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Isolation of selenium dependent glutathione peroxidase from disk abalone (*Haliotis discus discus*)

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Introduction

Reactive oxygen species (ROS) are critical deleterious compounds produced by various metabolic reactions in aerobic organisms. These compounds generate oxidative stress leading to detrimentaleffect on cell constituents like lipids, proteins and DNA. Hence, the prevention of harmful peroxidation is an essential process in all the aerobic organisms. There are several antioxidant defensemechanisms existed, including α-tocopherol, ascorbic acid, glutathione and β-carotene like non-enzymatic system and enzymatic antioxidant system depends on manganese superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx). GPx plays a main role via the glutathione system to protect the cells from oxidative damage by catalyzing the reduction of organic hydroperoxides, lipid hydroperoxides (ROOH) and hydrogen peroxide to lipid alcohol and waterin the presence of glutathione (Ho and Howard, 1992). GPx are selenoenzymes containing rare amino acid (selenocystein) with selenium (Se) that has been recognized as a key component of the active site (Schuklet *et al.*, 1991).

This study was conducted to isolate an antioxidant enzyme - glutathione peroxidase from the cDNA library constructed from disk abalone (*Haliotis discus discus*) digestive gland.

Materials methods

The cDNA fragment encoding GPx was selected from the abalone cDNA library and sequenced using internal primer 5'- CAGGACACTACTGGAGGAGAG -3' and poly (T) primer. The homogeneity of the full length sequence and the open reading frame was analyzed using the NCBI BLAST X program. Multiple sequence alignment was performed using CLUSTAL W program and the phylogenetic analysis was conducted by Neighbor-Joining method in MEGA 3.0 program.

Results and summery

The 1477 bp of full length sequence displayed 51% and 46% similarity to selenium dependent salivary glutathione peroxidase of black legged tick (*Ixodes scapularis*) and glutathione peroxidase of house rat (*Mus musculus*) respectively. 675 bp of coding sequence encodes a protein containing 225amino acids where 74th position indicates selenocystein. Selenocystein encoding TGA is the typical stop codon, which terminates the protein. To continue the amino acid encoding of the whole coding sequence, two RNA structures, the mRNA selenocystein insertion sequence (SECIS element) and a unique selenocysteyl tRNA are required.

In addition to this functional group, another six cystein residues are scatteredmainly in the N-terminal domain, which are responsible for the antioxidant activity. Although functionally related with members of the glutathione peroxidase clade, phylogenetic analysis of the abalone GPx showed the greatest identity (81%) with selenium dependant glutathione peroxidase of black legged tick (*Ixodes scapularis*).

Reference

Ho Y.S. and Howard G.J. (1992). Cloning and characterization of the rat glutathione peroxidase gene. *Federation of European Biochemical Societies.* 301: 5-9.

Schuckelt R., Brigelius-Flohe R., Maiorino M., Roveri A., Reumkens J., Strassburger W., Ursini F., Wolf B. and Flohe L. (1991). Phospholipid hydroperoxide glutathione peroxidase is a selenoenzyme distinct from the classical glutathione peroxidase as evident from cDNA and amino acid sequencing. *Free Radical Research Communication.* 14: 343-361.