

## An Imidazolate-bridged Heterobinuclear (Copper–Zinc) Complex of a Macrobicyclic Ligand, a Model of the Active Site of Superoxide Dismutase

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The first imidazolate-bridged heterobinuclear copper–zinc complex of a unique macrobicyclic ligand (cryptand) was prepared and characterized by X-ray diffraction; the complex-catalysed dismutation of superoxide anion survives in the presence of bovine serum albumin.

Copper–zinc superoxide dismutase (SOD) contains in its active site an imidazolate-bridged bimetallic centre [one copper(II) and one zinc(II) atom].<sup>1</sup> Several imidazolate-bridged dicopper complexes have been described with open-chain or monocyclic ligands<sup>2–12</sup> and the pioneering work of Lippard in this field must be emphasized. A few imidazolate-bridged heterobinuclear Cu–Zn complexes have been described.<sup>13–15</sup> They consist of two independent complexes, a Cu<sup>II</sup> and a Zn<sup>II</sup> complex respectively, connected by an imidazolate bridge between the copper and the zinc atoms; this implies that breaking the imidazolate bridge leads to three different molecules.

We describe here the first imidazolate-bridged copper–zinc complex [LCuZn(im)]<sup>3+</sup> of a unique macrobicyclic ligand L (L = 1,4,12,15,18,26,31,39-octaazapentacyclo[13,13,13,16,10,120,24,1,33,37]tetratetracontane-6,8,10,20,22,24,33,35,37-nonaene). We have reproduced the procedure described by Martell<sup>16</sup> leading to the free cryptand L (Fig. 1). A white monozinc complex [LZn](ClO<sub>4</sub>)<sub>2</sub> was prepared by adding one equivalent of zinc perchlorate to a solution of L in acetonitrile, and was characterized by FAB<sup>+</sup> mass spectrometry, <sup>1</sup>H and <sup>13</sup>C NMR. Upon addition of one equivalent of copper perchlorate in the same solvent to the monozinc complex, we obtained a blue solution {heterobinuclear complex [LZnCu](ClO<sub>4</sub>)<sub>4</sub>}. After 30 min., one equivalent of neutral imidazole (Him) in acetonitrile was added. The solution turned dark-blue and was stirred for 3 hours. The complex [LZnCu(im)](ClO<sub>4</sub>)<sub>3</sub> was precipitated in methanol (yield 70%) and recrystallized by slow diffusion of ethyl acetate in acetonitrile solution, giving suitable crystals for X-ray analysis.† The complex was characterized by elemental analysis and FAB<sup>+</sup> mass spectrometry.

The X-ray structure of the complex is depicted in Fig. 2. Each metal atom is five-coordinated with a trigonal bipyramidal arrangement (four nitrogen atoms from L and one from the imidazolate). As both metals were refined as Cu<sup>†</sup> the X-ray results are consistent with an average (Cu + Zn)/2 on each metal site [*i.e.* Cu(or Zn)–N(im) distance of 1.955 Å]. This distance is shorter than in SOD (2.06 Å), inducing a shorter Cu–Zn distance (6 Å instead of 6.3 Å).

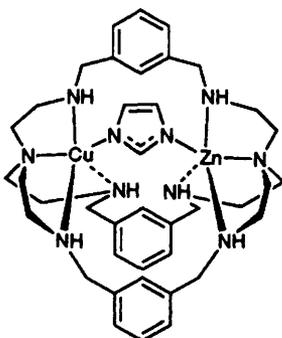


Fig. 1

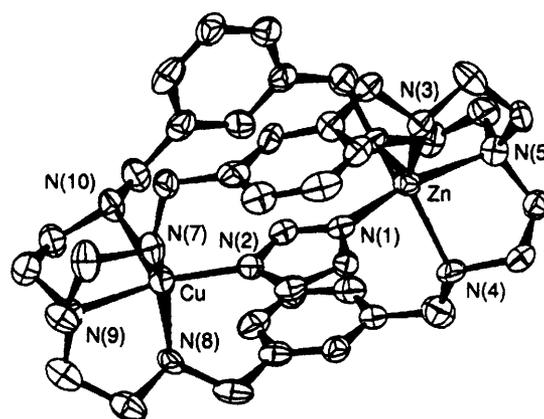


Fig. 2 Molecular structure of [LZnCu(im)](ClO<sub>4</sub>)<sub>3</sub>. Hydrogen atoms and perchlorate group have been omitted. Selected distances (Å): Zn–N(1) 1.97(1), Cu–N(2) 1.95(1), Zn–N(3) 2.12(1), Cu–N(7) 2.14(1), Zn–N(4) 2.15(1), Cu–N(8) 2.14(1), Zn–N(5) 2.12(1), Cu–N(9) 2.09(1), Zn–N(6) 2.11(1), Cu–N(10) 2.14(1).

Spectral characteristics of the complex in solution were examined. The electronic spectrum of a 10<sup>–3</sup> mol dm<sup>–3</sup> solution of [LZnCu(im)](ClO<sub>4</sub>)<sub>3</sub> in water showed the d–d transition absorption band at 700 nm ( $\epsilon = 220 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ). The spectrum was unchanged in the pH range 6.5–10. The EPR spectrum for a 10<sup>–3</sup> mol dm<sup>–3</sup> frozen solution (100 K) of [LZnCu(im)](ClO<sub>4</sub>)<sub>3</sub> in 1:1 (v:v) water–DMSO exhibited the usual line shape for mononuclear Cu<sup>II</sup> complexes, with  $g_{\parallel} > g_{\perp} > 2.03$ , indicating an axial symmetry. The measured values are:  $g_{\parallel} = 2.205$ ,  $g_{\perp} = 2.058$ ,  $A_{\parallel} = 1.45 \times 10^{-2} \text{ cm}^{-1}$  and  $g_{\parallel}/A_{\parallel} = 152 \text{ cm}$ . The spectrum is very similar to that of Cu<sub>2</sub>Zn<sub>2</sub>SOD<sup>17</sup> (with  $g_{\parallel}/A_{\parallel} = 162 \text{ cm}$ ) for which an arrangement around the copper ion intermediate between a trigonal bipyramid and a square pyramid has been postulated.

[LZnCu(im)](ClO<sub>4</sub>)<sub>3</sub> exhibits catalytic activity towards the dismutation of superoxide anion; superoxide was supplied enzymatically from the hypoxanthine–xanthine oxidase reaction to the evaluating system<sup>18</sup> and the SOD activity was evaluated by the NBT assay.<sup>19</sup> The SOD activity survives bovine serum albumin in solution. The IC<sub>50</sub> value, 0.5  $\mu\text{mol dm}^{-3}$ , is significantly lower than the value exhibited by natural SOD (0.04  $\mu\text{mol dm}^{-3}$ ), but of the same order as the values of the best SOD mimics described in the literature.

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### Footnote

† Crystal data: for complex [LZnCu(im)](ClO<sub>4</sub>)<sub>3</sub>; Monoclinic, space group P2<sub>1</sub>/a,  $a = 18.179(4)$ ,  $b = 20.334(5)$ ,  $c = 15.853(5)$  Å,  $\beta = 113.07(1)^\circ$ . A total of 4551 reflections were collected at room temperature on an Enraf-Nonius CAD-4 diffractometer using Mo–K $\alpha$

radiation. The structure was solved by the heavy atom method and refined by the full-matrix least-squares technique in the anisotropic approximation. All hydrogen atoms were placed in calculated and fixed positions with isotropic thermal parameters. Both metal centres were refined as Cu<sup>II</sup> ions. Convergence was obtained at  $R = 0.103$  and  $R_w = 0.099$ . Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Information for Authors, Issue No. 1.

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