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An Accurately-Constructed Structural Model for an Active Site of Fe-Containing Superoxide Dismutases (Fe-SODs)

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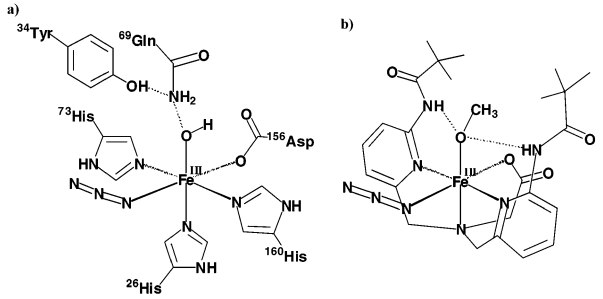
A novel structural model, [Fe(bpga)(N_3)(OCH_3)] (**1**) (BPGA: bis-(6-pivalamido-2-pyridylmethyl)(carboxy-methyl)amine), for an active site of Fe-containing SOD enzyme with N_3^- has been accurately designed and prepared, which has been characterized by X-ray structure analysis and UV–vis, ESR, and IR spectroscopies. Reaction of **1** with acid/base in methanol has shown that the superoxide-accessible site is the N_3^--coordination site in the equatorial plane.

Superoxide dismutases (SODs), which are an enzyme found in all cellular organisms, catalyze the disproportionation of superoxide anion to hydrogen peroxide and dioxygen with an uptake of two protons.[1] Three structurally-different types of SODs have been identified: Cu/Zn-, Mn- or Fe-, and Ni-containing enzymes.[1,2] For the SOD mechanisms, in which the iron ion alternates between the +3 and +2 oxidation states, two different mechanisms have mainly been proposed from several experimental results based on functional models: inner sphere (via binding for metal ions) and outer sphere ones (via coordinated water/hydroxide) for reaction of Fe-SOD with superoxide.[3,4] Recently, the crystal structure of the oxidized form of Fe-SOD (N_3-Fe-SOD) (from *Escherichia coil*) with azide as an inhibitor has been reported,[5] which has revealed that the iron center is coordinated with three histidine imidazoles, one aspartate carboxylate, one hydroxide, and one azide ligand (Chart 1a). In the paper, the equatorial site where azide anion coordinates has been suggested as the binding site of superoxide.

Chart 1



However, the accessible site of superoxide to the iron ion, hydroxide- or azide-coordination site (Scheme 1), has not been confirmed yet.[3−6]

Although some iron(III) complexes coordinated with ligands such as pyridine,[7] imidazole,[8] and carboxylate derivatives[9] have been reported as a reaction model for Fe-SOD, there is no report of an accurate structural model of the active site for Fe-SOD enzyme until now. We have now designed and prepared a new tripodal tetradentate ligand HBPGA (bis(6-pivalamido-2-pyridylmethyl)(carboxymethyl)-amine), which has two pyridine nitrogens, one amine nitrogen, and one carboxylate oxygen as the coordination sites and two pivalamido groups as the surrounding sphere around the active center. The HBPGA ligand can bind to the iron(III) center in a four-coordination mode, which provides two vacant coordination sites on the metal center to accommodate the fifth and sixth external ligands. Here,

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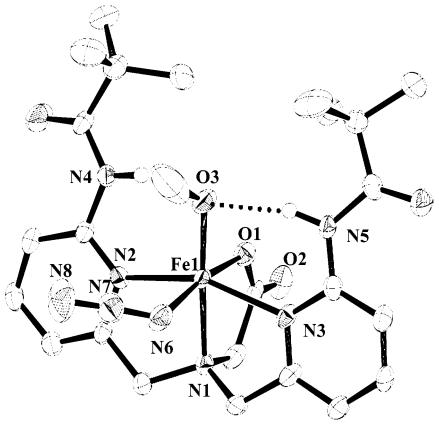

Figure 1. ORTEP view of **1**, showing 30% probability thermal elipsoids. The counteranions and hydrogen atoms are omitted except for NH hydrogens for clarity. Selected bond lengths (Å) and angles (deg): Fe1−O1 2.003(3), Fe1−O3 1.866(3), Fe1−N1 2.194(3), Fe1−N2 2.193(3), Fe1−N3 2.223(3), Fe1−N6 2.135(4); O3···N4 2.971(3); O3···N5 2.966(3); O1−Fe1−O3 90.3(1), O1−Fe1−N1 81.6(1), O1−Fe1−N2 87.0(1), O1−Fe1−N3 86.8(1), O1−Fe1−N6 170.7(1), O3−Fe1−N1 174.5(1), O3−Fe1−N2 103.8(1), O3−Fe1−N3 102.1(1), O3−Fe1−N6 96.3(1), N1−Fe1−N2 77.1(1), N1−Fe1−N3 76.7(1), N1−Fe1−N6 89.1(1), N2−Fe1−N3 153.7(1), N2−Fe1−N6 90.4(1), N3−Fe1−N6 91.6(1).

using the HBPGA ligand, we describe the preparation of [Fe(bpga)(N_3)(OCH_3)] (**1**) (Scheme 1b) and its structural characterization on the basis of X-ray structure analysis and UV−vis, ESR, and IR spectroscopies.

Complex **1** has been prepared by reaction of $Fe(ClO_4)_3 \cdot 6H_2O$ with equimolar amounts of HBPGA, KOH, and NaN_3 in methanol, and has been isolated as red crystals, which are stable for a few months under air at ambient temperature. The IR spectrum of **1** showed a characteristic N═N stretching vibration derived from N_3 ion at 2037 cm^{-1}, which was detected at a lower wavenumber than that of NaN_3 (2063 cm^{-1}). The crystal structure of **1** (Figure 1)[10] revealed a distorted octahedron where the iron atom was surrounded with the tripodal tetradentate BPGA ligand and two monodentate CH_3O^- and N_3^- ligands with a cis configuration. The coordination geometry and bond parameters for **1** are quite similar to those of N_3-Fe-SOD (Chart 1a). The bond lengths from Fe to the coordinating atoms in complex **1** are comparable to those in N_3-Fe-SOD: Fe−N(6) is 2.135(4) Å vs the Fe−azide distance of 2.12 Å in N_3-Fe-SOD, Fe−N(2) = 2.193(3), Fe−N(3) = 2.223(3), and Fe−N(1) = 2.194(3) Å vs Fe-histidine imidazole nitrogens in enzyme (2.13, 2.15, and 2.21 Å), Fe−O(1) = 2.003(3) Å vs Fe-aspartate carboxylato oxygen (2.03 Å), and Fe−O(3) = 1.866(3) Å vs Fe-aquo/hydroxo oxygen (2.00 Å), respectively.[5] Furthermore interestingly, complex **1**, which is similar to the case of natural N_3-Fe-SOD enzyme, exhibited some intramolecular hydrogen bonds between two NH groups of BPGA and methoxide oxygen (N4···O3 = 2.971(3) and N5···O3 = 2.966(3) Å). These hydrogen bonds might contribute to higher stability of the (methoxo)iron(III) core structure of **1**. Moreover, the sterically bulky

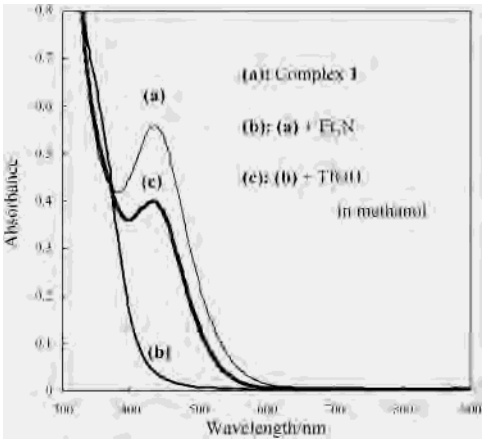

Figure 2. UV−vis spectra of **1** (a), after addition of 1.0 equiv of Et_3N into a solution of **1** (b), after addition of 1.0 equiv of trifluoromethane sulfonic acid (TfOH) into a solution of **1** containing 1.0 equiv of Et_3N (c).

pivalamido *tert*-butyl groups of BPGA prevent the dimerization of the complex.

The structure and reactivity of complex **1** in solution, judging from the redox potential value and ESR and electronic absorption spectra, are quite similar to those of natural Fe-SOD enzyme. A methanol solution of **1** demonstrated a characteristic ESR signal at $g = 4.23$ (at 4 K) suggesting a rhombic high-spin Fe^{III} center, which agrees well with that of N_3-Fe-SOD enzyme ($g = 4.27$).[11] Cyclic voltammetry of complex **1** revealed a quasi-reversible wave at $E_{1/2} = +386$ mV ($\Delta E = 140$ mV) vs NHE in methanol at ambient temperature, which corresponds to the Fe^{3+}/Fe^{2+} couple. This value agrees well with that of Fe-SOD enzyme (+220 mV vs NHE).[1,12] The UV−vis spectrum of complex **1** in methanol gave a distinct band at 436 nm ($\epsilon = 2300$ M^{-1} cm^{-1}), assignable to the LMCT band from azide to iron(III) (Figure 2a), which agrees well with that of N_3-Fe-SOD having a broad absorption band at 440 nm ($\epsilon = 1660$ M^{-1} cm^{-1} in H_2O).[6] Such similarities in structural, spectroscopic, and electrochemical properties between complex **1** and Fe-SOD enzyme indicate clearly that the model iron(III) complex **1** which we have constructed is important in the construction of the iron(III) active center of Fe-SOD.

The complex **1** exhibited the following interesting behavior upon addition of base/acid: Upon reaction of complex **1** with Et_3N as a base in methanol, a yellow complex was obtained, which was determined to be dimethoxo iron(III) complex, [Fe(bpga)(OCH_3)$_2$] (**2**), based on the results of UV−vis (Figure 2b) and ESI-mass spectroscopies. The LMCT band observed at 436 nm decreased upon addition of base (Figure 2b), and the ESI-mass spectrum gave a prominent feature at *m*/*z* 674.2 corresponding to $\{[Fe(bpga)(OCH_3)_2](Et_3NH)\}^+$ (**2** + Et_3NH^+). Complex **2** showed an ESR signal at $g = 4.19$ which is characteristic of a rhombic high-spin Fe^{III} center. In addition, the LMCT band at 436 nm has been regenerated by addition of acid (Figure 2c), and the crystal

(10) X-ray crystal data of **1**: $C_{26}H_{38}FeO_6N_8$, $M_w = 614.48$, monoclinic, space group $P2_1/c$, $a = 12.729(1)$, $b = 15.732(1)$, $c = 14.594(2)$ Å, $\beta = 92.578(5)°$, $V = 2919.5(5)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.398$ gcm^{-3}, μ(Mo Kα) = 5.70 cm^{-1}, $R_1 = 0.057$, $R_w = 0.177$.

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structure of the reproduced complex agrees completely with complex **1**. Therefore, it is clear that the reaction of complex **2** with trifluoromethane sulfonic acid as an acid source in azide anion-containing methanol solution has reproduced complex **1**. The above findings suggest that the methoxide anion *trans* to the carboxylate oxygen in complex **2** is easily substituted in comparison with another methoxide ion in the *cis* position. This implies that the methoxo ligand in the *cis* position for the carboxylate is maintained by hydrogen bonding and hydrophobic interactions of pivalamido groups and the binding/releasing site of azido ligand can be controlled by addition of acid/base, that is, by pH condition of the reaction solution. In the case of N_3-Fe-SOD from *E. coli*, the release of azide ion was significantly observed at high pH condition (pH 8.2),[13] in analogy with the reaction behavior of complex **1**.

Furthermore, to estimate the reactivity of complex **1** for superoxide, the reaction of complex **1** with KO_2 was performed in methanol solution at −78 °C. Interestingly, the solution color changed clearly from reddish brown to yellow, which was monitored by electronic absorption and ESR spectroscopies. The LMCT band from azide to iron(III) observed at 436 nm and the ESR signal at $g = 4.23$ decreased both upon addition of KO_2. Instead, ESR signals at $g = 2.07$, 2.00, which originated from an excess amount of superoxide radical, were observed, indicating the generation of Fe(II) species. In addition, the addition of $HClO_4$ as acid to this solution allowed the regeneration of the LMCT band at 436 nm and ESR signal at $g = 4.23$, and also the complex obtained from the solution showed completely the same crystal structure as complex **1**. The addition of acid into the reaction solution in the presence of reduced complex **1**(Fe(II)) will bring the π^* orbital of the remaining O_2^- down to give $HOO^\bullet$ with higher oxidation ability, which is thought that it oxidized from iron(II) to iron(III). These findings indicate that the complex **1** has the same SOD function as Fe-SOD enzymes.

In summary, we have succeeded in constructing a new Fe(III) complex (**1**) using the novel ligand HBPGA, which has been designed to mimic the active center of Fe-SOD. Complex **1** has formed a mer configuration for three N-donor ligands and the two pivalamido NH groups of BPGA hydrogen-bonded with the axially coordinated oxygen atom, which agrees well with the active site of N_3-Fe-SOD and its peripheral structures. This is the first accurate structural model for the active center of Fe-SODs. From the difference in the reaction behaviors between [(azido)(methoxo)(bpga)-iron(III)] (**1**) and [$(methoxo)_2$(bpga)iron(III)] (**2**), the methoxide anion in the trans position for carboxylate oxygen in complex **2** is accessible compared with that in the cis position. This suggests that the cis methoxide ion is stabilized by hydrogen bonds and hydrophobic sphere. Furthermore, the complex **1** is demonstrated to react catalytically for superoxide anion; the addition of KO_2 to complex **1** caused the reduction of Fe(III) to Fe(II), accompanied by release of N_3^-, and then the addition of acid caused consumption of excess O_2^- to reproduce the complex **1**. Our results suggest that not hydroxide but azide coordination site in Fe-SOD participates for attack of superoxide anion.

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Supporting Information Available: Crystallographic data including positional parameters, thermal parameters, and bond distances and angles (CIF). Figures S1 and S2 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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