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Characterization of two glutathione s-transferases from disk abalone (*Haliotis discus discus*)

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Introduction:

Glutathione S-transferases (GST) are a family of multi-functional isoenzymes that are widely found in prokaryotics and eukaryotics. GSTs mainly catalyze the conjugation of tripeptide glutathione (GSH) to a wide variety of electrophilic molecules of endogenous or exogenous origin. GSTs are active as dimers of either homogeneous or heterogeneous subunits. On the basis of primary and tertiary structure, substrate/inhibitor specificity and immunological cross-reactivities, cytosolic GSTs have been divided into over 10 different classes such as alpha, mu, pi and Omega *etc* (Hayes *et al.*, 1999).

In recent years, mollusks have been proposed as potential bioindicator species. They can be induced an increasing of GST expression and activity after exposure to heavy metals, organophosphorus pesticides or solvents. In mussels, GST expression is not affected by several abiotic and biotic factors such as temperature, season, sex, or age. Therefore, the induction of GST activity has been proposed as a potential indicator of exposure to environmental pollutants for mollusks. In the present study, two different glutathione s-transferases cDNA from disk abalone (*Haliotis discus discus*) were cloned and expressed in *E.coli* as a MBP fusion protein. Their activities of glutathione s-transferases were characterized using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate.

Materials and methods

Two cDNA clones HdGSTM1 and HdGSTO1 with expected function of glutathione s-transferase were selected from abalone cDNA library, with identities of 60% to mu class and identities of 50% to omega class, respectively. The plasmid DNA of these two putative GSTs were digested and subcloned into expression vector, pMAL-c2X. The recombinant enzymes were overexpressed in *E.coli* K12 (TB1) cells in the presence of IPTG and were purified using amylase resin. Purified proteins were

checked by 10% SDS-polyacrylamide gel electrophoresis (PAGE). GST activity was measured using CDNB and reduced GSH as substrates according to Habig *et al.*, (1974). The apparent K_m and V_{max} values were measured by Lineweaver-Burk method. Protein concentrations were calculated by the method of Bradford.

Results and summary

I. SDS-PAGE showed that the molecular weights of two clones were both 25 kDa approximately, which is matched with prediction result.

II. Phylogenetic analysis showed that HdGSTM1 and HdGSTO1 were placed in class mu and class omega, respectively with strong boot strap.

III. Both the alignment and three dimensional structure showed that N-terminal domains of HdGSTM1 and HdGSTO1 were very conserved structures, which were Thioredoxin-like structure. Both HdGSTM1 and HdGSTO1 showed activity towards CDNB, which are only 0.21 μmol/min/mg and 0.13 μmol/min/mg, respectively. The optimum pH of HdGSTM1 and HdGSTO1 were 8.0 and 9.0, respectively. The optimum temperature of HdGSTM1 and HdGSTO1 were 35℃ and 30℃, respectively. Both HdGSTM1 and HdGSTO1 showed wide range of pH stability and thermostability.

References:

Hayes J. D., and L. I. McLellan. 1999. Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. Free Radic Res 31(4),273-300.

Habig, W. H., M. J. Pabst and W.B. Jakoby. 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation, J. Biol. Chem. 249, 7130-7139