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Some novel features of glutathione transferase from juvenile catfish (*Clarias gariepinus*) exposed to lindane-contaminated water ☆

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Abstract
Catfish are hardy in nature and it is not known whether the presence of efficient detoxication enzymes is partly responsible for this trait. To investigate this, we have assessed induction of glutathione transferase (GST) in 10-week-old juvenile catfish (*Clarias gariepinus*) exposed to graded concentrations of lindane, an organochlorine insecticide, and characterised the purified enzyme from groups having the highest and statistically significant induction. Some of the unique properties observed for the purified enzyme are a high K_m (1.72 ± 0.21 mM) for the electrophilic model substrate, 1-chloro-2,4-dinitrobenzene (CDNB) and a very low catalytic rate ($V_{max} = 0.130 \pm 0.010$ units/mg protein). The k_{cat}/K_m being $55.4 \pm 0.2\ M^{-1} s^{-1}$. The enzyme is present in high concentration in the organism, the main isoform accounts for about 5.6% of the total soluble protein, probably to compensate for the observed kinetic imperfection. Since these properties are generally not known for a detoxication enzyme, we suggest that they may form part of the organism's own adaptation to its polluted environment.

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Contents

Introduction

Glutathione transferases, GSTs (E.C. 2.5.1.18) are phase II detoxication enzymes and are widely distributed in living organisms wherein they perform catalytic and noncatalytic functions. The noncatalytic properties of GST include among others, their roles in the binding and transport of some metabolites, e.g., steroids, haem, drugs, albumin, etc. (Lo and Ali-Osman, 2007; Yamamoto et al., 2011). The detoxication reactions (Scheme 1) often involve the conjugation of xenobiotic with the endogenous tripeptide, glutathione.

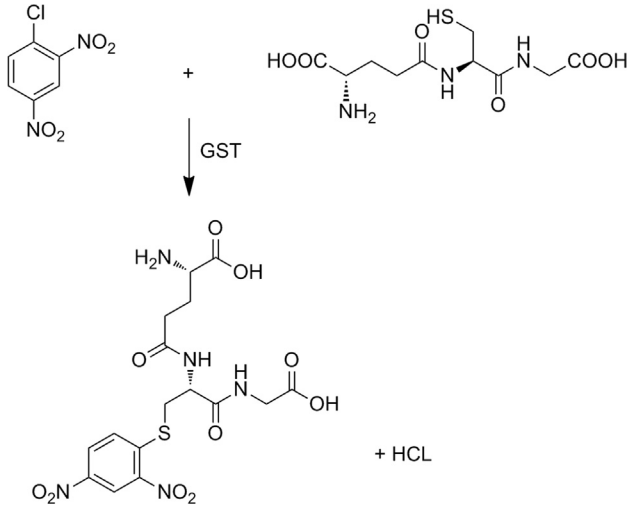
In principle, the conjugation ensures that the xenobiotic becomes more water soluble and this makes it easier to be removed from the body of the organism via mercapturic acid pathway (Lo and Ali-Osman, 2007; Yamamoto et al., 2011). In Scheme 1, the CDNB is a typical model substrate that is commonly used to assay for GST.

However, other model substrates also exist but organisms encounter herbicides, pesticides, chemical pollutants, drugs, etc. as their real xenobiotic substrates (Adewale and Afolayan, 2004). Part of the adaptation of living organisms to life includes the possession of efficient detoxication mechanisms. Such organisms with well-developed enzymes of detoxication are expected to be able to cope with a polluted environment (Sheehan et al., 2001).

When organisms encounter foreign chemical compounds, detoxication enzymes are often induced unlike housekeeping enzymes which are present in organisms at more or less the same level all the time. In the case of aquatic contamination, it has been suggested but not thoroughly investigated whether the induction of detoxication enzymes like GST for instance, could be used to measure the level of aquatic pollution (Havelková et al., 2008). Part of the problem includes the species-dependent nature of induction and the non-availability of studied organisms in other ecosystems.

It is therefore necessary to further investigate whether there is a connection between the level of pollution and the induction of enzymatic activity in an organism like catfish which are found in many parts of the world. Pollutants of interest in developing countries include organochlorine pesticides like lindane (γ-hexachlorobenzene). They have received the most attention partly because of the abuses to which they are subjected to and also due to their persistency in the environment which have resulted in many socio-economic and health-related challenges (Pesce et al., 2008).

In addition to their wide distribution, catfish endure polluted ponds, streams, or rivers better than many other species of fish. It was thought that the hardiness could be due to efficient detoxication enzymes like GST. We provide here some basic information on purified GST from catfish exposed to lindane as a first part of an ongoing investigation.



Scheme 1 GST-catalysed conjugation of 1-chloro-2,4-dintrobenzene (CDNB; the model substrate) with glutathione (GSH), forming S-2,4-dinitrophenylglutathione.

Response to exposure, enzyme induction and protein separation

There was no mortality in eight weeks old catfish exposed to less than 1 ppm of lindane. Mortality of 30%, 60% and 90% was recorded at 2.0, 3.0 and 4.0 ppm of lindane, respectively after 24 h of exposure. From the dose-response curve (Figure 1a), 24 h LC_{50} value of 2.7 ppm was obtained.

GST was induced in the treated fish when compared with the control group (Figure 1b). Statistical analysis showed that only the group of fish exposed to 2.0 ppm of lindane gave the highest GST expression which was significantly different from the control group. A comparison of the chromatographic profiles showed that exposure of juvenile catfish to lindane did not result in the formation of new isoenzymes, rather more quantity of the same isoenzymes were induced.

Two main activity peaks (Figure 2) were seen in both the control and the treated group. The amount of protein in A being four-times higher than the other. The activity of A is about 18 times higher than the activity of B. When A was