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Novel Tripeptide Model of Nickel Superoxide Dismutase

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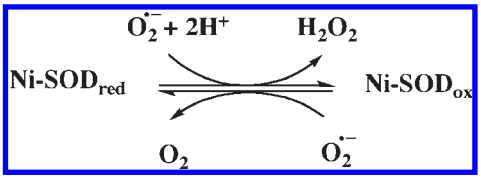
Nickel superoxide dismutase (Ni-SOD) catalyzes the disproportionation of superoxide to molecular oxygen and hydrogen peroxide, but the overall reaction mechanism has yet to be determined. Peptide-based models of the 2N:2S nickel coordination sphere of Ni-SOD have provided some insight into the mechanism of this enzyme. Here we show that the coordination sphere of Ni-SOD can be mimicked using the tripeptide asparagine−cysteine−cysteine (NCC). NCC binds nickel with extremely high affinity at physiological pH with 2N:2S geometry, as demonstrated by electronic absorption and circular dichroism (CD) data. Like Ni-SOD, Ni-NCC has mixed amine/amide ligation that favors metal-based oxidation over ligand-based oxidation. Electronic absorption, CD, and magnetic CD (MCD) data collected for Ni-NCC are consistent with a diamagnetic Ni^{II} center bound in square-planar geometry. Ni-NCC is quasi-reversibly oxidized with a midpoint potential of 0.72(2) V (vs Ag/AgCl) and breaks down superoxide in an enzyme-based assay, supporting its potential use as a model for Ni-SOD chemistry.

The radical species superoxide ($O_2^{\bullet -}$) and its reactive downstream products are known to cause oxidative damage to biological molecules, and the presence of superoxide in the body has been linked to many different diseases.[1] Superoxide dismutases (SODs) are oxidoreductases that catalyze the disproportionation of superoxide to hydrogen peroxide and molecular oxygen, therefore helping to protect biological systems from oxidative damage. These enzymes, classified by their metal center, include Cu/Zn-, Fe-, Mn-, and Ni-SODs.[2]

Ni-SOD is the most recently discovered form of the enzyme.[3,4] Its active site contains a mononuclear nickel center[5] coordinated in square-planar geometry at the N terminus of the enzyme via two cysteine sulfurs (Cys-2 and Cys-6), an amine nitrogen from the N terminus, and a deprotonated, anionic amide nitrogen from the peptide backbone (Cys-2).[6,7] Ni-SOD reacts with superoxide in a two-step reaction, involving oxidized and reduced forms of the enzyme (Scheme 1).[3,6−8]

Scheme 1. Reaction Catalyzed by Ni-SOD



As part of the redox reaction, the geometry of the bound nickel switches from square-planar Ni^{II} to square-pyramidal Ni^{III}. An additional ligand, an axial nitrogen donor, coordinates Ni^{III} in the resting state,[3] and the X-ray crystal structure revealed this species to be the imidazole nitrogen of His-1. Upon reduction of the enzyme, the histidine imidazole rotates away from the active site, leaving Ni^{II} coordinated in a 2N:2S, square-planar geometry.[6,7,9]

While much has been learned about the structure of the enzyme, only recently have studies begun to reveal more about the influence of the secondary coordination sphere.[10,11] While recent studies support an inner-sphere mechanism,[12,13] the catalytic mechanism is not yet fully elucidated. In order to further explore these issues, it has been of interest to develop model systems that mimic the activity of this enzyme. Within Ni-SOD, the metal is coordinated by ligands found within the first six residues of the N terminus.[6,7] Synthetic models with square-planar, 2N:2S geometries have appropriate redox potentials to act as SOD mimics, yet these do not exhibit

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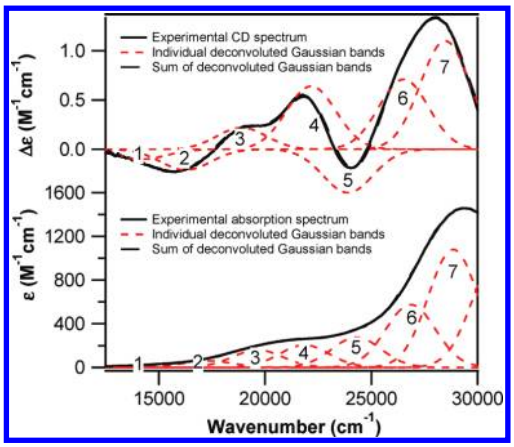

Figure 1. Deconvoluted CD (top) and absorption (bottom) spectra of 1.5 mM Ni-NCC in a 50 mM phosphate buffer, pH 7.4. Spectra were deconvoluted with Igor Pro (Wavemetrics).

Table 1. Comparison of Ni-NCC and Ni-SOD CD Transition Energies and Assignments. (Ni-SOD Values Were Measured by Fiedler et al.[21])

	transition assignment	Ni-NCC (cm^{-1})	Ni-SOD (cm^{-1})
1	Ni^{II} d−d	14 200	17 110
2	Ni^{II} d−d	16 270	18 430
3	Ni^{II} d−d	18 900	20 500
4	Ni^{II} d−d	22 170	22 240
5	S^{-}−Ni^{II} CT	23 900	24 970
6	S^{-}−Ni^{II} CT	26 520	27 650
7	S^{-}−Ni^{II} CT	28 475	29 220

SOD activity.[14−16] Peptide models based on the terminal sequence of the enzyme, however, have been shown to mimic SOD activity.[12,13,17−19] Here we present a novel tripeptide mimic, asparagine−cysteine−cysteine (NCC), that exhibits both quasi-reversible Ni^{II}/Ni^{III} transitions and SOD activity. NCC is unique because it is not derived from the sequence of the parent enzyme. Because of its small size, this tripeptide is likely to have better stability and a lower cost of production than larger peptide alternatives. Importantly, using an alternative simple model also has the potential to shed insight into the minimal geometric/electronic features necessary for SOD activity using nickel.

NCC binds nickel, apparently with extremely high affinity at physiological pH, and this system is thought to be a potential mimic of Ni-SOD based on similarities in coordination. To show the extent of incorporation of metal into the complex and assess changes in the protonation of the free versus bound peptide, electrospray ionization mass spectrometry (ESI-MS) operating in negative ion mode was used (Supporting Information). The data show full incorporation of the metal in a 1:1 ratio and suggest that no irreversible modifications to the peptide occur upon incorporation or release of the metal via acidification. These data further indicate that a tetra-deprotonated species binds the metal ion (Ni-NCC = 391.98).

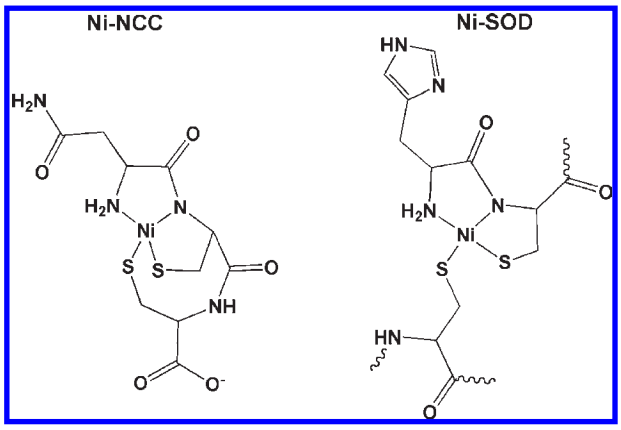

Figure 2. Metal coordination in Ni-SOD (right) and the proposed coordination in Ni-NCC (left). Wavy lines indicate the connection to the rest of the protein.

To probe the Ni^{II} geometry in more detail, electronic absorption, circular dichroism (CD), and variable-temperature magnetic CD (MCD) data were collected for Ni-NCC. Absorption spectra collected over a broad range of concentrations of the complex resulted in an increase in band intensity, indicating that there is one primary species present in solution. An iterative deconvolution of the absorption and CD spectra reveals seven electronic transitions between 14 000 and 29 000 cm^{-1} (Figure 1 and Table 1; Supporting Information).

With the exception of band 1, the energies of these transitions are within ∼1000−2000 cm^{-1} of those reported for Ni-SOD (Table 1), which is indicative of structural similarities between the Ni^{II} centers in these two systems. Bands 1−4 of Ni-NCC, which are relatively intense in the CD spectrum but carry only moderate absorption intensities, are assigned as the four d−d transitions expected for a square-planar Ni^{II} center. By analogy to Ni-SOD, we assign the higher-energy bands 5−7 as thiolate-to-nickel(II) charge-transfer (CT) transitions. Further support for the presence of a square-planar Ni^{II} center in Ni-NCC comes from the temperature independence of the MCD signals observed for Ni-NCC over the temperature range 2−15 K (Supporting Information). This behavior is indicative of a diamagnetic ($S = 0$) species, which is best rationalized in terms of a square-planar Ni^{II} center.[20]

On the basis of the data presented above, nickel is coordinated in a square-planar, 2N:2S geometry. In Ni-SOD, the thiolate ligands are cis and the nitrogen ligands include one amide and one amine.[21] Our current studies support a possible arrangement that parallels the coordination by Ni-SOD in which the two sulfur ligands are arranged cis and one nitrogen is derived from a backbone amide while the second is from the N-terminal amine (Figure 2). Density functional theory (DFT) computations[22] show that this proposed structure has the lowest total energy when compared with other potential structures with different nitrogen coordination environments and other cis and trans configurations (Supporting Information).

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To probe whether or not the Asn side chain is involved in Ni^{II} coordination, the tripeptide GCC was synthesized and transmetalated. The observation that the Ni-GCC spectra parallel those of Ni-NCC supports the structure proposed above, where the Asn side chain is not ligated to the Ni^{II} center. GGNCC and GGGCC peptides were also synthesized to further examine the identity of the nitrogen ligands in Ni-NCC. The spectral features of the pentapeptides are similar to each other but differ from those of the tripeptides (Supporting Information). This suggests the N-terminal amine participates in metal coordination in the tripeptides. Presumably, the N-terminal amine in the pentapeptides is too far removed from the Ni^{II} center to act as a ligand and the backbone amide nitrogen from the Asn substitutes for the terminal amine nitrogen to accomplish metal binding. Additionally, it is thought that mixed amine/amide coordination species are more stable to oxygenation than bis-amide species,[15] and the observation that Ni-NCC does not experience oxidation of the sulfur ligands is further evidence for the proposed mixed amine/amide coordination. Similarities in chemistry (vide infra) support the use of Ni-NCC as a model system for understanding the catalytic mechanism of Ni-SOD.

Because the studies above demonstrate that Ni-NCC serves as a reasonable structural mimic of Ni-SOD, the midpoint potential of Ni-NCC was measured using cyclic voltammetry (CV). CV data show that electron transfer is quasi-reversible, with a midpoint potential of 0.72(2) V versus Ag/AgCl (Supporting Information), which is similar to the 0.70(2) V measured for a peptide mimic based on the sequence of the parent enzyme with mixed amine/amide coordination and cis ligation ([$Ni(SOD)^{M1}$]).[17] Ni-SOD has a midpoint potential of 0.290 V (NHE; 0.093 vs Ag/AgCl).[10] The quasi-reversibility of the CV data indicates structural changes upon oxidation/reduction, which is rationalized in terms of the different geometries preferred by Ni^{II} and Ni^{III} centers. To confirm that electron transfer does not alter the peptide and the measured potential specifically reflects a change at the nickel ion, ESI-MS data were collected after bulk electrolysis. The mass is the same before and after oxidation, implying that the peptide is unaltered. Therefore, the redox potential measured corresponds to the Ni^{II}/Ni^{III} redox couple. Because the midpoint potential of the Ni-NCC complex falls between the reduction and oxidation potentials for superoxide, the ability of Ni-NCC to dismutate the reactive superoxide species was examined.

A standard SOD activity assay using xanthine oxidase was performed.[23,24] The data show that Ni-NCC does exhibit SOD activity, but it is slower than Ni-SOD. The IC_{50} for Ni-NCC ($4.1 \pm 0.8 \times 10^{-5}$ M) is comparable to those values reported for other peptide mimics, particularly the maquette with bis-amide nitrogen coordination (3×10^{-5} M).[18]

Because the reduction potential of Ni-NCC is similar to that of [$Ni(SOD)^{M1}$] but the activity of Ni-NCC is lower than this amine/amide maquette (2.1×10^{-7} M),[18] the rate may be decreased by the absence of a fifth ligand in the Ni-NCC complex. For Ni-SOD, it has been suggested that the axial imidazole ligand tunes the redox properties of the Ni^{III} species to be appropriate for superoxide dismutation.[19,21] In follow-up studies to be presented elsewhere, examination of the system in the presence of anions showed that Ni-NCC coordinates a transient, anionic fifth ligand, further supporting its similarity to Ni-SOD.

Ni-NCC exhibits features similar to those of Ni-SOD, and its midpoint potential and ability to break down superoxide support its use as a mimic of the enzyme. The sequence of this novel peptide mimic is unrelated to that of Ni-SOD and provides a complementary system for understanding how the identity and geometry of the supporting ligands influence not only the reduction potential but the functional electron transport.

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Supporting Information Available: Experimental procedures and ESI-MS, pH titration, MCD, DFT, CV, and control peptide data. This material is available free of charge via the Internet at http://pubs.acs.org.

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