

# A novel imidazolate-bridged copper–zinc heterodinuclear complex as a Cu, Zn–SOD active site model

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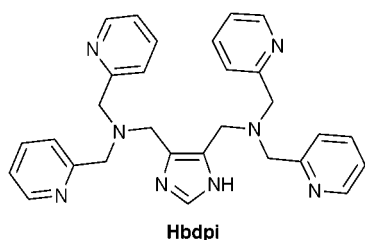
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A novel imidazolate-bridged Cu(II)–Zn(II) heterodinuclear complex of an imidazole derivative containing two metal-binding groups has been synthesized, and its structure and properties have been clarified.

Copper–zinc superoxide dismutase (Cu, Zn-SOD) contains an imidazolate-bridged Cu–Zn heterodinuclear metal center in its active site.<sup>1–3</sup> This enzyme catalyzes the dismutation of highly toxic superoxide anion produced as a byproduct of dioxygen and hydrogen peroxide in a sequence of dispersion-controlled reactions.<sup>4</sup> Since the enzyme structure was determined by X-ray analysis,<sup>1</sup> the unique active site structure attracted much attention, and a number of imidazolate-bridged complexes, such as the Cu(II)–Cu(II) homodinuclear complexes by Lippard *et al.*,<sup>5</sup> have been reported as mimics of the metal site and provided valuable insights into the structure and properties of the SOD active site. In contrast to this, only a few imidazolate-bridged Cu(II)–Zn(II) heterodinuclear model complexes have so far been reported.<sup>6</sup> Because such model complexes are often constructed by self-assembly of one or two complex units by incorporating a bridging imidazole ring, the labile nature of Cu(II) and Zn(II) complexes and dissociation of self-assembled structures made it difficult to study in detail their structures in solution and SOD functions. We here report the first imidazolate-bridged Cu(II)–Zn(II) heterodinuclear complex derived from a new imidazole derivative, Hbdpi {Hbdpi = 4,5-bis[di(2-



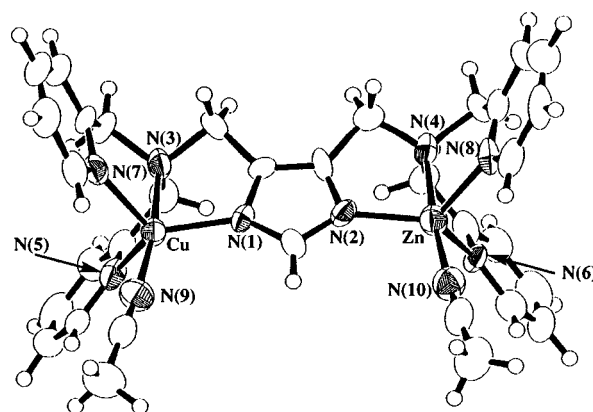
pyridylmethyl)aminomethyl]imidazole}, which has two sets of 3N donor groups tethered to the 4 and 5 positions of the bridging imidazole ring.

Hbdpi was prepared from imidazole-4,5-dialdehyde<sup>7</sup> and bis(2-pyridylmethyl)amine by reductive aminomethylation using sodium cyanoborohydride in methanol, and was characterized by <sup>1</sup>H and <sup>13</sup>C NMR.<sup>‡</sup> The heterodinuclear Cu(II)–Zn(II) complex of bdpi, [CuZn(bdpi)(MeCN)<sub>2</sub>](ClO<sub>4</sub>)<sub>3</sub>·2MeCN **1**, was obtained as crystals by mixing Hbdpi and equimolar amounts of Cu(II) and Zn(II) in methanol and subsequent addition of triethylamine, and the composition of the dried sample of **1** was determined by elemental analysis, ICP, and ESI-mass spectral measurements. The corresponding Cu(II)–Cu(II) homodinuclear complex, [Cu<sub>2</sub>(bdpi)(MeCN)<sub>2</sub>](ClO<sub>4</sub>)<sub>3</sub>·2H<sub>2</sub>O **2** was prepared in a similar manner using two equivalents of Cu(II).

The molecular structure of complex **1** was determined by X-ray analysis§ and is depicted in Fig. 1. As expected, the complex

has an imidazolate-bridged dinuclear structure. In line with the previous observation,<sup>6d</sup> the copper and zinc sites are very similar to each other and indistinguishable, because the complex has a C<sub>2v</sub>-like axis and both metal sites are statistically disordered. The metal ions are both in an approximately trigonal bipyramidal geometry with four nitrogen atoms of bdpi and a nitrogen atom of the solvent MeCN coordinated. The coordination site occupied by MeCN may be susceptible to ligand substitution, providing a possible binding site for substrate superoxide. The Cu(II)–Zn(II) distance of 6.197(2) Å in **1** is the same as that of native Cu, Zn-SOD (6.2 Å).<sup>1</sup>

The electronic spectrum of an acetonitrile solution of **1** (1 mM) showed two broad d–d bands at 645 and 880 nm ( $\epsilon = 60$  and 220 M<sup>-1</sup> cm<sup>-1</sup>, respectively). This spectrum is very close to that of [Cu(tpa)Cl]PF<sub>6</sub> [tpa = tris(2-pyridylmethyl)amine], indicating a trigonal bipyramidal structure at the copper site.<sup>8</sup> In addition, it exhibited an imidazolate-to-Cu(II) charge transfer (CT) band at 320 nm ( $\epsilon = 1500$  M<sup>-1</sup> cm<sup>-1</sup>). The intensities of the d–d and CT bands for **1** were half as strong as those observed for **2** at 645, 880 and 320 nm ( $\epsilon = 110$ , 420 and 3000 M<sup>-1</sup> cm<sup>-1</sup>, respectively). The EPR spectrum of a solution of **1** (0.5 mM) in 1:1 MeCN–MeOH at 77 K ( $g_{\parallel} = 2.10$ ,  $g_{\perp} = 2.24$  and  $|A_{\perp}| = 12.0$  mT) showed a line shape characteristic of mononuclear trigonal bipyramidal Cu(II) complexes,<sup>9</sup> but was very different from that of **2** having a single symmetrical derivative ( $g = 2.13$ ), which is similar to the spectrum of the imidazolate-bridged Cu(II)–glycylglycinate complex<sup>10</sup> and other imidazolate-bridged Cu(II)–Cu(II) dinuclear complexes.<sup>11</sup> These results as well as the ESI-mass spectrum in MeCN demonstrate that **1** in solution retains its imidazolate-bridged



**Fig. 1** X-Ray structure of complex **1** (perchlorate ions and the MeCN molecule of crystallization are omitted for clarity, and the Cu and Zn sites were so assigned that smaller  $R$  and  $R_w$  values could be obtained). Selected bonds (Å) and angles (°): Cu(1)–N(1) 2.001(7), Cu(1)–N(3) 2.104(7), Cu(1)–N(5) 2.074(7), Cu(1)–N(7) 2.105(8), Cu(1)–N(9) 2.004(9), Zn(1)–N(2) 2.005(7), Zn(1)–N(4) 2.112(7), Zn(1)–N(6) 2.076(8), Zn(1)–N(8) 2.084(7), Zn(1)–N(10) 2.023(10), N(1)–Cu–N(3) 82.5(3), N(1)–Cu(1)–N(5) 122.5(3), N(1)–Cu(1)–N(7) 118.3(3), N(3)–Cu(1)–N(9) 172.9(3), N(2)–Zn(1)–N(4) 83.0(3), N(2)–Zn(1)–N(6) 120.3(3), N(2)–Zn(1)–N(8) 120.1(3), N(4)–Zn(1)–N(10) 174.8(3).

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Cu(II)–Zn(II) heterodinuclear structure and is not a 1:1 mixture of Cu(II)–Cu(II) (**2**) and Zn(II)–Zn(II) complexes. Whereas the cyclic voltammogram of **2** in MeCN exhibited two reversible redox waves at  $E_{1/2}$   $-0.09$  and  $+0.19$  V (*vs.* NHE), **1** showed only one reversible Cu(I)/Cu(II) redox couple at  $E_{1/2}$   $+0.19$  V (*vs.* NHE), confirming that it has one copper ion site in the molecule. In this connection, an imidazolate-bridged asymmetric dinuclear Cu(II) complex showed one broad peak in the anodic and the cathodic wave,<sup>12</sup> and several mononuclear trigonal bipyramidal Cu(II) complexes of tripodal ligands containing pyridine and imidazole donors have been reported to have  $E_{1/2}$  values between  $-0.187$  and  $-0.290$  V *vs.* NHE.<sup>13</sup> The positive shift of the  $E_{1/2}$  values of **1** as compared with other mononuclear Cu(II) complexes may therefore be attributed to the electronic effect of the imidazolate-bound Zn(II), which could be an important factor for the efficient catalytic reaction of Cu, Zn–SOD. The SOD activities of **1** and **2** have been investigated by the cytochrome *c* assay<sup>14</sup> using the xanthine oxidase reaction as the source of superoxide. The IC<sub>50</sub> values for **1** and **2** were determined to be 0.24 and 0.32  $\mu\text{M}$  [*vs.* Cu(II) ion], respectively, which are higher than the value reported for native Cu, Zn–SOD (0.04  $\mu\text{M}$ )<sup>15</sup> but comparable with the values of some structurally established model complexes.<sup>6d,12,15</sup> In addition, the IC<sub>50</sub> value for [Cu(tpa)(MeCN)](ClO<sub>4</sub>)<sub>2</sub> was determined to be 1.4  $\mu\text{M}$ . The difference in IC<sub>50</sub> among **1**, **2** and [Cu(tpa)(MeCN)](ClO<sub>4</sub>)<sub>2</sub> may be ascribed to the imidazolate-bridge and zinc ion. The effect of zinc ion was recently reported for a Cu–Zn heterodinuclear complex prepared *in situ*, which was not structurally established.<sup>16</sup>

In conclusion, the new imidazole derivative containing two metal-binding groups incorporated Cu(II) and Zn(II) to form a heterodinuclear SOD model complex which was stable in solution, and the SOD activity measurements exhibited the effects of the imidazolate bridge and zinc ion on the SOD activity. Studies on the details of the SOD function and mechanism are now under way.

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## Notes and references

† *Experimental*: imidazole-4,5-dialdehyde (1.0 g, 8 mmol) was dissolved in methanol (100 ml), to which bis(2-pyridylmethyl)amine (3.2 g, 16 mmol) and a small amount of acetic acid were added. Sodium cyanotrihydroborate (1.0 g, 15.5 mmol) was then added dropwise to the mixture, and after the resulting solution had been stirred for three days at room temperature, it was acidified with concentrated HCl and concentrated almost to dryness under reduced pressure. The residue was dissolved in a saturated aqueous solution of Na<sub>2</sub>CO<sub>3</sub> (50 ml) and extracted with three 50 ml portions of CHCl<sub>3</sub>. The combined extracts were dried over Na<sub>2</sub>SO<sub>4</sub> and after removal of the solvent gave a brown oily product, which was purified by silica gel column chromatography with CHCl<sub>3</sub>–MeOH as eluent to give Hbdpi (3.16 g, 80.0%); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  3.65 (s, 4H), 3.75 (s, 8H), 7.12 (m, 4H), 7.47 (dt, 4H), 7.61 (td, 4H), 7.66 (s, 1H), 8.51 (dq, 4H). <sup>13</sup>C NMR (CDCl<sub>3</sub>; 100 MHz)  $\delta$  48.2 (CH<sub>2</sub>), 59.2 (CH<sub>2</sub>), 121.7 (Py), 122.0 (Im), 123.2 (Py), 133.8 (Im), 136.2 (Py), 148.4 (Py), 158.9 (Py). Complex **1** was prepared by mixing Hbdpi (0.50 g, 1.0 mmol) with a MeOH solution of Cu(ClO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O (0.370 g, 1.0 mmol) and Zn(ClO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O (0.372 g, 1.0 mmol) and adding NEt<sub>3</sub> (138.6  $\mu\text{l}$ , 1.0 mmol). After filtration, the filtrate was allowed to evaporate slowly in the open air to give [CuZn(bdpi)(MeCN)<sub>2</sub>](ClO<sub>4</sub>)<sub>3</sub>·2MeCN **1** as green crystals in 67.0% yield. Elemental analysis and ICP measurement were performed for the dried sample which lost the coordinated MeCN. The ratio of Cu(II) to Zn(II) was determined by ICP (SEIKODENKO SPS-700) with the use of the standard solutions (1000 ppm) of Cu(II) and Zn(II) obtained from Nacal Tesque. Anal. Calc. for [CuZn(bdpi)](ClO<sub>4</sub>)<sub>3</sub>·0.25MeCN·3H<sub>2</sub>O (C<sub>29.5</sub>H<sub>35.75</sub>N<sub>8.25</sub>Cl<sub>3</sub>O<sub>15</sub>CuZn): C, 36.11; H, 3.67; N, 11.78; Cu, 6.48; Zn, 6.66. Found: C, 35.88; H, 3.56; N, 11.77; Cu, 6.46; Zn, 6.65%. ESI-mass (MeCN):  $m/z$  816 [(M – ClO<sub>4</sub>)<sup>+</sup>]. [Cu<sub>2</sub>(bdpi)(MeCN)<sub>2</sub>](ClO<sub>4</sub>)<sub>3</sub>·2H<sub>2</sub>O **2** was prepared in a similar manner by

using Hbdpi and 2 equiv. of Cu(ClO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O. Elemental analysis and ICP measurement were performed for the dried sample. Anal. Calc. for [Cu<sub>2</sub>(bdpi)](ClO<sub>4</sub>)<sub>3</sub>·MeCN (C<sub>31</sub>H<sub>32</sub>N<sub>9</sub>O<sub>12</sub>Cl<sub>3</sub>Cu<sub>2</sub>): C, 38.94; H, 3.37; N, 13.18; Cu, 13.29. Found: C, 38.95; H, 3.32; N, 13.35; Cu, 13.27%. Effective magnetic moment  $\mu_{\text{eff}} = 3.46 \mu_{\text{B}}$  (300 K).

§ *Crystal data*: **1** (green): [CuZn(bdpi)(MeCN)<sub>2</sub>](ClO<sub>4</sub>)<sub>3</sub>·2MeCN, C<sub>37</sub>H<sub>41</sub>N<sub>12</sub>O<sub>13</sub>Cl<sub>3</sub>CuZn,  $M = 1083.09$ , triclinic, space group  $P\bar{1}$ ,  $a = 14.447(1)$ ,  $b = 14.561(1)$ ,  $c = 12.214(1)$  Å,  $\alpha = 108.002(7)$ ,  $\beta = 106.920(7)$ ,  $\gamma = 89.069(7)^\circ$ ,  $V = 2330.3(4)$  Å<sup>3</sup>,  $Z = 2$ ,  $D_c = 1.508$  g cm<sup>-3</sup>, 5067 reflections collected, 4821 independent reflections, 3209 reflections used, 596 variables,  $R = 0.076$ ,  $R_w = 0.110$  [ $I > 2.00\sigma(I)$ ]. The crystal was poorly diffracting. Rigaku AFC-5R four-circle automated diffractometer with graphite monochromated Cu-K $\alpha$  radiation,  $\lambda = 1.54178$  Å, and rotating anode generator. The structures were solved by the heavy-atom method and refined anisotropically for non-hydrogen atoms by full-matrix least-squares calculations. Hydrogen atoms for the structure were located from difference Fourier maps, and their parameters were isotropically refined. All the calculations were performed by using the teXSan crystallographic software package from the Molecular Structure Corporation. CCDC 182/1462. See <http://www.rsc.org/suppdata/cc/1999/2393/> for crystallographic files in .cif format.

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