E315 Identification of Sulfur ligands of NiSOD from *S. seoulensis*using site directed mutagenesis

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S. seoulensis SOD is an α_4 tetramer composed of 13 kDa-subunit and contains one Ni atom per subunit. X-ray absorption spectroscopic studies coupled with EPR of the Ni center show that the Ni in the oxidized enzyme is in a five-coordinate site composed of three S-donor ligands, one N-donor, and one other O- or N-donor. The sequence reveals that there are two cysteine residues (Cys16 and Cys20) and one methionine residue (Met42) in the amino acid sequence of the mature enzyme. Site directed mutagenesis by PCR was used to prove the identity of S-donor ligands in genetic level. Two cysteine residues near the N-terminus was changed to serine (C16S, C20S). The recombinant and mutant proteins of C16S, C20S were overexpressed in *sodN*-disrupted *S. coelicolor* as detected by Western hybridization analysis, but the mutant enzymes showed no detectable activity of NiSOD. Met42 considered as the only other S-donor ligand in the enzyme was substituted to serine (M42S) and cysteine (M42C). While M42S almost did not show the enzyme activity, M42C showed the activity of the similar level to the SOD-overexpressing strain. the experimental results suggest that NiSOD may be have mononuclear nickel-center, rather than dinuclear center.

E316 Physiological characterization of NiSOD from *Streptomyces seoulensis* and overexpression of *sodN* in *E. coli*, *S. coelicolor* and *S. seoulensis*

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The activity of NiSOD in *S. seoulensis* was increased when the cells enter into the exponential phase and in differentiaional processes in liquid culture and in plate culture, respectively. Western hybridization analysis showed that the increase in SOD activity was consistent with the increase in protein concentrations, especially in the concentration of the processed form. SOD activity and protein concentrations were dependent on the added nickel and were saturated at about 200 μM-added nickel. At more higher concentration, growth inhibition was observed. Also, in Bennett plate, differentiational retardation was observed at the nickel concentration of SOD induction. In addition of EDTA or 8-hydroxyquinoline, the decrease of SOD was observed not only enzyme activity but also protein concentrations. NiSOD overexpression in *E. coli* was not active regardless of the addition of nickel in medium, but immunologically reactive with anti-NiSOD antibody. To test functional expression of *sodN in vivo*, *sodN* was cloned in pIJ702 (pIJSODN) and overexpressed in *S. coelicolor* and *S. seoulensis*. Activity staining and Western hybridization showed that nickel incorporation into protein is a limiting step for producing of the active enzyme and *sodN* expression was regulated by nickel. The pIJSODN-containing *S. seoulensis* was different from wild type strain on R2YE medium in colony morphology.