CHARACTERIZATION OF HUMAN SUPEROXIDE DISMUTASE 1 
OVEREXPRESSED IN THE METHYLOTROPHIC YEAST PICHIA PASTORIS

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Introduction and Objectives:
The cytosolic Cu,Zn-superoxide dismutase 1 (SOD1) enzyme dismutates superoxide to oxygen and hydrogen peroxide, and displays a bicarbonate-dependent peroxidase activity. Mutations identified in human SOD1 (hSOD1) have been implicated in the development of amyotrophic lateral sclerosis (ALS). Similar to other neurodegenerative diseases, protein aggregation appears to contribute to disease pathogenesis. Our group and others have demonstrated that the bicarbonate-dependent peroxidase activity of hSOD1 can lead to oxidation of Trp32 and promotes aggregation. To circumvent some of the problems associated with SOD1 overexpression in E. coli and lengthy purification process we have utilized the yeast Pichia Pastoris to overexpress and secrete wild-type hSOD1 (hSOD1WT) and mutants hSOD1G93A, hSOD1W32F, and hSOD1W32F/G93A.

Materials and Methods:
The hSOD1WT gene was subcloned into the pPICZαA expression vector with a cleavable 6-histidine tag. Mutations were made using whole plasmid mutagenesis. Plasmids were transformed into P. Pastoris X33 cells by electroporation. Multicopy transformants were selected by growth on medium containing 1 mg/mL Zeocin. Clones were screened for hSOD1 expression, and hSOD1 was purified using affinity chromatography. Enzyme purity was assessed by SDS-PAGE. Metal content was determined spectrophotometrically using the PAR assay, superoxide dismutase activity was monitored spectrophotometrically using the cytochrome c method, and bicarbonate-dependent peroxidase activity was measured spectrophotometrically following DHR123 oxidation.

Results and Conclusions:
The overexpression of hSOD1WT in P. pastoris yields ~10 mg/L that can be rapidly purified to greater than 95% purity by affinity chromatography. The as-isolated enzyme contains nearly stoichiometric amounts of copper and zinc, displays a superoxide dismutase activity of ~4000 U/mg, and a bicarbonate-dependent peroxidase activity of 0.01 U/mg. We have also successfully overexpressed mutants hSOD1G93A, hSOD1W32F, and hSOD1W32F/G93A. We conclude that P. pastoris is a viable alternative to the bacterial expression system and will prove useful in unraveling the mechanism of protein aggregation.

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Key Words: superoxide dismutase 1, Pichia pastoris, amyotrophic lateral sclerosis

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