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Characterization of Cu, Zn-Superoxide dismutase from disk abalone (*Haliotis discus discus*)

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Introduction

Superoxide dismutase (SOD) are metalloenzymes in aerobic organisms, which play a crucial role in protecting organisms against the toxic wastes caused by reactive oxygen species (ROS), in particular superoxide radicals (O2-). It catalyses the dismuation of superoxide radicals to molecular oxygen and hydrogen peroxide. SODs are classified into several forms and can be distinguished by their primary structure, cellular compartmentalization, primary function and the metal required for activity (Wright *et al.* 2002). Three distinct groups depending on the metals identified in their active sites are Cu,Zn-SOD, Mn-SOD and Fe-SODs are widely distributed in eukaryotes, where as Mn-SOD and Fe-SOD enzymes are predominantly found in mitochondria or prokaryotes (Fridovich, 1986).

Coding sequence for Cu,Zn-SOD of *Haliotis diversicolor supertexta* is available. However, limited studies on cloning of Cu,Zn-SOD from Molluscs led us to clone, sequence and express aCu,Zn-SOD in *E. coli* and compare with other known Cu,Zn-SOD sequences.

Materials and methods

A clone with expected function of Cu,Zn-SOD was selected from disk abalone cDNA library. The full length sequence was determined by sequencing reactions from 3′ end using oligo dT primer. After deriving the full length, the sequence was compared against the National Center for Biotechnology Information (NCBI) databases.

The coding region with the signal peptide was amplified with two design primers and ligated into pMAL c2× (New England Biolabs, USA). The recombinant clone was transformed into *E. coli* K12 (TB1) and produced recombinant protein by IPTG

(Isopropyl-D-thiogalactopyranoside). Recombinant protein was analyzed using 12 % SDS polyacrylamide gel electrophoresis. Xanthine oxidase method was used to determine the activity of aCu,Zn-SOD. Further, optimal pH and temperature were also examined.

Results and summary

The full length of aCu,Zn-SOD contained 1027 bp, with an ORF of 465 bp coding for 154 amino acids with a pI value of 5.5. The expression of gene in *E. coli* K12 (TB1) resulted in a soluble protein of 16 kDa. The purified protein exhibited 2461 unit/mg activity when induced with 0.5 mM of IPTG. The optimum temperature of the enzyme was 37℃ and it was active in a rage of acidic pH from 3.5 to 6.5. The enzyme was heat inactivated after 70℃. When compared with other Cu,Zn-SOD, it was revealed that 48 amino acid residues were conserved in all species. Based on the structural analysis, it was found that 4 amino acid residues (Gly45, Gly62, Pro75 and Gly83) were conserved.

References

Wright, A. C., H. Ahmed, J. D. Gauthier, A. M. Silva and G. R. Vasta. 2002. cDNA cloning and characterization of two iron superoxide dismutase from the oyster parasite Perkinsus marinus. Mol. Biochem. Parasitol. 123, 73-77.

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