

Characterization of imidazolate-bridged Cu(II)–Zn(II) heterodinuclear and Cu(II)–Cu(II) homodinuclear hydroperoxo complexes as reaction intermediate models of Cu,Zn–SOD

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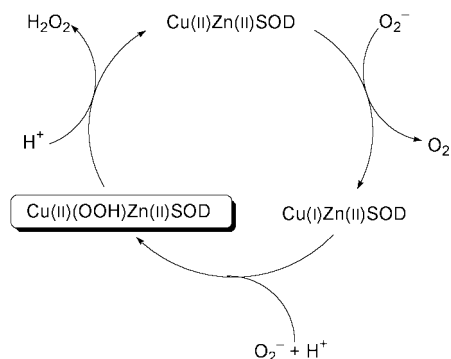
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Imidazolate-bridged Cu(II)–Zn(II) heterodinuclear and Cu(II)–Cu(II) homodinuclear hydroperoxo complexes are generated in the reactions between imidazolate-bridged heterodinuclear and homodinuclear complexes and H₂O₂ in the presence of triethylamine base and characterized spectroscopically as reaction intermediate models of Cu,Zn–SOD.

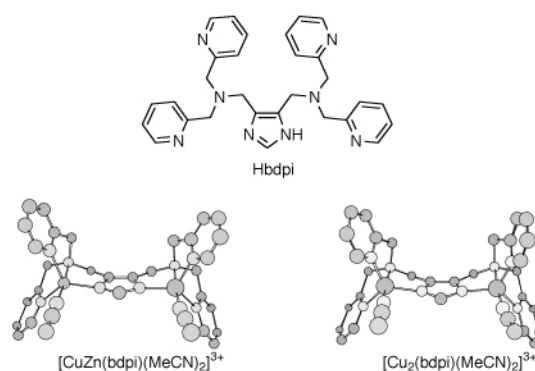
Copper–zinc superoxide dismutase (Cu,Zn–SOD) contains an imidazolate-bridged Cu(II)–Zn(II) heterodinuclear metal center in its active site.^{1–6} This enzyme catalyses a very rapid two-step dismutation of superoxide to dioxygen and hydrogen peroxide through an alternate reduction and oxidation of the active-site copper ion as shown in Scheme 1.^{5–7} An outer-sphere electron transfer from superoxide to the Cu(II) center occurs to produce O₂ and a Cu(I) center which may be oxidized by another molecule of superoxide in the presence of protons to produce H₂O₂ via a hydroperoxo–Cu(II) species (Scheme 1).^{6,7} The hydroperoxo–copper(II) species is a key intermediate in biological oxidations catalyzed by copper enzymes including SOD. However, hydroperoxo–copper(II) complexes have been rarely characterized,⁸ and there has been no report on the characterization of SOD model hydroperoxo–copper(II) complexes.

Several imidazolate-bridged heterodinuclear complexes have so far been reported as models of Cu,Zn–SOD using two independent mononuclear complexes and a bridging imidazolate ring.⁹ However, the lack of binding site of O₂^{•−} in these dinuclear complexes has precluded the characterization of the hydroperoxo–copper(II) intermediate. We have recently prepared imidazolate-bridged Cu(II)–Zn(II) heterodinuclear and Cu(II)–Cu(II) homodinuclear complexes with a newly designed dinucleating ligand, Hbdpi {Hbdpi = 4,5-bis[di(2-pyridylmethyl)aminomethyl]imidazole}.¹⁰

These SOD model complexes have a pentacoordinate structure at each metal ion including a solvent molecule which



Scheme 1



can be readily replaced by a substrate. We report herein the first characterization of SOD model hydroperoxo–Cu(II) intermediates generated by the reactions of hydrogen peroxide with imidazolate-bridged Cu(II)–Zn(II) heterodinuclear and Cu(II)–Cu(II) homodinuclear complexes.

The addition of a large excess of hydrogen peroxide to a MeOH solution of [CuZn(bdpi)(MeCN)₂](ClO₄)₃·2MeCN **1** or [Cu₂(bdpi)(MeCN)₂](ClO₄)₃·MeCN·3H₂O **2** in the presence of a base such as triethylamine at –80 °C resulted in slight color change from greenish blue to green. The absorption spectra of the resulting solutions are shown in Fig. 1, where an intense band at 360 nm is observed in each case.

No decay of these bands was observed at –80 °C but they disappeared at room temperature. The absorbance of the

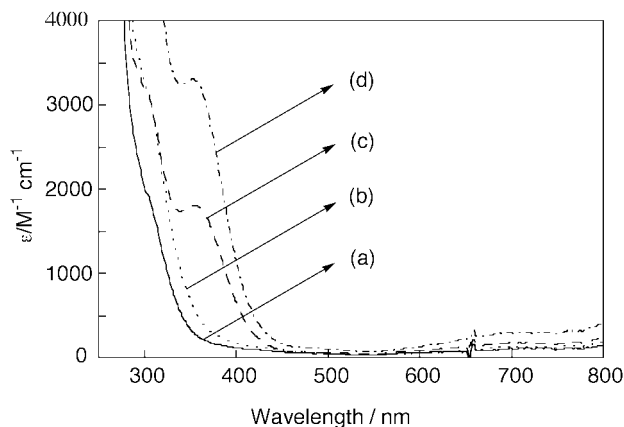


Fig. 1 Absorption spectra of (a) complex **1** (0.2 mM) and (b) **2** (0.2 mM) and those observed upon addition of a large excess of H₂O₂ to a MeOH solution of (c) **1** and (d) **2** in the presence of triethylamine at –80 °C.

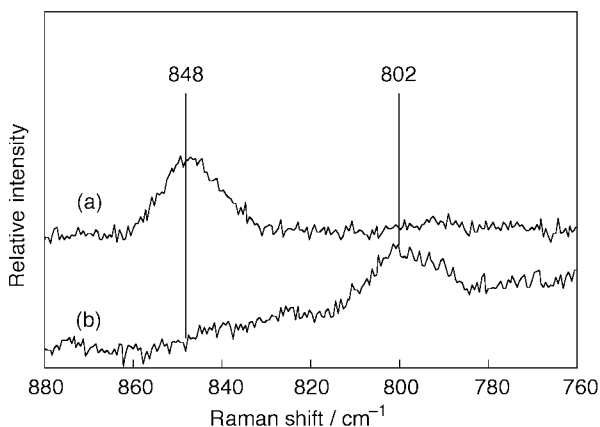


Fig. 2 Resonance Raman spectra obtained with 351 nm excitation of (a) a large excess $\text{H}_2^{16}\text{O}_2$ and (b) $\text{H}_2^{18}\text{O}_2$ in a MeOH solution of complex **1** in the presence of triethylamine at -80°C .

intermediate **3** derived from complex **1** is about half that of intermediate **4** derived from the same concentration of **2**.

The resonance Raman spectrum of **3** in MeOH measured at -80°C (laser excitation wavelength 351 nm) shows (Fig. 2) a strong resonance-enhanced Raman band at 848 cm^{-1} , which shifted to 802 cm^{-1} ($\Delta\nu = 46\text{ cm}^{-1}$) when ^{18}O -labeled H_2O_2 was used. The resonance Raman spectrum of **4** was essentially the same as that of **3** ($\text{H}_2^{16}\text{O}_2$: 848 cm^{-1} , $\text{H}_2^{18}\text{O}_2$: 802 cm^{-1} , $\Delta\nu = 46\text{ cm}^{-1}$). The ν value (848 cm^{-1}) of **3** is slightly lower than the value (856 cm^{-1}) reported for the mononuclear hydroperoxo-Cu(II) complex $[\text{Cu}(\text{II})(\text{bppa})(\text{OOH})](\text{ClO}_4)$ [bppa = bis(6-pivalamide-2-pyridylmethyl)(2-pyridylmethyl)amine], which is the only example of a structurally characterized hydroperoxo-Cu(II) complex.^{8c} The $\Delta\nu$ value of **3** corresponds exactly to that of $[\text{Cu}(\text{II})(\text{bppa})(\text{OOH})](\text{ClO}_4)$ (46 cm^{-1}) in $\text{H}_2^{18}\text{O}_2$.^{8c} The hydroperoxo-Cu(II) complex also has an intense absorption band at 380 nm which is slightly red-shifted as compared to **3** (Fig. 1).^{8c} The intense absorption band has been assigned to the charge-transfer transition of the hydroperoxo group to copper(II) ion (LMCT).^{8c}

The resonance Raman and absorption spectra in comparison with those of $[\text{Cu}(\text{II})(\text{bppa})(\text{OOH})](\text{ClO}_4)$ strongly indicate formation of hydroperoxo-Cu(II) intermediates, $[\text{CuZn}(\text{bdpi})(\text{OOH})]^+$ **3** and $[\text{Cu}_2(\text{bdpi})(\text{OOH})_2]^+$ **4** in the reactions of **1** and **2** with H_2O_2 . This assignment is consistent with the half intensity in the absorption band at 360 nm of **3** as compared to **4** (Fig. 1), since **4** contains two Cu-OOH moieties.

Formation of hydroperoxo-Cu(II) intermediates was further confirmed by the ESI mass spectra of **3** and **4** in MeOH at -80°C , which exhibited signals at m/z 651 and 681, respectively.¹¹ The observed mass and isotope patterns corresponded to the ions $[\text{CuZn}(\text{bdpi})(\text{OOH})]^+$ and $[\text{Cu}_2(\text{bdpi})(\text{OOH})_2]^+$, respectively. The use of $\text{H}_2^{18}\text{O}_2$ instead of $\text{H}_2^{16}\text{O}_2$ resulted in the expected change of mass numbers of **3** and **4** to m/z 655 and 685, respectively.

The EPR spectrum of **3** was typical of a trigonal-bipyramidal copper complex ($g_{\parallel} = 2.09$, $g_{\perp} = 2.22$, $A_{\parallel} = 8.9\text{ mT}$ and $A_{\perp} = 11.3\text{ mT}$ at 77 K in MeOH). These EPR parameters are quite close to those of **1** ($g_{\parallel} = 2.10$, $g_{\perp} = 2.25$, $A_{\parallel} = 10.4\text{ mT}$ and $A_{\perp} = 11.5\text{ mT}$ at 77 K in MeOH) and the X-ray structure of **1** shows a trigonal bipyramidal coordination environment of the

copper ion.¹⁰ Thus, the copper coordination site of **1** occupied by a solvent molecule may be substituted with hydroperoxide in the reaction with H_2O_2 in the presence of triethylamine base without changing the copper coordination geometry. The EPR spectrum of **4** gave a fairly broad signal centered at $g \cong 2.13$ without hyperfine structure, and this spectral feature is the same as that of **2**. This also indicates that the structure of the intermediate **4** maintains the imidazolate-bridged structure.

These results clearly demonstrate that hydroperoxo-Cu(II) complexes **3** and **4** are generated from imidazolate-bridged Cu(II)-Zn(II) and Cu(II)-Cu(II) SOD model complexes **1** and **2** which utilize their binding sites by reaction with H_2O_2 in the presence of triethylamine base. The hydroperoxo-Cu(II) complexes characterized in this study could serve as SOD intermediate models, providing valuable insight into the SOD catalytic mechanism.

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Notes and references

- 1 I. Fridovich, *J. Biol. Chem.*, 1989, **264**, 7761; I. Fridovich, *Annu. Rev. Biochem.*, 1995, **64**, 97.
- 2 I. Bertini, L. Banci and M. Piccioli, *Coord. Chem. Rev.*, 1990, **100**, 67.
- 3 J. A. Tainer, E. D. Getzoff, K. M. Beem, J. S. Richardson and D. C. Richardson, *J. Mol. Biol.*, 1982, **160**, 181.
- 4 J. A. Tainer, E. D. Getzoff, J. S. Richardson and D. C. Richardson, *Nature*, 1983, **306**, 284.
- 5 P. J. Hart, M. M. Balbirnie, N. L. Ogihara, A. M. Nersissian, M. S. Weiss, J. S. Valentine and D. A. Eisenberg, *Biochemistry*, 1999, **38**, 2167.
- 6 E. M. Fielden, P. B. Roberts, R. C. Bray, D. J. Lowe, G. N. Mautner, G. Rotilio and L. Calabrese, *Biochem. J.*, 1974, **139**, 49.
- 7 L. M. Ellerby, D. E. Cabelli, J. A. Graden and J. S. Valentine, *J. Am. Chem. Soc.*, 1996, **118**, 6556.
- 8 (a) K. D. Karlin, R. Ghosh, R. W. Cruse, A. Farooq, Y. Gultneh, R. R. Jacobson, N. J. Blackburn, R. W. Strange and J. Zubieta, *J. Am. Chem. Soc.*, 1988, **110**, 6769; (b) R. R. Tahir, N. N. Murthy, K. D. Karlin, N. J. Blackburn, S. N. Shaikh and J. Zubieta, *Inorg. Chem.*, 1992, **31**, 3001; (c) A. Wada, M. Harata, K. Hasegawa, K. Jitsukawa, H. Masuda, M. Mukai, T. Kitagawa and H. Einaga, *Angew. Chem., Int. Ed.*, 1998, **37**, 798.
- 9 M. Sato, S. Nagae, M. Uehara and J. Nakaya, *J. Chem. Soc., Chem. Commun.*, 1984, 1661; Q. Lu, Q. H. Luo, A. B. Dai, Z. Y. Zhou and G. Z. Hu, *J. Chem. Soc., Chem. Commun.*, 1990, 1429; M. Zongwan, C. Dong, T. Wenxia, Y. Kaibei and L. Li, *Polyhedron*, 1992, **11**, 191; J.-L. Pierre, P. Chautemps, S. Refaif, C. Beguin, A. E. Marzouki, G. Serratrice, E. Saint-Aman and P. Rey, *J. Am. Chem. Soc.*, 1995, **117**, 1965; Z.-W. Mao, M.-Q. Chen, X.-S. Tan, J. Liu and W.-X. Tang, *Inorg. Chem.*, 1995, **34**, 2889.
- 10 H. Ohtsu, Y. Shimazaki, A. Odani, O. Yamauchi, S. Itoh and S. Fukuzumi, *J. Am. Chem. Soc.*, 2000, **122**, in press.
- 11 The flow pass (fused silica capillary, ca. 400 mm long, 0.075 mm inner diameter) in the system was precooled by flushing with MeOH cooled at -80°C in an acetone/dry ice bath prior to measurements. A solution of **3** or **4** cooled at -80°C was delivered to the sprayer through the fused silica capillary under a constant Ar pressure (0.1 MPa). The sprayer was held at a potential of -4.5 kV , and compressed N_2 was employed to assist liquid nebulization. The orifice potential was maintained at -25 V . The positive ion ESI mass spectra were measured in the range m/z 100–1000.