C18

Structural Characteristics of NiSOD from *Streptomyces seoulensis*

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The heterologous expression of *sodN* gene from *Streptomyces seoulensis* in *Streptomyces lividans* together with the gel filtration and sedimentation equilibrim data indicated that the quaternary structure of NiSOD is homohexamer, which is novel among SODs, not the previously reported homotetramer. The EPR spectrum of ^{61}Ni (I = 3/2) substituted NiSOD showed a clear resolved hyperfine structure at g = 2.016, unambiguously identifying that the EPR signal from NiSOD is due to Ni. When the EPR spectrum was taken from the ^{15}N (I = 1/2)-enriched NiSOD, the three prominent lines in the g_z region of native NiSOD was changed to two prominent lines, indicating that the original triplet was originated from ^{14}N (I = 1) superhyperfine splitting. The ENDOR and ESEEM spectroscopy indicated that the N-donor ligand is a N_ε of histidine imidazole. EPR spectrum of ^{33}S enriched NiSOD showd distinct line broadening in g_z region resulted from superhyferfine interaction with ^{33}S nucleus (I = 3/2), directly showing that sulfur act as a ligand for Ni. The crystal structure of NiSOD showed that NiSOD is a hexameric enzyme consisting of four-helix-bundle subunits. The hexamer exhibited a threefold symmetry axis with three two fold axes perpendicular to the threefold axis. The subunit structure which comprises 117 residues in the mature enzyme revealed a four-helix bundle in the canonical all-antiparallel topology. The crystal structures of the resting NiSOD revealed that each Ni(III) ion is coordinated by the amino group of His1, the amide group of Cys2, and two thiolate groups, Cys2 and Cys6.