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Short communication

Dioxygen and superoxide stability of metallopeptide based mimics of nickel containing superoxide dismutase: The influence of amine/amidate vs. bis-amidate ligation

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ABSTRACT

Nickel containing superoxide dismutase (NiSOD) is a metalloenzyme that catalyzes the disproportionation of $O_2^{\bullet-}$. In its reduced state, the Ni(II) ion is coordinated by two *cis*-cysteinates, an amine nitrogen and an amidate nitrogen atom. It thus bears a resemblance to the distal bis-cysteinate bis-amidate ligated nickel center of acetyl coenzyme A synthase. Using metallopeptide based NiSOD mimics derived from the first 12 residues of the NiSOD sequence we demonstrate that altering the primary coordination sphere from a bis-thiolate amine/amidate motif to a bis-thiolate bis-amidate motif changes the O_2 and ROS stability of the metallopeptide. Using FT-IR, ESI-MS and S K-edge XAS we show that the bis-amidate bis-thiolate ligated metallopeptide {$Ni^{II}(SOD^{m1}$-Ac)} (SOD^{m1}-Ac = AcHN-HCDLPCGVYSPA-COOH) undergoes oxidation at one thiolate ligand in the presence of O_2, converting it into a coordinated sulfinate. Upon exposure of {$Ni^{II}(SOD^{m1}$-Ac)} to $O_2^{\bullet-}$ the metallopeptide undergoes extensive sulfur oxidation. This can be contrasted with the unacylated metallopeptide {$Ni^{II}(SOD^{m1})$} which does not undergo sulfur based oxidation under these conditions. The biological implications of these findings are discussed.

Nickel containing superoxide dismutase (NiSOD) is a bacterial enzyme that catalyzes the disproportionation of $O_2^{\bullet-}$ into O_2 and H_2O_2 [1–4]. In its reduced form NiSOD contains Ni(II) within an N_2S_2 coordination motif utilizing two cysteinate residues (Cys2 and Cys6), an amidate nitrogen and the *N*-terminal amine nitrogen (His(1); Scheme 1) to coordinate nickel [5]. As such, the NiSOD active site bears a resemblance to the bis-amidate ligated distal nickel site of acetyl coenzyme A synthase (ACS; Scheme 1) [6]. Upon oxidation of NiSOD the Ni(III) ion ligates the His(1) imidazole forming a five-coordinate structure (Scheme 1). Thus, all of the coordinating residues to nickel in NiSOD are contained within the first six residues from the *N*-terminus. We, and others, have constructed SOD active mimics of NiSOD using short nickel containing metallopeptides based on the NiSOD protein sequence [7–12].

Previously we compared the NiSOD metallopeptide based mimic {$Ni(SOD^{m1})$} (SOD^{m1} = H_2N-HCDLPCGVYSPA-COOH) to a derivative where the *N*-terminus was acylated ({$Ni(SOD^{m1}$-Ac)}; SOD^{m1}-Ac = AcHN-HCDLPCGVYSPA-COOH) [13]. The two metallopeptides differ only in the amine/amidate ({$Ni(SOD^{m1})$}) vs. bis-amidate ({$Ni(SOD^{m1}$-Ac)}) nitrogen donors. It was found that the two peptides displayed similar physical properties with subtle differences attributed to the change in the nitrogen ligands to nickel. Most interesting was that the two peptides had Ni^{III}/Ni^{II} couples that indicated that both, theoretically, could disproportionate $O_2^{\bullet-}$. A modified xanthine/xanthine oxidase assay demonstrated that {$Ni(SOD^{m1}$-Ac)} is several orders of magnitude less active than {$Ni(SOD^{m1})$} and other NiSOD-based peptide mimics [7,11,13]. Considering that SOD activities obtained by such methods are unreliable [9,14], we utilized stopped-flow kinetics to measure the direct decomposition of $O_2^{\bullet-}$. Stopped-flow studies unambiguously demonstrate that {$Ni(SOD^{m1}$-Ac)} is *not* catalytically active [15]. The reasons for this were not well understood at the time. Herein it will be shown that the cysteinates of {$Ni(SOD^{m1}$-Ac)} rapidly oxidize upon exposure to O_2 and $O_2^{\bullet-}$ rendering the metallopeptide catalytically nonviable (Scheme 2 [16]).

FT-IR monitoring of aerobic solutions of {$Ni^{II}(SOD^{m1}$-Ac)} indicates cysteinate modification. Upon addition of O_2(g) to samples of {$Ni^{II}(SOD^{m1}$-Ac)} there is the appearance of bands at 1059 and 1179 cm^{-1} (Fig. 1). The frequencies of these bands are similar to the symmetric and asymmetric vibrational modes of the S═O_2 oscillators found in structurally related sulfinate-ligated $Ni^{II}N_2SS(O_2)$ complexes [17]. Use of $^{18}O_2$(g) in place of air confirms this supposition. Oxidation of the coordinated cysteinates with $^{18}O_2$(g) yields bands red-shifted to 1032 and 1144 cm^{-1}, close to the 30 and 33 cm^{-1} shift predicted for the O═S═O oscillator upon $^{16}O/^{18}O$ isotopic substitution. FT-IR analysis does not indicate any S-based oxidation of {$Ni^{II}(SOD^{m1})$} exposed to O_2.

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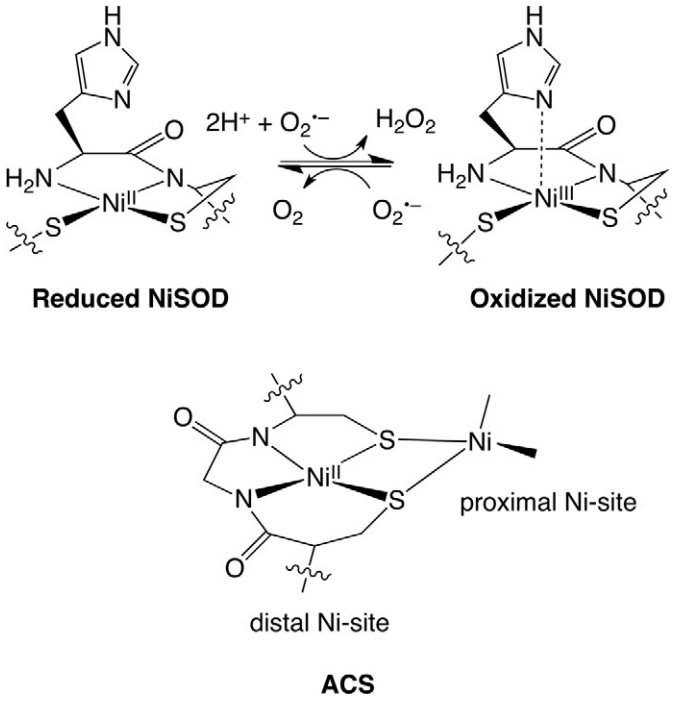
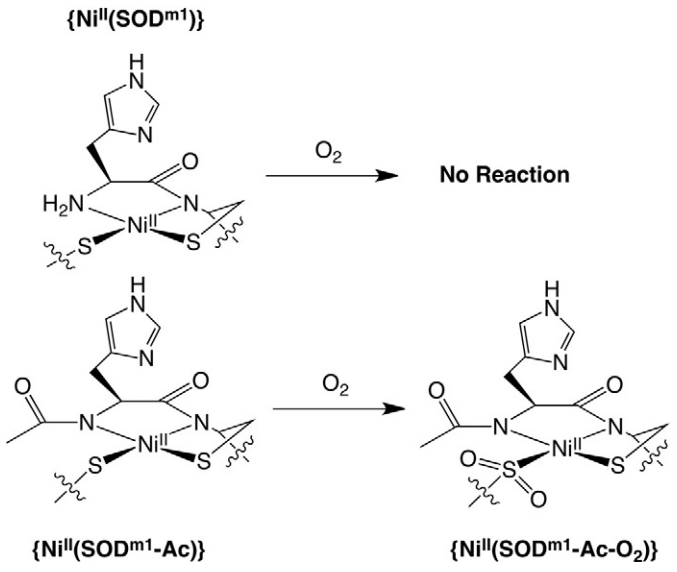


Scheme 1. NiSOD and ACS active sites.

Full oxidation of the cysteinate in O_2 saturated water (O_2 concentration ~610 μM) appears to be complete within 45 min (Fig. 1). Plotting the change in absorbance of the O═S═O symmetric vibrational mode vs. time yielded a second order rate constant of $k = 0.3(1)\ M^{-1}\ s^{-1}$ for the conversion of {$Ni^{II}(SOD^{m1}$-Ac)} to {$Ni^{II}(SOD^{m1}$-Ac-O_2)} by O_2 at 20 °C. No further oxidation of the cysteinates under these conditions is noted, even after prolonged exposure to air. The fact that only one set of vibrational modes is observed upon O_2(g) exposure to {$Ni^{II}(SOD^{m1}$-Ac)} suggests that only one of the cysteinate residues is being oxidized under these conditions. This is supported by the ESI-MS data of aerobically matured {$Ni^{II}(SOD^{m1}$-Ac)}, which demonstrates the incorporation of two oxygen atoms into the {$Ni^{II}(SOD^{m1}$-Ac-O_2)}



Scheme 2. Reaction of dioxygen with {$Ni^{II}(SOD^{m1}$)} and {$Ni^{II}(SOD^{m1}$-Ac)}.

metallopeptide product (Fig. 2). Similar behavior has been noted for other NiN_2S_2 complexes that undergo sulfur oxidation; subsequent oxidation of the $Ni^{II}N_2SS(O_2)$ species requires harsher oxidative conditions than simple O_2 exposure [17,18].

Further evidence that only one cysteinate is being oxidized within {$Ni^{II}(SOD^{m1}$-Ac)} is given by S K-edge X-ray absorption spectroscopy (Fig. 3). The S K-edge X-ray absorption spectrum results from the promotion of a S(1s) electron into bound, quasi-bound and unbound states. As this is an atomic spectroscopic technique individual S-atoms can be probed. Upon oxidation of an organosulfur compound from a thiolate (RS^-) to a sulfinate (RSO_2^-) the oxidation state of the S changes from a formal S^{2-} to an S^{2+}, which will result in an ~5–6 eV blue-shift of the edge energy [19,20].

The S K-edge spectrum of {$Ni^{II}(SOD^{m1}$-Ac)} prior to air exposure displays a small peak at 2471.2(1) eV and an intense band at 2472.9(1) eV. These bands are attributed to the S(1s) → Ni(3d)/S(3p) and S(1s) → (C-S(σ)*) transitions. Following 1 h of air exposure the spectrum demonstrates a marked change. First, the S(1s) → Ni(3d)/S(3p) transition (now at 2469.8(1) eV) gained intensity relative to the S(1s) → (C-S(σ)*) transition. This is likely due to a coordinated Ni-Cys(H) deprotonation event occurring upon the oxidation of one of the cysteinates [10]. Second, a new series of transitions at 2476.2(1) eV and 2479.0(2) eV have appeared. These can be attributed to the SO_2(1s) → Ni(3d)/SO_2(3p) and SO_2(1s) → (C-S(σ)*/O-S(σ)*) transitions. The fact that transitions for both thiolate and sulfinate ligands are observed suggests that only one cysteinate is oxidized upon air exposure. As with the FT-IR studies, no further change of the S K-edge spectrum is noted after prolonged air exposure.

As {$Ni^{II}(SOD^{m1}$-Ac)} is far less stable toward O_2 than {$Ni^{II}(SOD^{m1}$)} it was reasoned that {$Ni^{II}(SOD^{m1}$-Ac)} would be far less stable toward $O_2^{\bullet-}$ (and the H_2O_2 produced in the disproportionation reaction) than {$Ni^{II}(SOD^{m1}$)} as well. The addition of 10 eq. of KO_2 (in DMSO) to aqueous solutions of {$Ni^{II}(SOD^{m1}$)} followed by ESI-MS analysis demonstrates no evidence of metallopeptide modification (Fig. 4). Although {$Ni(SOD^{m1}$)} is reactive toward H_2O_2 [7] oxidation under these conditions is likely not observed because of the short period of time between KO_2 addition and analysis coupled with the relatively low concentration of KO_2 used in the reaction [21]. In stark contrast, the addition of 10 eq. of KO_2 to aqueous solutions of {$Ni^{II}(SOD^{m1}$-Ac)} yields extensive metallopeptide modification as evidenced by ESI-MS (Fig. 4). In addition to {$Ni^{II}(SOD^{m1}$-Ac-O_2)}, one can also observe other metallopeptide-based products with varying degrees of oxygen atom insertion.

Previously we demonstrated that the combination of amine and amidate donors within Ni(II)N_2S_2 complexes protected the thiolate ligands from oxidative damage [22]. Changing the amine/amidate ligand set to a bis-amidate ligand set resulted in the activation of the filled Ni(3dπ)/S(3pπ)* orbital. It was reasoned that this would make the thiolate ligands more susceptible to electrophilic attack by O_2 and reactive oxygen species (ROSs). Grapperhaus reached a similar conclusion in a previous DFT study [23]. These results support this notion; bis-amidate ligands within a NiSOD peptide scaffold lead to relatively rapid S-ligand oxidation under both aerobic conditions and conditions relevant for SOD catalysis. This is contrasted with the amine/amidate donor-set, which yields a remarkably stable NiN_2S_2 center under identical conditions. It therefore seems reasonable to suggest that nature does not utilize an ACS-like coordination motif within NiSOD because of the high susceptibility of the coordinated cysteinates within such a metalloenzyme toward oxidation upon exposure to O_2 and ROSs. Such oxidation events would yield a catalytically dead "SOD."

Abbreviations

ACS	acetyl coenzyme-A synthase
ESI-MS	electrospray ionization mass spectrometry