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Harnessing Scorpionate Ligand Equilibria for Modeling Reduced Nickel Superoxide Dismutase Intermediates

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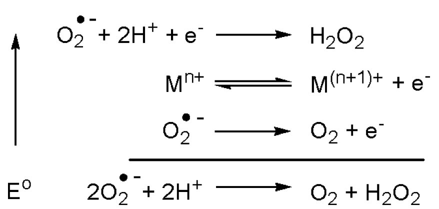
Hydrotris(3-phenyl-5-methylpyrazoyl)boratonickel(II) complexes with organoxanthate or dithiocarbamate coligands equilibrate between κ^2- and κ^3-chelation modes of the scorpionate ligand in solution, connecting N_2S_2 square-planar and N_3S_2 pyramidal ligand fields and a spin crossover. The complexes also exhibit quasi-reversible oxidations at low anodic potentials, thus modeling the structure, dynamics, and redox reactivity of the reduced NiSOD active site.

Organisms inhabiting aerobic environments have evolved multiple layers of defense against oxidative stress caused by the partial reduction of dioxygen.[1] Prominent among these are superoxide dismutases (SODs).[2] These enzymes are particularly interesting from a mechanistic perspective. While any metal ion exhibiting a fast one-electron couple poised between the redox half-reactions of superoxide can, in principle, catalyze its disproportionation (Scheme 1), low metal concentrations in the biological milieu necessitate tight binding of metal ions by SODs and control of the redox reactivity without the introduction of kinetic barriers that would obviate fast reactivity with the $O_2^{\bullet-}$ flux. In a striking example of convergent evolution,[3−5] nature has obtained disparate active sites to carry out this reaction, utilizing Cu^I/Cu^{II}, Mn^{II}/Mn^{III}, Fe^{II}/Fe^{III}, or Ni^{II}/Ni^{III} couples. Understanding how the distinct active site structures and dynamics lead to common SOD activity thus gains significance.

Scheme 1



X-ray structures of NiSOD were recently reported (Figure 1).[4,5] The active site supports an unusual Ni^{III} ion in a square-pyramidal $N_3S_2^{3-}$ ligand field comprised of the N-terminal amine, an axial 1-histidine imidazole, an adjacent backbone amide, and 2- and 6-cysteine thiolates (Figure 1, left). Electron paramagnetic resonance spectra are also consistent with low-spin ($S = {}^1/_2$) Ni^{III} and an axial nitrogen donor.[6,7] Photoreduction of this resting state by X-rays prompts axial imidazole dissociation, generating a classical square-planar Ni^{II} center (Figure 1, right). The free imidazole rotates toward flanking backbone carbonyls, suggestive of protonation.[4,5] Thus, the reductive half-reaction to H_2O_2 may be driven by proton transfer from His-1 and recoordination to Ni^{III},[6] analogous to the mechanistic role of the bridging imidazole in Cu/ZnSOD.[8] However, superoxide approaches the active site from the opposite face of the N_2S_2 equatorial plane, precluding direct proton transfer to nascent peroxide.[5] S K-edge X-ray absorption spectroscopy studies indicate ligation of equatorial thiolates in photoreduced enzymes but protonated thiols in peroxide-reduced samples.[9] A density functional theory (DFT) study alternatively proposed five-coordinate, high-spin ($S = 1$) Ni^{II} as a reduced catalytic intermediate, with transient protonation of the 2-cysteine thiolate adjacent to an inner-sphere peroxide; this gives a calculated turnover barrier of only 12 kcal/mol.[10] Others favor outer-sphere turnover with only weak axial imidazole

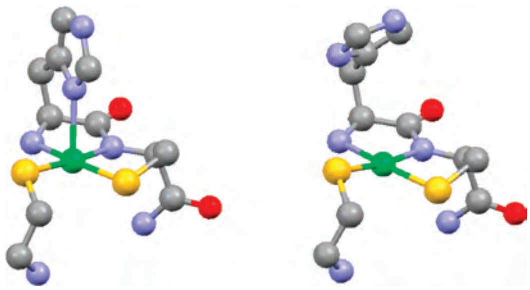


Figure 1. X-ray structures of the active-site ligand field in oxidized (left) and photoreduced (right) NiSOD (1t6u.pdb).[5]

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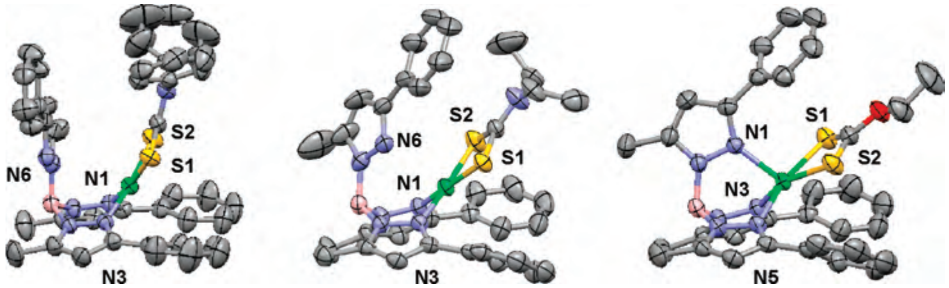

Figure 2. X-ray crystal structures of $Tp^{Ph,Me}NiS_2CNPh_2$ (left), $Tp^{Ph,Me}NiS_2CNEt_2$ (center), and $Tp^{Ph,Me}NiS_2COEt$ (right) drawn with 50% thermal ellipsoids. Only one configuration of each disordered dithiocarbamate substituent is shown. Coordinate bond lengths (Å) for $Tp^{Ph,Me}NiS_2COEt$: Ni−S1, 2.201(1); Ni−S2, 2.183(1); Ni−N1, 1.910(2); Ni−N3, 1.911(1); Ni···N6, 3.541. For $Tp^{Ph,Me}NiS_2CNEt_2$: Ni−S1, 2.199(1); Ni−S2, 2.193(1); Ni−N1, 1.929(1); Ni−N3, 1.934(1); Ni···N6, 2.805. For $Tp^{Ph,Me}NiS_2COEt$: Ni−S1, 2.399(1); Ni−S2, 2.379(1); Ni−N3, 2.042(2); Ni−N5, 2.078(1); Ni−N1, 2.052(2).

binding at diamagnetic Ni^{II}.[11] A significant mechanistic role for the axial His-1 base is indicated by loss of activity in an H1Q mutant;[7] manipulation of the axial bond may constitute a mechanism for redox tuning of NiSOD.[10−12]

Intrigued by the interplay of geometry, spin state, protonation, and redox reactivity at this unique active site and the consequent mechanistic uncertainties in NiSOD turnover, we modeled salient features of the ligand field into synthetic complexes. We utilized the hydrotris(3-phenyl-5-methylpyrazolyl)borate ligand (i.e., $Tp^{Ph,Me}$) to mimic the monoanionic facial array of nitrogen donors;[13] such "scorpionate" ligands can adopt variable κ^2- and κ^3-chelate modes on diamagnetic $4d^8$ and $5d^8$ metal ions (e.g., Rh^I, Ir^I, Pt^{II}), albeit without spin crossover.[14] 1,1-*S*,*S′*-chelating dithiocarbamates ($R_2NCS_2^-$, R = Et, Ph)[15] and organoxanthate ($EtOCS_2^-$)[16] were used as coligands to mimic the dithiolate motif; their predominant zwitterionic resonance form affords κ^2 chelation of anionic sulfurs and charge neutrality at Ni^{II}. These coligands also provide a range of sterics (i.e., $NPh_2 > NEt_2 > OEt$) and donor strength (i.e., $NEt_2 > NPh_2 > OEt$).

Complexes were prepared by metatheses of *S*,*S′*-chelate sodium salts with $Tp^{Ph,Me}NiCl$.[17] The complexes exhibited ν(B−H) modes at 2545 cm^{-1} in green solutions, indicating κ^3-$Tp^{Ph,Me}$ ligation,[14] as well as UV−visible spectra consistent with five-coordinate, high-spin Ni^{II}.[17,18] Absorption bands near 425 nm ($\epsilon = 1.3{-}1.6$ mM^{-1} cm^{-1}) and 655 nm ($\epsilon = 0.1$ mM^{-1} cm^{-1}) weakly blue-shifted with increasing *S*,*S′*-chelate donor strength, consistent with ligand-field character, while a near-UV band ($\epsilon = 13{-}17$ mM^{-1} cm^{-1}) strongly red-shifted, consistent with ligand-to-metal charge-transfer character. ^1H NMR spectra were obtained with paramagnetic shifting of resonances that were, nonetheless, consistent with the formulations. For example, the spectrum of $Tp^{Ph,Me}NiS_2CNEt_2$ in $CDCl_3$ at 295 K contained scorpionate ligand resonances due to 5-Me substituents at 1.6 ppm, the 3-Ph groups at 7.1 (para), 6.8 (meta) and 4.7 (ortho), the 4-pyrazolyl ring protons at 52.7 ppm, and the borohydride at −8.5 ppm; the *N*-ethyl signals were found at 34.0 (CH_2) and 1.1 ppm (CH_3). Analogous results were found for $Tp^{Ph,Me}NS_2COEt$ and $Tp^{Ph,Me}NiS_2CNPh_2$, although the respective 4-pyrazolyl ring proton paramagnetic shifts varied noticeably, falling at 63.2 and 54.4 ppm. Equivalent resonances were consistent with κ^3-$Tp^{Ph,Me}$ coordination and rapid chelate site exchange in all three complexes.

Despite common solution spectra, divergent solid-state structures were obtained. Red crystals of the dithiocarbamate complexes exhibited ν(B−H) modes at 2480 cm^{-1} in KBr pellets, indicative of κ^2 chelation,[14] while green xanthate crystals retained the mode at 2545 cm^{-1}. This implies distinct four- and five-coordinate geometries. X-ray crystallography confirmed square-planar N_2S_2 ligand fields for the dithiocarbamates (Figure 2, left and center) and a square-pyramidal N_3S_2 field ($\tau = 0.12$)[19] for the xanthate (Figure 2, right), with κ^2- and κ^3-scorpionate ligands, respectively. The *S*,*S′*-chelates occupy cis equatorial sites in the structures; the $Tp^{Ph,Me}$ ligand also occupies two equatorial sites, leaving the third arm to variably interact with an axial site. The square planes were distorted by the constrained *S*,*S′*-chelate bites; the cis S−Ni−S angles were 78.84(2)° and 78.06(2)° for $Tp^{Ph,Me}NiS_2CNPh_2$ and $Tp^{Ph,Me}NiS_2CNEt_2$, respectively, while N−Ni−N angles were square [90.34(6)° and 90.32(6)°]. Equatorial S−Ni−S and N−Ni−N angles of the xanthate complex were 74.75(2)° and 87.54(7)°.

Compared to the square-planar dithiocarbamate complexes, the square-pyramidal organoxanthate exhibits elongated

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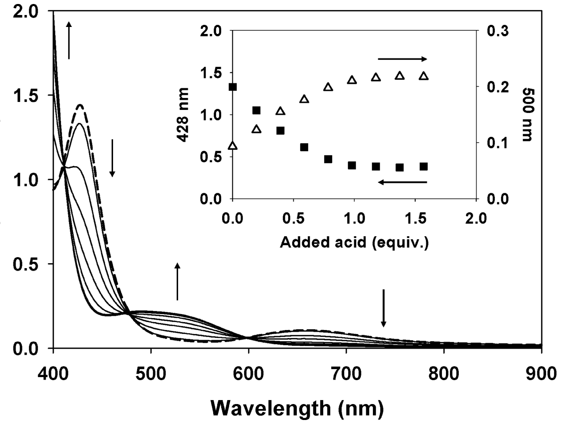

Figure 3. Titration of $Tp^{Ph,Me}NiS_2CNPh_2$ with CF_3CO_2H in CH_2Cl_2 at 20 °C. The dashed line shows the final spectrum after the addition of 2.0 equiv of NEt_3. The inset shows extinctions against added proton equivalents at 428 nm (■, left axis) and 500 nm (Δ, right axis).

coordinate bonding consistent with paramagnetic ($S = 1$) Ni^{II}, resulting from transfer of one electron from the destabilized axial d_{z^2} lone pair into the equatorial $d_{x^2}-_{y^2}$ orbital. A similar structure was reported for a nickel(II) xanthate complex cation supported by a neutral triazamacrocycle.[20] Despite the chemical approximations in our modeling of the $N_3S_2^{3-}$ ligand field, Ni−N and Ni−S coordinate bond lengths in our complexes are comparable to those of the corresponding isomers of reduced NiSOD; the dithiocarbamate complexes compare to photoreduced NiSOD,[4,5,7] and the xanthate structure to DFT calculations on proposed paramagnetic square-pyramidal catalytic intermediates.[10,11] In contrast, more strongly σ-donating equatorial ligands can enforce diamagnetism in an elongated square pyramid:[11] $[NEt_4][Tp^{Me,Me}Ni(CN)_2]$ has short equatorial Ni−N bond lengths of 1.934(2) and 1.936(2) Å and a long axial bond of 2.389(2) Å;[21] $TpNi(PMe_3)C_6H_5$ has respective bond lengths of 1.938(8), 1.979(9), and 2.57(1) Å.[22]

Crystallization of the model complexes in two different geometries is suggestive of a facile axial equilibrium in solution. Solution magnetic moments, determined by the Evans NMR method[23] in $CDCl_3$ solutions at 295 K, fell in a range of $\mu_{eff} = 2.5-2.7\ \mu_B$, less than the spin-only limit. Titration of $Tp^{Ph,Me}NiS_2CNPh_2$ with equivalent CF_3CO_2H in CH_2Cl_2 changed the solution color from green to red with the appearance of a new ligand-field band at 490 nm ($\epsilon = 220$ M^{-1} cm^{-1}; Figure 3); the starting spectrum was recovered by subsequent addition of NEt_3. Similar spectral changes were elicited by simply heating a CH_3CN solution of $Tp^{Ph,Me}NiS_2CNPh_2$; thermodynamic parameters were determined for this proton-independent axial base equilibrium, $\Delta H° = 2.1(1)$ kcal mol^{-1} and $\Delta S° = +5.6(3)$ cal mol^{-1} K^{-1}. These values reflect a favorable entropy of pyrazole dissociation, offsetting the unfavorable combined enthalpies of the Ni−N bond rupture and spin crossover. Comparative values calculated for the corresponding axial base equilibrium in reduced NiSOD are $\Delta H° = 1.9$ kcal mol^{-1} and $\Delta S° = -11$ cal mol^{-1} K^{-1}.[10] Cyclic voltammetry of a NiSOD maquette in an aqueous solution gave $\Delta G° = 1.6(5)$ kcal mol^{-1} for the axial His-1 equilibrium at Ni^{II}.[11] The equilibrium favors the green, paramagnetic square-pyramidal structure, with the classical square-planar red form accumulating toward higher temperatures. This is reversed from the related ring-slip equilibrium of Cp*Ni(acac).[24]

The crystal structures together correlate three motions germane to the equilibrium: axial pyrazole rotation about its B−N bond, tilting of the equatorial plane, and inversion of Ni^{II} through this plane. For $Tp^{Ph,Me}NiS_2CNPh_2$, $Tp^{Ph,Me}NiS_2CNEt_2$, and $Tp^{Ph,Me}NiS_2COEt$, respectively, H−B−N−N torsion angles into the axial pyrazole (i.e., using the B−H bond as a center line) are 83.5°, 133.2°, and 175.1°. Coincident with inward pyrazole rotation, the N_2S_2 equatorial plane is tilted; dihedral angles defined by a second plane of the four nitrogen atoms in the equatorial pyrazoles are 130.44°, 140.02°, and 146.01°, respectively. Ni^{II} is displaced through the N_2S_2 equatorial plane by distances of −0.121, −0.013, and +0.304 Å. The axial pyrazole of $Tp^{Ph,Me}NiS_2CNEt_2$ seems disposed to minimize overlap of the d_{z^2} lone pair with the pyrazole σ lone pair, as well as the π orbital on the adjacent nitrogen proximal to boron. This is not possible in the bulkier $Tp^{Ph,Me}NiS_2CNPh_2$, which exhibits both a short separation of this proximal nitrogen from nickel (2.997 Å) and short C···H−C contacts between the axial pyrazole and the opposing dithiocarbamate (e.g., C8−H43b, 2.779 Å).

Given the unusual Ni^{III} ion of oxidized NiSOD and the presumption of a fast one-electron couple supporting a ping-pong mechanism (Scheme 1), we also investigated the redox properties of our complexes. All three complexes exhibited quasi-reversible ($\Delta E \leq 100$ mV) one-electron couples by cyclic voltammetry in CH_2Cl_2 solutions, with observed $E°'$ values reflecting the relative donor strengths of the *S*,*S*′-chelates: $Tp^{Ph,Me}NiS_2CNEt_2$, −0.08 V (vs external Fc/Fc^+); $Tp^{Ph,Me}NiS_2CNPh_2$, +0.05 V; $Tp^{Ph,Me}NiS_2COEt$, +0.26 V. These couples fall in a range suitable for SOD activity, suggesting our complexes might function as catalysts under appropriate solvent conditions. Regardless, our model complexes do exhibit facile, tuneable oxidation and an axial equilibrium coupled to spin crossover, which together mimic key properties of reduced NiSOD intermediates.[4,5,10,11]

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Supporting Information Available: Experimental details, spectroscopic and analytical data, details of the X-ray crystallography, and data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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