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COMMUNICATIONS

Published on Web 03/15/2002

Hydrogen-Bond-Mediated Tuning of the Redox Potential of the Non-Heme Fe Site of Superoxide Dismutase

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Received May 17, 2001

The highly homologous proteins of Fe-containing superoxide dismutase (FeSOD) and MnSOD from *Escherichia coli* nonetheless exert very different redox tuning on the active site metal ion.[1] This has been proposed to stem from different hydrogen bonding between the protein and the metal ion's coordinated solvent molecule.[1,2] We now present density functional theory (DFT) calculations on Fe^{2+} and Fe^{3+} bound to models of both FeSOD and MnSOD. The calculations support an important role for the conserved second sphere Gln side chain in destabilizing H_2O coordinated to Fe^{2+} vs OH^- coordinated to Fe^{3+}. Indeed mutation of this Gln to Glu, which should increase the stability of Fe^{2+}-bound H_2O, significantly raises the reduction potential, E_m.

The E_m is a crucial thermodynamic determinant of redox reactivity. Thus, redox-active enzymes' E_ms are precisely tuned by the active site environment. The FeSOD and MnSOD of *E. coli* provide a striking example of this, as the Mn-specific protein, (Mn)-SOD, depresses the E_m of the bound metal ion significantly more than does the Fe-specific protein, (Fe)SOD,[1] although the two active sites are almost superimposible.

The active site Fe or Mn in FeSOD and MnSOD is coordinated in a trigonal bipyramid by three His residues, one Asp^-, and a solvent molecule[3] (Figure 1). Since metal ion reduction is intimately coupled to proton uptake in SOD,[4] the degree to which protonation of coordinated solvent is favored in each oxidation state contributes significantly to the observed E_m. Thus, stronger hydrogen bond donation to the metal-ion-bound solvent has been proposed to strongly depress the E_m of the bound metal ion in the (Mn)SOD protein.[1,2] We now test this proposal at an atomic level to obtain insight into the role of the second sphere in modulating the properties of the active site.

Figure 1 shows active site models for oxidized and reduced FeSOD (left) and derivatives thereof in which the second-sphere Gln and Tyr have been positioned as in the MnSOD site (right). In the oxidized state, the decrease in optimized distance between the Gln amide hydrogen and the oxygen of the Fe^{3+}-bound OH^-, from 2.70 Å in FeSOD to 1.94 Å in the Fe(Mn)SOD model, suggests formation of a stronger hydrogen bond (Figure 1, top right). However, protonation of the solvent ligand upon Fe reduction results in considerable steric interference between the Gln and the H_2O protons in the Fe(Mn)SOD model, leading to rotation of the coordinated H_2O and weakening of its bond to Fe^{2+} (Figure 1, bottom right). This effect is less pronounced yet still substantial in FeSOD, whose Gln amide N is ≈0.6 Å further from the metal ion[3] and is much more weakly coupled to Fe^{2+}.[2] Thus, DFT results indicate that the closer active site Gln side chain in (Mn)SOD than

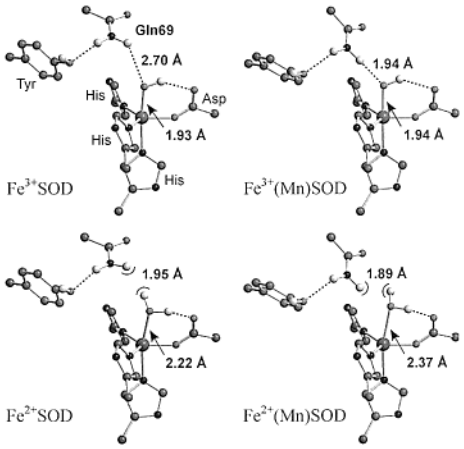

Figure 1. Active site models for oxidized and reduced FeSOD (left) and a modification thereof in which the Gln and Tyr side chains have been moved to the positions characteristic of MnSOD to generate a model of Fe(Mn)SOD (right). Only protons involved in the conserved H-bonding network including Tyr, Gln, and the solvent ligand are shown. The positions of these protons and the solvent oxygen were obtained through DFT energy minimizations. Calculations were performed using the Amsterdam Density Functional (ADF) 2000.02 software package.[6]

in (Fe)SOD destabilizes coordinated H_2O vs OH^-, thus strongly favoring the Fe^{3+} state and lowering the E_m in this protein.[5]

The above findings are consistent with the several hundred mV lower E_m displayed by Fe(Mn)SOD than FeSOD, as well as the much lower E_m of MnSOD than Mn(Fe)SOD.[1,7] They suggest that the (Fe)SOD and (Mn)SOD proteins can tune their E_ms through precise positioning of the second-sphere Gln. This Gln can affect the E_m by modulating the proton affinity of the metal-bound solvent molecule that acquires a proton upon metal ion reduction. This mechanism predicts that replacement of the H-bond donating Gln with an H-bond acceptor should result in a considerable increase in E_m because this mutation would greatly increase the stability of coordinated H_2O relative to coordinated OH^-.

To test this hypothesis, we have constructed the Gln69 to Glu mutant of FeSOD. Glu is isosteric and isoelectronic with Gln but differs in lacking a single H atom when Glu is neutral or lacking two H nuclei and placing a negative charge near coordinated solvent when Glu is ionized. Regardless of pH, Glu is a better H-bond acceptor than Gln. Based on our model this should increase the E_m of the site. Alternately, to the extent that Glu is ionized and electrostatic interaction with Fe instead of H-bonding to coordinated solvent is dominant, substitution of Glu for Gln should lower E_m.

Comparison of the NMR spectra of the active sites of Q69E- and WT-Fe^{2+}SOD, and in particular the resonances of the three ligand histidines near 90 and 40 ppm, demonstrates that the coordination environment of the Fe^{2+} is very similar in the two

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10.1021/ja011220v CCC: $22.00

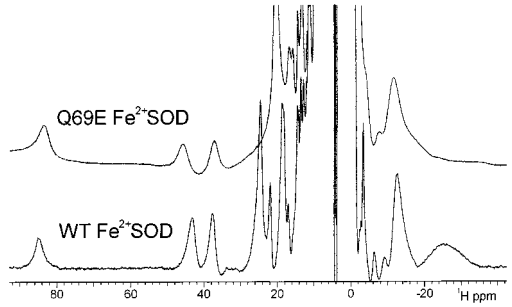

Figure 2. ^{1}H NMR spectra of Q69E-Fe^{2+}SOD and dithionite-reduced WT-Fe^{2+}SOD active site resonances, at pH 7.4.

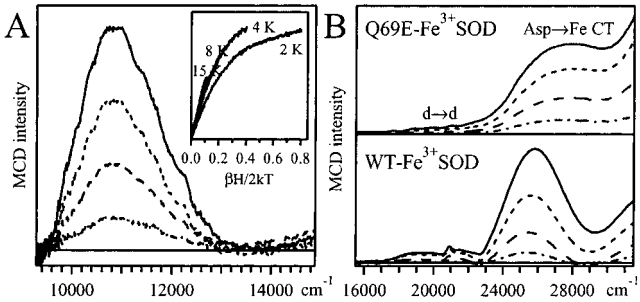

Figure 3. (A) MCD spectra at 4.5 K and 0.5, 1.5, 3.5, and 7 T of as-isolated Q69E-Fe^{2+}SOD. The VTVH behavior of the signal at 10695 cm^{-1} is shown in the inset. (B) MCD spectra at 4.5 K of $KMnO_4$-oxidized Q69E-Fe^{3+}SOD (top) and as-isolated WT Fe^{3+}SOD (bottom) both at pH 7.0.

cases (Figure 2), based on the similar line widths and paramagnetic chemical shifts (indicative of the relative energies of electronic states and the unpaired electron distribution). This conclusion is supported by near-infrared magnetic circular dichroism (MCD) spectra of Q69E-Fe^{2+}SOD (Figure 3A). Both the single feature at ~11 000 cm^{-1} (characteristic of a five-coordinate ferrous site[8]) and the large nesting of the variable-temperature variable field (VTVH) MCD data obtained at 10695 cm^{-1} (reflecting the Fe^{2+} d orbital splitting pattern) agree well with published data for reduced WT-Fe^{2+}SOD.[9]

Variable-field MCD spectra at 4.5 K of native WT-Fe^{3+}SOD and $KMnO_4$-oxidized Q69E-Fe^{3+}SOD (Figure 3B) both show a broad band at ~26 000 cm^{-1} (385 nm) that coincides with an intense shoulder in the absorption spectrum (ϵ = 1500−2000 M^{-1} cm^{-1}), which we assign to an Asp^--to-Fe^{3+} charge-transfer transition. The similar position and MCD saturation behavior of this feature (indicative of the Fe^{3+} ligand environment) reveal that the Q69E mutation also has little effect on the nature of the ferric center. Nonetheless, the complete and native-like binding of Fe to Q69E-FeSOD represents a fascinating contrast with the analogous Q146E mutant of *E. coli* MnSOD that does not bind Fe or Mn significantly.[10]

Our spectroscopic studies rule out gross disruption of the active site, and the fact that oxidized Q69E-Fe^{3+}SOD binds N_3^- to form a complex similar to that formed by WT Fe^{3+}SOD (Xie et al.[11]) argues against inactivity's being due to inability to bind substrate. However, Q69E-FeSOD is completely inactive (Table 1). We ascribe this to Q69E-FeSOD's several hundred mV higher E_m than WT-FeSOD's (Yikilmaz et al., unpublished) which is manifested by the mutant's being fully reduced as isolated (Table 1).

The fact that Q69E-SOD's E_m is raised instead of lowered argues against predominance of direct electrostatic interaction of Glu69 with the Fe itself, and our spectroscopy indicates that electronic contributions to this redox tuning are rather minor. Instead, replacement of Gln69 by Glu has the qualitative effect predicted by the computations and thus supports our model that destabilization of coordinated H_2O and stabilization of coordinated OH^- by Gln

Table 1. SOD Types, Metal Ion Content, Activity, and Oxidation State As Isolated

	content[a]			oxidation state	
species	Fe	Mn	activity[b] (units/mg)	Fe	Mn
WT-FeSOD	0.9	0	6073	+3	
Q69E-FeSOD	>0.95	0	3	+2	
Fe(Mn)SOD	0.9	0	0	+3	
WT-MnSOD	~0.05	>0.9	6900		+2/+3

[a] Quoted on a per site basis based on colorimetric assay, atomic absorption, EPR after acidification, and FeSOD's extinction coefficient at 280 nm. [b] Based on the xanthine oxidase assay of McCord and Fridovich.

are important in lowering the metal ion's E_m in MnSOD's active site. Indeed, computations on a Q69E-Fe^{2+}SOD active site model indicate that Glu69 is ionized and the stability of coordinated H_2O relative to OH^- is significantly increased over the same in the WT-Fe^{2+}SOD active site model. Thus, residue 69 could modulate the E_m via its destabilization or stabilization of the proton whose uptake accompanies reduction, via hydrogen bonding.

Since proton uptake accompanies reduction of the metal ion in SOD, the E_m reflects not only the redox equilibrium of the metal ion but also the proton equilibrium of the metal-bound solvent that acquires the proton. Thus, engineering modified proton donation and H-bonding to groups involved in redox-coupled proton uptake may provide routes to mutant enzymes with modified E_ms and thereby novel reactivities.

Acknowledgment. A.-F. M. thanks the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research under ACS-PRF 33266-AC4,3. A.-F. M. is grateful to the N.I.H (GM55210), and N.S.F. for financial support (MCB9728793) and aid in acquiring an NMR spectrometer (CRIF award CHE-9974810). T.C.B. is pleased to acknowledge the University of Wisconsin for generous support. We also thank A. J. Lind for preliminary CD spectroscopy.

Supporting Information Available: Experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA011220V