



Superoxide interaction with nickel and iron superoxide dismutases

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ABSTRACT

Interaction of superoxide with the metal active sites of nickel and iron-containing superoxide dismutases is investigated with density functional theory. Outer-sphere mechanisms for both the reduction and oxidation of superoxide are proposed to be the common feature of these structurally unrelated SODs.

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1. Introduction

Superoxide is a key component of oxidative stress *in vivo*, and its removal by specialized metalloenzymes, superoxide dismutases (SODs) and superoxide reductases (SORs), is essential for living organisms [1]. The mechanisms of enzymatic superoxide reduction have been studied extensively but remain incompletely understood. Three main classes of SODs are known. The Fe/MnSODs feature a five-coordinate Fe or Mn center, and are structurally (and presumably, mechanistically) extremely similar to each other. A second, more recently discovered, class of SODs contains Ni at their active sites [1]. Proposed catalytic mechanisms for the Fe- and NiSODs are shown in Fig. 1. While no metal-superoxide adducts have been demonstrated as catalytic intermediates for any SOD so far, such adducts have often been proposed [2]. For Ni-SOD, a proposed catalytic cycle (cf. Fig. 1) [2] involves superoxide binding to Ni(II), to form a Ni(II)-superoxo adduct, which undergoes a proton-dependent decay to Ni(III) and H₂O₂. Subsequent binding of superoxide to the Ni(III) center leads to a Ni(III)-superoxo adduct, which decays to Ni(II), liberating dioxygen. Initial structural data on NiSOD indicated the Ni coordination in the resting state to be square planar, as shown in Fig. 1 [3]; however, more recent crystallographic work indicates additional ligation of a histidine side-chain to the Ni(III) state (cf. Scheme 1) [2]. Although particularly weak (the minimum Ni-His distance found by

crystallography is 2.6 Å, 0.5 Å longer than typical Ni–nitrogen bond lengths) [2], this latter interaction occurs *trans* to the putative superoxide binding position and has therefore been proposed to promote dioxygen release; axial His ligation/deligation may in principle also help bring the Ni(III/II) redox potential into the range required for outer-sphere superoxide dismutation. The third known class of SODs contain a zinc–copper binuclear active site; here again outer-sphere and inner-sphere mechanisms have been proposed, with the latter recently shown to be feasible computationally [1,4–6].

Here, density functional calculations are reported on the interaction of superoxide with the mononuclear active sites of nickel and iron-containing SODs, aimed to identify possible underlying common features of enzymatic superoxide dismutation. Siegbahn and co-workers have also reported a computational study on NiSOD [7]. An inner-sphere mechanism possibly requiring protonation of a cysteine ligand was proposed [7]; although our results also support the possibility of superoxide-metal ligands (possibly driven by protonation), we find that Ni-SOD activity can be rationalized without invoking such adducts, and that indeed catalytically competent superoxide oxidation/reduction may be achieved by the nickel center without proper formation of a nickel–oxygen bond. These conclusions appear to place the FeSOD mechanism in a new light.

2. Methods

Fig. 1 illustrates the structures of the basic models used in the present work. We note that with a pK_a of 4.9, superoxide (OOH⁰), is

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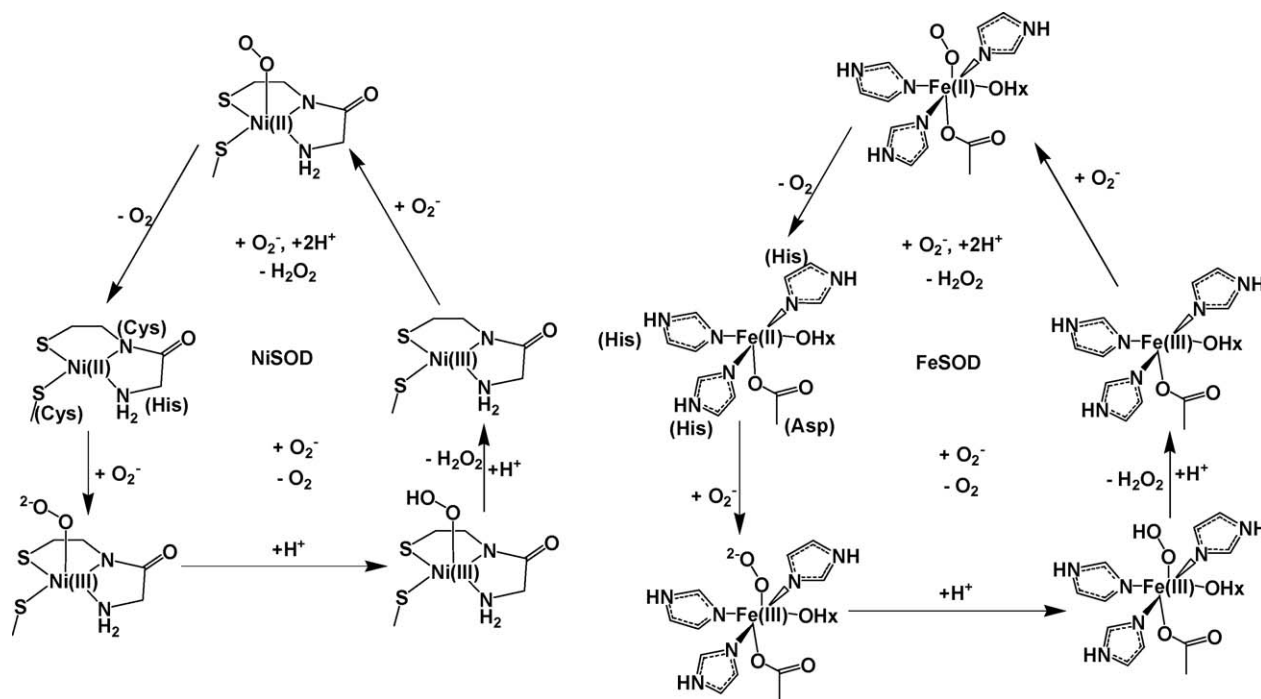
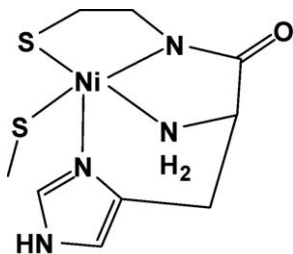


Fig. 1. Proposed catalytic cycles for NiSOD and FeSOD. Straight arrows indicate inner-sphere mechanisms, including putative metal-superoxide adducts. Curved arrows indicate outer-sphere mechanisms. The active sites are drawn as employed in the models used in the present study. The identity of protein residues providing metal ligands is indicated in parentheses.

essentially entirely deprotonated at physiological pH (O_2^-), and therefore the models examined here are the products of putative reactions between metal centers and O_2^- , rather than OOH^0 (though subsequent protonation of the metal-OO species is still investigated). The starting coordinates for NiSOD models were taken from the crystal structure of the active NiSOD (pdb code 1T6U) [3]. Geometries for NiSOD models were optimized without any constraints. The starting coordinates for FeSOD models were taken from the crystal structure of resting FeSOD (pdb code 1BSM [8]). Geometries for FeSOD models were optimized by freezing the coordinates of the heavy atoms within the protein-derived iron ligands, in order to avoid massive rearrangement of the imidazole and carboxylate ligands to relative orientations that would impose steric and electronic constraints not present and thus not directly relevant to the FeSOD active site; such rearrangement does not occur in the NiSOD models. This approach for treating enzyme active sites has been verified with a number of related models; it has the advantage of allowing a relaxation of the iron coordination sphere (as the iron and the water/hydroxo and superoxo ligands are free to move) to accommodate changes in charge and spin state, while also preserving geometrical features of the active site which are clearly imposed by the larger protein/solvent environment [9–11]. An alternative to this approach [4,11] would be to

include in the model atoms found further away from the metal, such as the alpha carbons of aminoacids coordinated to the metal, freeze the coordinates of these distant atoms during the geometry optimization procedure, and allow the rest of the coordination sphere to optimize freely. As pointed out before, this approach does not conserve key geometrical features of the active site, such as dihedral angles between imidazole and carboxylate planes, and their relative orientations towards the metal [10–12]. Unless otherwise specified, imidazole rings are assumed monoprotonated (neutral), while carboxylates and cysteinates are assumed anionic (deprotonated). Metal and peroxo/superoxo oxidation states of each model are listed in figure legends and table.

The UBP86 functional, which uses the gradient-corrected exchange functional proposed by Becke [13], the correlation functional by Perdew [14], and the DN** numerical basis set (comparable in size to 6-31G**) were used as implemented in Spartan [15]. For the SCF calculations, a fine grid was used, and the convergence criteria were set to 10^{-6} (for the root-mean square of electron density) and 10^{-8} (energy), respectively. For geometry optimization, convergence criteria were set to 0.001 au (maximum gradient criterion) and 0.0003 (maximum displacement criterion). Net charges and spin states on the models are shown. Partial atomic charges and spin densities were derived from Mulliken population analyses after DFT geometry optimization. Activation energies were not explicitly calculated, as most of the processes for which these would be sought have already been calculated by Siegbahn and co-workers, who found them to be very low and hence the processes would be quite facile [7]. The results do come with a caveat, in that the active sites models employed here include only the first coordination sphere of the metal in a gas-phase, and do not account directly for the larger protein and solvent environment. Nevertheless, our previous experience with models of similar chemistry, charge and/or size suggest that geometries obtained at this level can be reliable, and that trends in energy differences will be conserved after applying solvent corrections [9–11,16–20].



Scheme 1.

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