme that is very important in effective detoxification of radicals causing hidrogen peroxide and radiation. In the detection of aquatic pollution, to determine of the Glutathione reductase activity is very important. In this study, the effect of the lead, a heavy metal, on the Glutathione reductase enzyme activity in *Gammarus pulex,* an aquatic amphipod, is investigated; Glutathione reductase was exposed to lead acetate EC_{50} concentrations, in order to get the changes of the Glutathione reductase activity after 4, 8, 16, 32, 64 and 96 hours. Different concentrations (1μM, 10μM, 50μM, 100μM and 500μM) of Cu^{+2}, Mg^{+2}, Zn^{+2} ions on the enzyme activity is analysed. And also, the optimum pH for this enzyme is observed and with the activity assay of different NADPH concentrations, K_m is determined 129,03 μM.

Keywords: Gammarus pulex, *Glutathione reductase, Lead Acetate*

Introduction

For long years, fresh water amphipods have been used as test organisms in aquatic toxicology. *Gammarus pulex*, is one of the most popular living organisms among the fresh water amphipods used in toxicological studies. Besides, its advantages like being wide spread throughout many regions in the world, being accessible and causing no problems in laboratory conditions; It's also a sensitive organisms against toxic substances (Kutlu M., 1996).

Glutathione reductase, is a member of pyridine nucleotide-disulfate oxidoreductase including lipoamide dehydrogenase and thioredoxine reductase that is dimeric each (Takeda T. et al, 1993; Leister B. and Perham R., 1994).

Glutathione reductase enzyme is a very significant enzyme in the effective detoxification of radicals that cause hydrogen peroxidase and radiation (Marvin B. and Duvel D.L., 1988). The analyses of the modifications at GSH (reducted glutathione), which is accomplished by toxic compounds, gives important information about the toxicity mechanism of a compound (Adams J.D et. al, 1983).

Glutathione fractions are like anti oxidant and takes part in the removement of the hydroperoxides in the ascorbate-glutathione circulation and free toxic radicals; air pollution and detoxification of xenobiotics. Glutathione reductase is in every place of living organisms. This enzyme mates directly with Glutathione peroxidase in the animals; mates indirectly with ascorbate peroxidase via dehydroascorbate reductase in the hight plants including euglena, green alges and cyanobacteria (Takeda T. et al, 1993).

Glutathione reductase catalyses the reaction $NADPH + GSSG + H^+ \rightarrow NADP^+ + 2GSH$ (Lehninger A.L. et. al,

1993; Zubay G., 1988; Yenson M., 1984; Goldstein M., 1983). Especially this enzyme plays an important role in the production of deoxyribonucleotide and in the control of oxidative stress (Nordhoff A. et. al, 1993; Stryer L., 1988).

Reduced glutathione carries an non-specific reductive feature. The number of enzymes activating bound to sulphydril groups on prostatic group surfaces, is high in this organisms. In the case that can unite with sulphydril, they become inactivated and gain their activities by entering into a reaction with GSH.

Glutathione reductase, provides the GSH and GSSG amounts to be in the right percentage in the cells (Marvin B. and Duvel D.L., 1988; Colak O, 1990).

In this study, it is determined that the activity of glutathione reductase enzyme in the *Gammarus pulex* has increased up to a 64 hour period as result of its treatment with lead in 4, 8, 16, 32, 64 and 96 hour periods and then has decreased in the 96 hour period. At the end of the experiments carried out with different concentrations of Cu^{+2}, Mg^{+2} and Zn^{+2} ions (1μM, 10μM, 50μM, 100μM ve 500μM), a decrease is observed in the activity of glutathione enzyme in *Gammarus pulex* and the decreasing effect of these ions on the activity is determined successively as $Zn^{+2} > Mg^{+2} > Cu^{+2}$.

Materials and Methods

Providing Living Organisms Used in Experiments:

Gammarus pulex used in the experiments were picked up from Porsuk River (Eskisehir, around Regulator) and brought to the laboratory.

The water quality parameters for *Gammarus pulex* were similar for all tests with the heat, excluded (12 °C ± 1).

The familiarities of the laboratory conditions were provided with the air flow and appropriate heat (adjusted to 10-12 °C) prepared by water and organic material taken from their natural environment. The living organisms, were fed with organic food given and from the soil taken from their own environment. Organic food was given to the living organisms once in 20-25 days.

5-8 mm living organisms were used in the experiments and the ones kept for the experiment were exposed to the conditions of the experiment in 1 litter aquariums. No food is given to the living organisms before the experiment. During the experiment the moving parts of the living organisms were observed every day for 15 minutes and the non-moving parts were not included in the experiment.

Lead Doses Used in the Experiment:

The prepared stock lead solvent was used like lead acetate being solved in pure-water. The stock solvent; was prepared 0,1 mg/ml and EC_{50} values were applied to it.

Determination of Glutathione Reductase Activity:

Gammarus pulex that are found suitable for the experiment (between 5-8 mm) were homogenized in the 500 μl potassium phosphate buffer (0,2 mM with EDTA, pH: 7.2), in the ice, using the Janke&Kunkel Ultra-Turrax T25 homogenizator with low turn. The homogenated Herraus Sepatec Biofuge 20 RS was centrifuged in cooling centrifuge at 20000 rpm for 15 minutes at 0°C. As an enzyme source for the glutathione reductase activity, the supernatant, gained at this stage are used.

Glutathione reductase is a flavoprotein which catalyses the NADPH-bounded reduction of GSSG (Glutathione desulphate) to glutathione (GSH).

$$NADPH + GSSG + H^+ \rightarrow NADP^+ + 2GSH$$

The reaction is required in observing the glutathione level. In the processes of oxidation and reduction, glutathione takes a basic part like a reductant in the detoxification and some other important cellular functions.

1 unit glutathione reductase activity is the amount of the enzyme that reducts 1 micro mole NADPH in 1 minute. The oxidation of NADPH

is monitored as 340 nm in the spectrophotometer (Carlberg I. and Mannervik B., 1985).

Results and Discussion

Glutathione reductase plays an important role in the detoxification mechanism that provides aquatic living organisms to continue their lifes by detoxification the toxic substances smeared to their environment.

Natural antioxidants, free radical scavengers as glutathione reductase, catalase and glutathione are considered for aging mechanisms (Fıskın K., 1994). It's well known that reduced glutathione (GSH) is catalysed by Glutathione reductase that detoxifies the toxic substance in the environment having toxic substances, transforms into oxidized glutathione (GSSG) in the detoxification reactions. The GSH required to make the toxic substances, in the cell, ineffective, is created by glutathione reductase enzyme. Inner-cell reduced glutathione comes from two sources. The first one is the normal GSH synthesis that is from amino acids at the erythrocytes, and the second one is oxidized glutathione that is reduced by the reaction that glutathione reductase catalyses (Paniker N.V. et al, 1970).

In this study, the toxicity of the lead , a heavy metal, on aquatic living organisms and the changes on the activity of glutathione reductase enzyme are examined.

Glutathione reductase present in *Gammarus pulex* chosen as a test organism, is used as an indicator in the measurement of water pollution. As a result of the application the EC_{50} values of lead in 4, 8, 16, 32, 64 and 96 hour periods to *Gammarus pulex*, it is monitored that activity of glutathione reductase enzyme at the 64 hour period according to control groups is increased and after this period the activity is decreased.

When *Gammarus pulex* is exposed to lead acetate, the tendency of *Gammarus pulex* to decrease GSSG that accumulates in the cell by detoxificating lead, can be explained best by the increase in the glutathione reductase enzyme activity in this period.

The glutathione reductase activity is proved to be higher in the human breast and in the prenoplastical and neoplastical tissues of rat breast glands, when that is compared to other tissues. It can be estimated that the increase of glutathione reductase in the breast cancer is an adaptive response when enzyme increases in the case that GSH is exhausted (Ilio C.D. et. al, 1985).

When its exposure to lead is increased to 96 hours; after 64 hours exposure, the decrease in the enzyme activity can be explained by a corruption that can exist in the structure of the enzyme (Figure 1.).

This apparent decrease in the glutathione reductase activity, is explained with the inactivation of sulphydril groups in the structure of enzymes and at the active centres, with superoxide and hydroxyl radicals that exits with the effect of radiation (Erden M., 1992).

In a study carried out with rats, the glutathione reductase activity that was gained after experiment animals had been radiated with 300 rad (gamma radiation), showed a significant decrease when compared to the control value (Erden M., 1992).

It's observed that GSH amount in *Gammarus pulex*, on aquatic amphipod, is constant since it has more glutathione reductase enzyme when compared to developed backboned living organisms. The decrease after 64 hour period can be explained with the decrease in the

resistance of the living organism because of the conditions of the environment after the corruption that can occur in the structure of the enzyme.

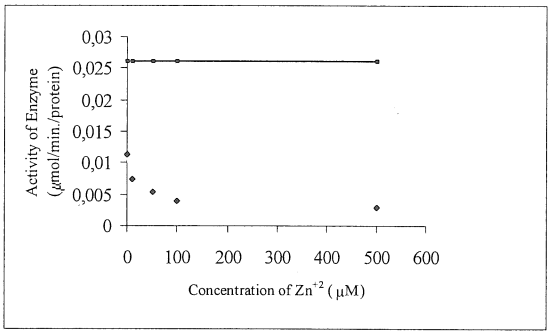

Fig. 1. The graphic of EC_{50} of the Glutathione reductase activity in *Gammarus pulex* according to the lead changing within hours (4, 8, 16, 32, 64 and 96.) (——Control group, ♦ Test group).

Some certain ions like Hg^{+2} and Cu^{+2} and thiol inhibitors inhibited glutathione reductase enzyme activity (Takeda T. et al, 1993).

The effects of Cu^{+2}, Mg^{+2} and Zn^{+2} ions on glutathione reductase activity in *Gammarus pulex* was examined and it's seen that those ions being added to reaction environment in different concentrations, inhibited enzyme activity. The decreasing effect of those ions found successively as $Zn^{+2} > Mg^{+2} > Cu^{+2}$ (Figure 2., 3., 4.).

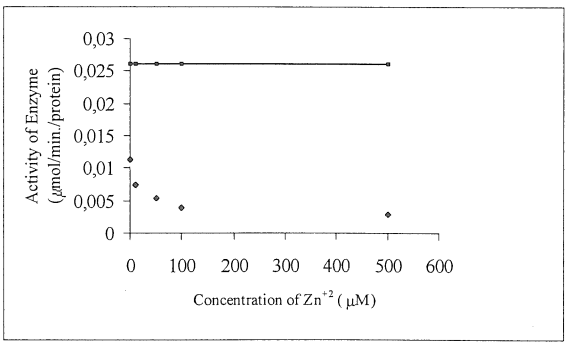

Fig. 2. Glutathione reductase activity for Zn^{+2} ion (—— Control group, ♦ Test group).

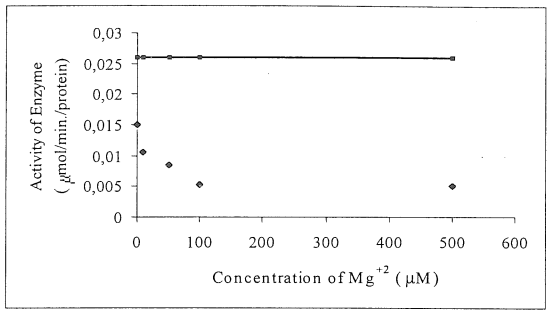

Fig. 3. Glutathione reductase activity for Mg^{+2} ion (——Control group, ♦ Test group).

In the study also the effects of three metal salts and N-trityl-morpholine (Triphenmorph) and two aquatic amphipod species were examined. In the experiments carried out in eucalcic and oligocalcic to detect LC_{50}, the toxicity state in eucalcic water was found as similar for both kinds (Triphenmorph > $Cu^{+2} > Zn^{+2} > Ba^{+2}$). It's indicated that in oligocalcic water, $CuCl_2$ was more toxic than Triphenmorp on *Gammarus pulex* (Vincent M. et. al., 1986).

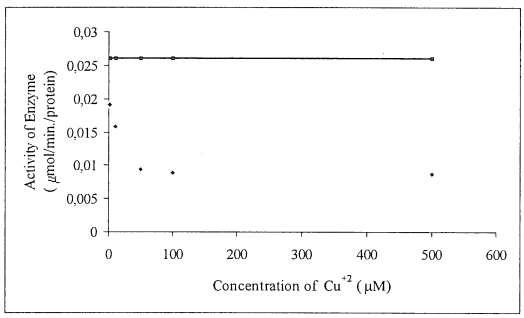

Fig. 4. Glutathione reductase activity for Cu^{+2} ion (—— Control group, ♦ Test group).

The lethal toxicities of the four pollutants 3,4-dichloroaniline (DCA), atrazine, copper, and lindane were determined for the 2nd larval instar of the insect *Chironomus riparius* Meigen and the juvenil stage (2nd or 3rd moult) of the crustacean *Gammarus pulex* (L.). Median lethal concentrations (LC50) were determined over a 240 h test period. The order of toxicity of the test

chemicals is different for each species. *For C. riparius*, lindane was the most toxic, followed by copper, DCA and atrazine. During the first 96 h of exposure, the order for *G. pulex* was copper, lindane, then DCA and atrazine with similar LC50 values. Howewer, at 240 h lindane replaced copper as the most toxic chemical to *G. pulex* (Taylor E.J. et. al, 1991).

The glutathione reductase activity at the *Chlamydomonas reinhardtii* at 49°C and pH: 8.2 was found as maximum. No recycled reaction was observed with GSH and $NAD(P)^+$ in the internal of pH: 4.8-8.2 (Takeda T. et. al, 1993).

The optimum pH was detected as 7,0 in the tests carried out on rats for glutathione reductase (Carlberg I., Mannervik B., 1985).

The optimum pH of glutathione reductase in *Gammarus pulex* was found as 7.0-7.2 (Figure 5).

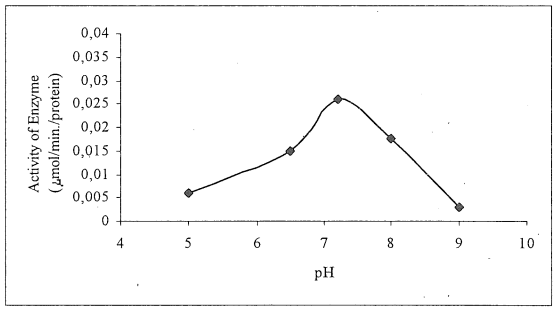

Fig. 5. The activity of Glutathione reductase in different pH.

Km values of glutathione reductase enzyme for NADPH and GSSG at *Chlamydomonas reinhardtii* were determined successively as 10,6 µM and 54,1 µM (Takeda T. et. al, 1993).

The 1/V vs 1/[S] graphic was drawn according to different NADPH amounts of glutathione reductase at *Gammarus pulex* and 129,03 was found as its K_m value (Figure 6).

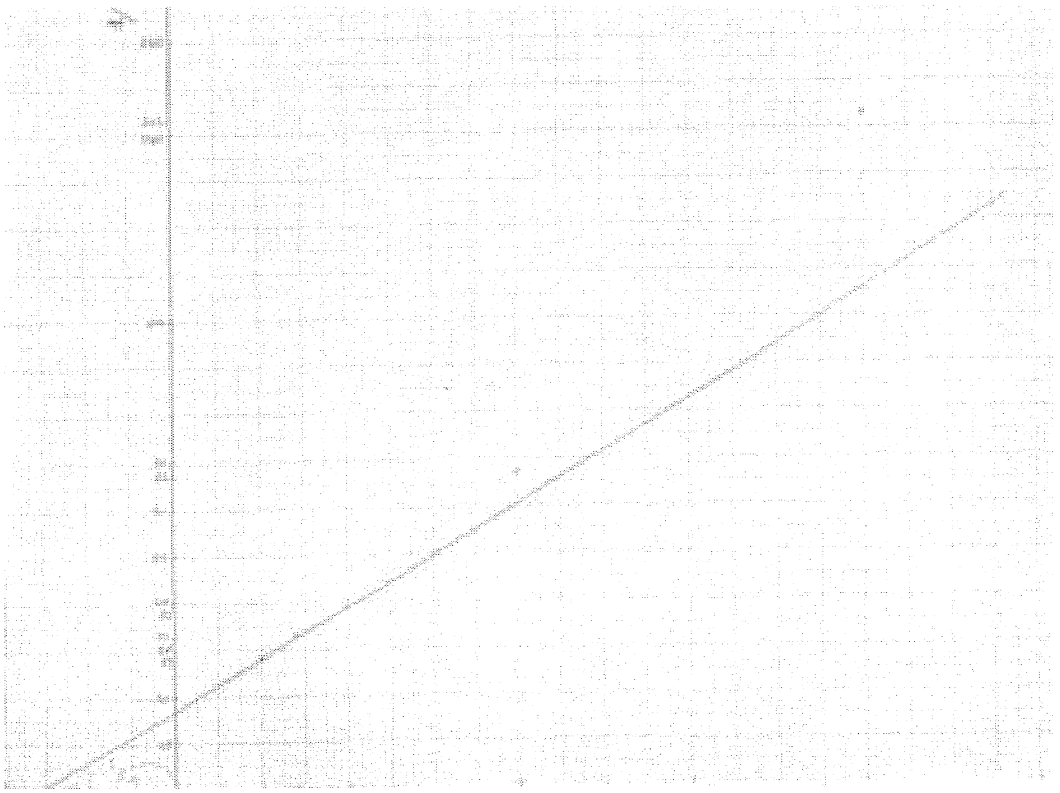

Fig. 6. Km graphic; 1/v vs 1/[S]

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