

S K-Edge X-ray Absorption Spectroscopic Investigation of the Ni-Containing Superoxide Dismutase Active Site: New Structural Insight into the Mechanism

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Superoxide dismutases (SODs) protect cells from the toxic effects of reactive oxygen species derived from superoxide.¹ The enzymes remove superoxide by converting it to O₂ and H₂O₂, a disproportionation that requires a metal ion with a one-electron redox potential between that of the two half-reactions (~200 mV). Nickel-containing superoxide dismutases² (NiSODs) are novel members of the SOD family of metalloenzymes found in several *Streptomyces* species³ and cyanobacteria.⁴ Despite a highly similar catalytic rate constant and pH dependence,⁵ NiSOD is distinct from Mn-, Fe-, or Cu/Zn-containing SODs in amino acid sequence and metal ligand environment.^{5,6}

S. seoulensis and *S. coelicolor* NiSODs are homohexamers^{2,6} with subunits containing Cys residues in positions 2 and 6 that constitute two of the ligands involved in coordinating the nickel centers on the basis of Ni K-edge EXAFS.⁵ The remaining three ligands in the five-coordinate Ni(III) site include an axial N-donor responsible for the hyperfine observed by EPR. Upon dithionite reduction, an EPR-silent, four-coordinate Ni(II) site arises from the concomitant loss of the axial N-donor.

The EXAFS fits were consistent with either a mono- or binuclear active site, where the former featured methionine coordination. Recent studies on the M28L variant of *S. coelicolor* NiSOD revealed no change in activity or in the EPR spectrum due to the mutation, which argues against any significant catalytic or structural role of the Met28 residue.⁶ The fact that there are only three S-containing residues per subunit, the EPR spin quantitation, and the redox chemistry of model complexes⁷ supported a binuclear active site with bridging thiolates.

Sulfur K-edge XAS offers a direct probe of S coordination to metal centers. We report here the analysis of S K-edge spectra obtained from a series of models with various S ligands and from NiSOD samples.⁸ These studies elucidate the types of Ni–S interactions found in oxidized and reduced NiSOD. The results clearly indicate the presence of terminal and the absence of bridging thiolate bound to Ni(III) in the oxidized enzyme, and the absence of terminal thiolate coordination to Ni(II) in peroxide-reduced samples. The striking change in the S ligands in oxidized and reduced protein samples suggests a new mechanistic role for the thiolate(s).

The schematic structures of model compounds are displayed in Figure S1 (Supporting Information). The S K-edge spectra of octahedral and four-coordinate planar and tetrahedral mononuclear Ni(II/III)–thiolate complexes are shown in Figure 1A. The pre-edge features (2469–2472 eV) are resolved, and each is characteristic of a specific type of Ni–S interaction. These features involve

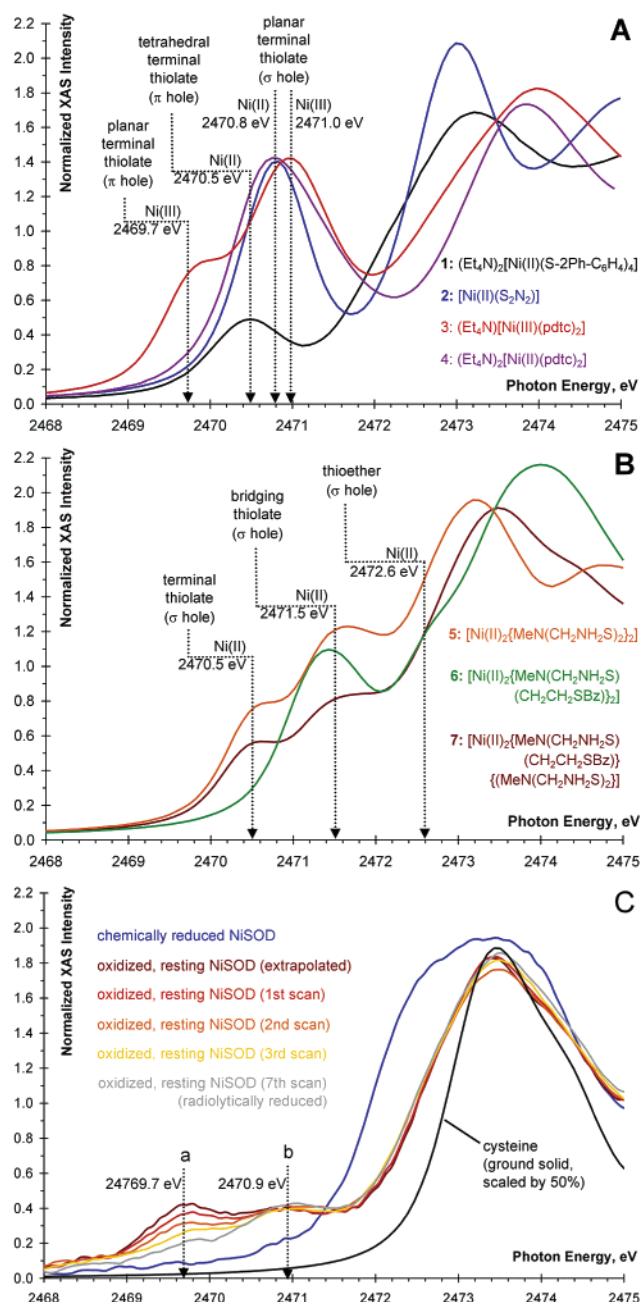


Figure 1. S K-edge spectra of (A) mononuclear and (B) binuclear model complexes, and (C) oxidized and reduced NiSOD protein samples.

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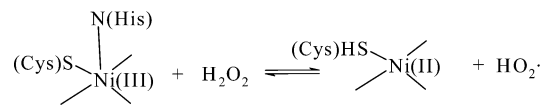
transitions originating from the S 1s orbital of terminal thiolate ligands (S^t) to the unoccupied, antibonding combination of a S-based ligand and Ni orbitals. There are two such orbitals resulting from out-of-plane and in-plane S 3p components. In Ni(III), the additional electron hole is in an out-of-plane, singly occupied Ni–S orbital (also probed by EPR) and gives rise to a transition at 2469.7 eV in **3** that is not observed for the analogous Ni(II) complex **4**. The corresponding transition involving the in-plane Ni(III)–S^t orbital occurs at 1.3 eV higher energy (2471.0 eV). The pre-edge features corresponding to transitions into in-plane Ni(II)–S^t orbitals in **2**, **3**, and **4** appear at a similar energy. These intense pre-edge features correspond to high covalency⁹ and thus the extent of ligand participation in the redox chemistry. The Ni(II)–S^t bond in complex **1** shows a significantly weaker pre-edge feature at lower energy (2470.5 eV) due to the difference in covalency between tetrahedral and tetragonal Ni(II) complexes.¹⁰

In the binuclear complexes (Figure 1B), the pre-edge features (2470–2473 eV) overlap more, but they still can be uniquely assigned. The pre-edge feature at 2470.5 eV is present in **5** and **7** and absent in **6**; thus, it can be unambiguously assigned to the Ni(II)₂–S^t interaction. This pre-edge feature is shifted down in energy by ~0.3 eV in the binuclear complexes compared to the mononuclear Ni(II) complexes, due to the lowering of the Ni 3d manifold energy that results from a weaker ligand field. All three binuclear compounds show a pre-edge feature at around 2471.5 eV, which can be assigned to the Ni(II)-bridging thiolates (Ni(II)₂–S^b). The energy of this feature varies the most (±0.2 eV) among all the Ni–S bonds studied, which can be explained by its weaker bonding to two Ni centers compared to the Ni–S^t bonding. As expected from the increased effective nuclear charge of a thioether-S relative to a thiolate-S, the pre-edge features for Ni(II)–thioether (Ni(II)₂–S^e) bonds appear at the higher energy (~2472.6 eV, most resolved in **6**) and greatly overlap the rising-edge features (S^t 1s → C–S σ* transitions).

The S K-edge spectra obtained for resting oxidized NiSOD shows two pre-edge features (a, 2469.7 eV and b, 2470.9 eV, Figure 1C) that are consistent with terminal thiolate(s) bound to Ni(III) (see above, peak b can have a small Ni(II)–S^t contribution). The lower energy feature gradually disappears with continuous X-ray beam exposure as the Ni(III) center is photoreduced. This process, similar to radiolytic reduction of NiSOD by a ⁶⁰Co source (i.e., solvated electron), gives a strikingly different S K-edge spectrum (gray line) from peroxide-reduced NiSOD (blue line). The pre-edge feature around 2470.9 eV for the radiolytically reduced active site is consistent with the presence of a Ni(II)–S^t bond. The presence of a thiolate-bridged binuclear site in the resting form can be ruled out due to the absence of characteristic Ni₂–S^b features at 2471.5 and 2470.2 eV for the Ni(II) and Ni(III) (est. from Figure 1A) sites, respectively.

The spectrum of the peroxide-reduced NiSOD (blue line, Figure 1C) shows a broad, structureless S K-edge.¹¹ The intensity at 2470.9 eV is significantly decreased from that of the photoreduced form, indicating that there is less Ni(II)–S^t interaction in the peroxide-reduced form. The broad rising edge feature between 2471.8 and 2472.8 eV could correspond to a Ni(II)₂–S^b or a Ni(II)₂–S^e bond. The latter can be ruled out, as the M28L variant shows no structural or reactivity difference,⁶ and the presence of a bridging thiolate is not likely, as this is absent in the oxidized form. Alternatively, this broad feature would be consistent with the presence of protonated thiolate(s) (black line, Figure 1C) coordinated to the Ni(II) ion. Electron donation from the Cys-SH ligands to the Ni would give rise to a pre-edge feature at about 2472.6 eV, similar to that of the Ni(II)₂–S^e bond.

Scheme 1. Proposed Mechanism for Peroxide/Superoxide Redox Reaction of NiSOD



Scheme 1 illustrates a proposed mechanism for the superoxide/peroxide redox reaction based on the S K-edge XAS results. In the oxidized resting form, the Ni(III) sites have terminal thiolate(s). Upon peroxide reduction, an electron is transferred to the Ni(III) site and the terminal thiolate is protonated, providing an efficient mechanism for proton-coupled electron transfer. Similar roles for Ni–S(Cys) protonation/deprotonation in determining the redox potential of the Ni center and as a proton source for catalysis have been suggested by computational studies of NiFe hydrogenases.¹²

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Supporting Information Available: Schematic structures of models (Figure S1), second derivative spectra of binuclear complexes (Figure S2), and summary of transition energies (Table S1). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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