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Molecular cloning, expression and characterization of Mn-superoxide dismutase from disk abalone (*Haliotis discus discus*)

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Introduction

SODs are classified into four groups depending on their metal cofactors namely cytosolic CuZn-SOD containing copper and zinc, Mn-SOD containing manganese, Fe-SOD containing iron and Ni-SOD containing nickel. The mitochondrial enzyme manganese-superoxide dismutase (Mn-SOD) is one of the antioxidant enzymes involved in cellular defense against oxidative stress and catalyzes the conversion of $O_2^{\cdot-}$ into the stabler H_2O_2. Mn-SOD is particularly important as it is located in mitochondria and represents the first line of defense against superoxide radicals produced as byproducts ofoxidative phosphorylation (Beyer *et al.*, 1991). Mn-SOD has been shown to play a major role in promoting cellular differentiating and tumorgenesis (St.Clair *et al.*, 1994) and in protecting cells against hyperoxia-induced pulmonary toxicity.

In this study, we cloned cDNA for Mn-SOD from disk abalone (*Haliotis discus discus*) and expressed and characterized the protein. The isolated sequence was compared with other Mn-SODs available in the public database and attempts were made to build structure-functional relationship of the amino acid sequence.

Materials and methods

A clone with expected function of Mn-SOD was selected from disk abalone cDNA library. The full length sequence was determined by sequencing reactions 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

A putative gene encoding Mn-SOD from disk abalone (*Haliotis discus discus*) (aMn-SOD) was cloned, sequenced, expressed in *E. coli*. Sequencing resulted ORF of 682 bp, which corresponded to 226 amino acids. The protein was expressed in soluble form with molecular weight of 25 kDa + 42.5 kDa maltose binding protein. The predicted Isoelectric point of aMn-SOD was 6.5 and activity of the fusion protein was 2781 U/mg. The optimum temperature of the enzyme was 37 ℃ and it was active in a range of acidic pH from 3.5 to 6.5. The enzyme activity was reduced to 50% at 50 ℃ and completely heat inactivated at 80 ℃. The alignment of aMn-SOD amino acid sequence with known Mn-SODs revealed that the enzymes are conserved among animals with higher than 30% identity. In comparison with human Mn-SOD, all manganese-binding sites are also conserved in aMn-SOD (H28, H100, D185 and H189). aMn-SOD amino acid sequence was closer to that of *Biomphalaria glabrata* in phylogenetic analysis.

References

Beyer, W., Imlay, J. and Fridovich, I. 1991. Superoxide dismutases. Prog. Nucleic Acid Res. Mol. Biol. 40, 221-253.

St.Clair, D.K., Oberley, T.D., Muse, K.E., StClair, W.H. 1994. Expression of manganese superoxide dismutase promotes cellular differentiation. Free Radic. Biol. Med. 16(2), 275-282.