

C301 Characterization of Nickel-ligands in NiSOD
from *Streptomyces seoulensis* by EPR Spectroscopy

Jin-Won Lee*, Yang-In Yim, In-Kwon Kim, Hwan Youn,
and Sa-Ouk Kang

*Laboratory of Biophysics, Department of Microbiology, College of
Natural Sciences and Research Center for Molecular Microbiology,
Seoul National University*

The nickel-containing superoxide dismutase(NiSOD) has been purified from cells of the *Streptomyces seoulensis* grown on a medium containing several stable nuclear spin isotopes (^{61}Ni , ^{15}N , ^{33}S) and characterized by EPR spectroscopy. The nuclear spin ($I=3/2$) of ^{61}Ni induced well-resolved hyperfine structure in the EPR spectra of the purified enzyme, unambiguously identifying the observed signal as a Ni(III) species. The nuclear spin ($I=1/2$) of ^{15}N induced doublet instead of original triplet hyperfine structure, identifying the g_z triplet was originated from the nitrogen atom. And the enzyme prepared from the ^{14}N -histidine and ^{15}N -Ammonium sulfate enriched media showed normal g_z triplet, implying that the g_z triplet was due to histidine nitrogen. The nuclear spin ($I=3/2$) of ^{33}S induced apparent line broadening in the g_z region, strongly suggesting that one or more S atoms are also act as Ni ligand. And the investigation and simulation of EPR spectra of enzymes prepared from the ^{32}S -methionine, ^{32}S -cysteine and ^{33}S -sulfate showed possibility that the methionine as well as cysteine can acts as a sulfur ligand.