

The syntheses, crystal structure and magnetic behavior of μ -imidazolato-dicopper(II) complexes with the dinucleating hexaazamacrocycles

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Summary

The synthesis, crystal structure and magnetic properties of the imidazolate-bridged dinuclear copper(II) complex $[\text{LCu}_2(\text{Im})](\text{ClO}_4)_3(\text{H}_2\text{O}) \cdot 1/2(\text{MeCN})$, (ImH = imidazole, L = bis-*p*-xylylBISDIEN) have been studied. Single crystal X-ray diffraction determination reveals the distorted square planar geometries of the imidazolate bridged dicopper(II) center are incorporated within the dinucleating macrocycle. The Cu—Cu separation in the complex is 6.005 Å. Magnetic measurements reveal an antiferromagnetic exchange interaction with a coupling constant of $J = -26.52 \text{ cm}^{-1}$. The enzymatic activity of the title complex is 5.9 percent of that of the protein.

Introduction

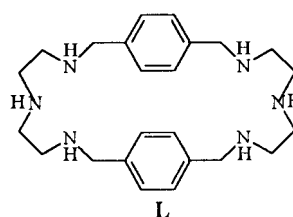
The superoxide anion radical (O_2^-) is a highly toxic species in many biological systems. Superoxide dismutase (SOD) serves as an important means of defense against oxygen toxicity, and catalyzes O_2^- dismutation very efficiently. The structure of Cu, Zn-SOD has been determined by X-ray crystallography⁽¹⁾. Each of the two identical subunits contains an imidazolate-(histidine)-bridged bimetallic active site incorporating one zinc(II) and one copper(II) atom. Of particular interest is the catalytically active four-copper form of the protein ($\text{Cu}_2\text{Cu}_2\text{SOD}$), in which the zinc atoms have been replaced by copper⁽²⁾. Antiferromagnetic coupling of the two copper atoms in each subunit through the imidazolate bridge results in characteristic features in the electron spin resonance (e.s.r.) spectrum, providing a sensitive indicator of the bridged, dinuclear copper(II) center.

As models for the $\text{Cu}_2\text{Cu}_2\text{SOD}$ active site, several imidazolate-bridged dicopper complexes have been prepared and characterized. Simple complexes such as $[(\text{DTMA})_2\text{Cu}_2(\text{Im})](\text{ClO}_4)_3$ ⁽³⁾ proved to be insufficient as models because their imidazolate bridge in aqueous solution is stable only over a very narrow pH range ($8.0 < \text{pH} < 9.5$). Below pH 8, the imidazolate group becomes protonated, breaking the bridge, while above pH 9.5 hydroxide ion competes with imidazolate binding.

In order to obtain imidazolate-bridged dicopper(II) complexes that more faithfully mimic the solution, magnetic, and structural properties of $\text{Cu}_2\text{Cu}_2\text{SOD}$, dinucleating macrocyclic polyamine ligands⁽⁴⁾ have been used to stabilize the $[\text{M—Im—M}]^{3+}$ unit. These dinucleating macrocycles provide a stable environment for the $(\text{Cu—Im—Cu})^{3+}$ or $[(\text{Cu—Im—Zn})^{3+}]$ ion. It

remains intact over a wide pH range, exhibiting a stability similar to that of the imidazolate bridge in the enzyme. The magnetic properties of imidazolated dicopper(II) complexes with these ligands also match those of $\text{Cu}_2\text{Cu}_2\text{SOD}$.

In order to obtain the model compounds whose properties are closer to those of the native enzyme, we employed a dinucleating 26-membered hexaazamacrocyclic ligand L (L = bis-*p*-xylylBISDIEN)⁽⁵⁾ as a ligand for the imidazolate bridged dinuclear copper atoms. Herein we report the synthesis, structure, magnetic behaviour and SOD-like activity of a new copper, zinc-superoxide dismutase model compound.



Experimental

Materials

Reagents were used as obtained without further purification. Solvents were purified by standard methods before use.

L · 6HBr

L · 6HBr was prepared by a procedure similar to that described by Martell⁽⁵⁾. The related macrocyclic-polyamine was obtained according to the following method: L · 6HBr (4.45 g) and NaOH (2 g) were dissolved in H_2O (5 cm^3) and cooled. CH_2Cl_2 (50 cm^3) was then added to the mixture in order to extract the product. The organic phase was evaporated to dryness and the residual solid was recrystallized from the mixture solution of MeOH and MeCN. White crystals (1.70 g) were obtained. The 500 MHz ^1H n.m.r. in D_2O of L gave (ppm from DSS) 3.66, (8H, Benzene— CH_2), 7.31 (aromatic H), 2.59 (16H, NH—CH_2) in a 1:1:2 ratio.

$\text{LCu}_2(\text{Im})(\text{DNSO})_2(\text{ClO}_4)_3$

Under N_2 to a MeOH solution (5 cm^3) of $\text{Cu}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ (186 mg, 0.5 mmol) was added dropwise, during

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Table 1. Elemental analyses of the complexes

| Complexes | Yield (%) | Found (Calcd.) % C | H | N | M |
|---|-----------|-----------------------|----------|------------|------------|
| [LCu ₂ (DMSO) ₂](ClO ₄) ₃ C ₃₁ H ₅₃ N ₈ O ₁₄ Cl ₃ S ₂ Cu ₂ | 55 | 34.9(35.1) | 5.0(5.0) | 10.9(10.5) | 11.7(12.0) |
| [LCu ₂ (Im)](ClO ₄) ₃ (H ₂ O) · 1/2(MeCN) C ₂₈ H _{44.50} Cl ₃ Cu ₂ N _{8.5} O ₁₃ | 32 | 35.5(35.7) | 4.9(4.7) | 12.1(12.6) | 13.2(13.5) |

10 min, a solution of imidazole (34 mg, 0.5 mmol) in MeOH (5 cm³). The resulting blue solution was stirred for 0.5 h, and was then added dropwise to L (103 mg, 0.25 mmol), in MeOH (10 cm³). The resulting solution was then stirred for a further 0.5 h and led to a blue viscous oil, which was dissolved in DMSO (1 cm³) and recrystallized by slow diffusion of MeOH. 65 mg blue prism crystals were obtained.

[LCu₂(Im)](ClO₄)₃(H₂O) · 1/2(MeCN)

The complex was prepared by the same procedure to that used for LCu₂(Im)(DMSO)₂(ClO₄)₃. The resulting blue viscous oil was dissolved in MeCN (5 cm³) and recrystallized by slow diffusion of Et₂O. Blue prism crystals were obtained.

The elemental analyses of the above compounds are listed in Table 1.

Crystal and molecular structure determination

A crystal (0.43 × 0.36 × 0.28) of [LCu₂(Im)](ClO₄)₃(H₂O) · 1/2(CH₃CN) was mounted on a Siemens P4 diffractometer equipped with graphite-monochromated MoKα (λ = 0.71073) radiation. Accurate cell parameters were derived from least-squares fitting of the setting angles of 25 independent reflections in the 5.5–14° range. The relevant crystal data and structural parameters are summarized in Table 2. The intensities were collected at 296 K using 2θ scan mode with a variable scan speed 5.0 to 50.0 degrees per minute in ω. The data were corrected for Lorentz and polarization effects during data reduction using XSCANS⁽⁶⁾. Of the 6838 measured independent reflections, 6722 were observed with I ≥ 2σ, and were used for the structure refinements. The structure was solved by direct methods and refined on F² by full-matrix least-squares methods using SHELXTL version 5.0⁽⁷⁾. All the non-hydrogen atoms were refined anisotropically. All the hydrogen atoms were placed in calculated positions assigned fixed isotropic thermal parameters at 1.2 times the equivalent isotropic U of the

Table 2. Summary of crystal data, data collection and structure refinement for [LCu₂(Im)](ClO₄)₃(H₂O) · 1/2(MeCN)

| | |
|------------------------------------|---|
| Formula | C ₂₈ H _{44.50} Cl ₃ Cu ₂ N _{8.5} O ₁₃ |
| fw | 941.65 |
| crystal system | monoclinic |
| space group | C2/c |
| color | blue |
| a/Å | 35.484(6) |
| b/Å | 8.747(4) |
| c/Å | 26.905(7) |
| β(deg) | 113.56(3) |
| V/Å ³ | 7655(4) |
| Z | 8 |
| T/°C | 295(2) |
| D _c /g cm ⁻³ | 1.634 |
| λ/Å | 0.71073 |
| No. of unique reflections | 6838 |
| No. of observed reflections | 6722[I ≥ 2σ(I)] |
| F(000) | 3880 |
| Crystal size | 0.43 × 0.36 × 0.28 |
| linear abs/mm ⁻¹ | 1.393 |
| R ₁ | 0.0695 |
| R _w | 0.1143 |

atoms to which they are attached and allowed to ride on their respective parent atoms. The contribution of these hydrogen atoms were included in the structure-factors calculations. The O(11), O(12), O(13), O(14), O(21), O(22), O(23), O(24), O(32), O(33) and O(34) atoms of the perchlorate ions were found disordered and the site occupancy factor was fixed at 0.5 for O(11), O(11'), O(12), O(12'), O(13), O(13'), O(14), O(14'), O(21), O(21'), O(22), O(22'), O(23), O(23'), O(24), O(24'), O(32), O(32'), O(33), O(33'), O(34) and O(34'). Analytical expressions of neutral-atom scattering factors employed and anomalous dispersion corrections were incorporated. Selected bond lengths and angles are reported in Table 3.

Physical measurements

¹H n.m.r. spectra were obtained at 319 K on a Bruker AM 500 spectrometer using an Aspect-3000 computer

Table 3. Selected bond distances (Å) and bond angles (°) for [LCu₂(Im)](ClO₄)₃(H₂O) · 1/2(MeCN).

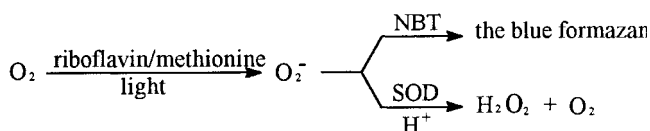
| | | | |
|-----------------------|------------|-----------------|------------|
| <i>Bond distances</i> | | | |
| Cu(1)—N(7) | 1.956(3) | Cu(1)—N(1) | 2.105(3) |
| Cu(1)—N(2) | 2.014(3) | Cu(1)—N(3) | 2.114(3) |
| Cu(2)—N(8) | 1.963(3) | Cu(2)—N(6) | 2.027(3) |
| Cu(2)—N(5) | 2.019(4) | Cu(2)—N(4) | 2.052(3) |
| <i>Bond angles</i> | | | |
| N(7)—Cu(1)—N(2) | 174.53(12) | N(7)—Cu(1)—N(1) | 94.51(11) |
| N(2)—Cu(1)—N(1) | 82.58(12) | N(7)—Cu(1)—N(3) | 101.87(12) |
| N(2)—Cu(1)—N(3) | 81.62(12) | N(1)—Cu(1)—N(3) | 162.25(13) |
| N(8)—Cu(2)—N(5) | 168.4(2) | N(8)—Cu(2)—N(6) | 101.88(13) |
| N(5)—Cu(2)—N(6) | 82.53(14) | N(8)—Cu(2)—N(4) | 97.05(11) |
| N(5)—Cu(2)—N(4) | 81.80(13) | N(6)—Cu(2)—N(4) | 156.00(13) |

* Symmetry transformations used to generate equivalent atoms: #1-x + 2, y, -z + 3/2.

data system. Magnetic measurements on a powder sample were carried out in the 77–300 K range at 7000 G with a CAHN-2000 Faraday-type magnetometer. The molar susceptibilities were corrected for the diamagnetism of the constituent atoms by using Pascal constants.

SOD activity determination

The SOD activities were evaluated by the classical nitro blue tetrazolium (NBT) assay⁽⁸⁾. The principle is as follows:



Superoxide anions were produced from the riboflavin/methionine system. The indicator utilized, in this case, is NBT, which reacts with O_2^- to form blue formazan. Cells containing 3.3×10^{-6} M riboflavin, 0.01 M methionine, 4.6×10^{-5} M NBT, and 0.05 M phosphate buffer at pH 8, and 10^{-7} – 10^{-6} M model complex or 2×10^{-9} – 2×10^{-8} M native Cu, Zn-SOD, were illuminated. The absorbance at 560 nm increased linearly with the illumination time. The reduction of NBT, during 2.5 min of illumination, was measured in terms of increased absorbency at 560 nm on a Shimadzu UV-240 spectrophotometer. All photoinduced reactions were performed at 30 °C.

Results and discussion

Crystal structure description of $[\text{LCu}_2(\text{Im})](\text{ClO}_4)_3(\text{H}_2\text{O}) \cdot 1/2(\text{MeCN})$

The structure of the complex consists of a cation, $[\text{LCu}_2(\text{Im})]^{3+}$, three ClO_4^- anions, half an uncoordinated MeCN molecule and a lattice water molecule. The structure of the cation $[\text{LCu}_2(\text{Im})]^{3+}$ is displayed in Figure 1. Two copper atoms, having similarly square-planar geometry, are present. The Cu(1) atom is four-coordinated by three nitrogen atoms from the framework of the macrocycle and one nitrogen atom from the imidazolate-

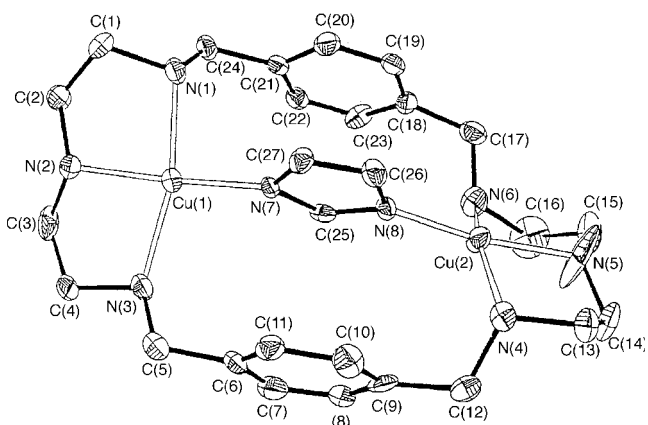
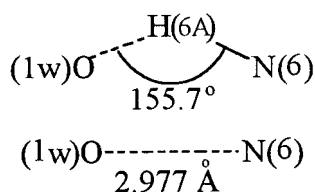


Figure 1. The cation structure of $[\text{LCu}_2(\text{Im})](\text{ClO}_4)_3(\text{H}_2\text{O}) \cdot 1/2(\text{MeCN})$.

bridge. N(1), N(2), N(3), N(7) and Cu(1) constitute the plane with a deviation of 0.089 Å. The N(7)–Cu(1)–N(2) (174.53°) and N(1)–Cu(1)–N(3) (162.25°) angles deviate from 180°, and the bond lengths of Cu(1)–N(1) (2.105 Å) and Cu(1)–N(3) (2.114 Å) are significantly longer than those of Cu(1)–N(2) (2.014 Å) and Cu(1)–N(7) (1.956 Å), which indicate that the coordination geometry around Cu(1) is a slightly distorted square-plane. N(4), N(5), N(6) (from the macrocycle) and N(8) (from the bridged imidazolite) constitute the coordination plane of Cu(2), and the metal is 0.200 Å out-of-the-plane. The N(8)–Cu(2)–N(5) (168.4°) and N(6)–Cu(2)–N(4) (156.00°) angles severely deviate from 180°, indicating that the coordination sphere around Cu(2) is a greatly distorted square-plane. The intramolecular Cu···Cu separation is 6.005 Å, which is shorter than 6.3 Å of Cu–Zn distance in the native Cu, Zn-SOD. This is due to the shorter bonds of copper-imidazolite in the title compound, but is longer than in $[(\text{Cu}(\text{Im})\text{Cu})\text{L}](\text{ClO}_4)_3$ ⁽⁴⁾ (5.95 Å) and that in $[\text{LCu}_2(\text{Im})(\text{DMSO})_2](\text{ClO}_4)_3(\text{DMSO})$ ⁽⁹⁾ (5.93 Å).

The angle between plane N(1)N(2)N(3)N(7) and plane N(4)N(5)N(6)N(8) is 62.9°. The macrocycle adopts a twist stretched conformation, and has no unusual bond lengths or angles. It is interesting to note that the three aromatic rings in each molecule are almost parallel to each other. The angles between the imidazole ring and the phenyl rings are 2.5° with one containing C(6)–C(11) atoms and 1.6° with the other involving C(18)–C(23) atoms. The two phenyl rings are at the angle of 2.9°.

There are several intra- and inter-molecular hydrogen bonds. Unfortunately, they are difficult to describe due to the disorder of the related atoms. A clearly stated intramolecular hydrogen bond is shown in following Scheme:



Magnetic susceptibility of $[\text{LCu}_2(\text{Im})](\text{ClO}_4)_3(\text{H}_2\text{O}) \cdot 1/2(\text{MeCN})$

The temperature dependence of the magnetic susceptibility for the title compound was measured between 77 and 300 K. The magnetic behavior is depicted in Figure 2 in the form of a χ_m^{-1} versus T plot. When the temperature is increased, the χ_m^{-1} value increases continuously. This behavior, together with a monotonous decreasing of $\chi_m T$ upon cooling, is characteristic of an antiferromagnetic exchange interaction between two copper(II) ions. The susceptibility data were analyzed by a modified Bleaney-Bowers equation:⁽¹⁰⁾

$$\chi_\mu = \frac{[2Ng^2\beta^2/kT][3 + \exp(-2J/kT)]^{-1}(1 - \rho)}{+ Ng^2\beta^2/(4kT)^{-1}\rho}$$

where the parameters have their usual meanings. ρ is the mole fraction of paramagnetic impurity. A good fit, as indicated by the solid curve in Figure 2, was achieved with $g = 2.19$, $J = -26.52 \text{ cm}^{-1}$, $\rho = 0.010$ (Coefficient of determination, $R = 0.9998$). The J value

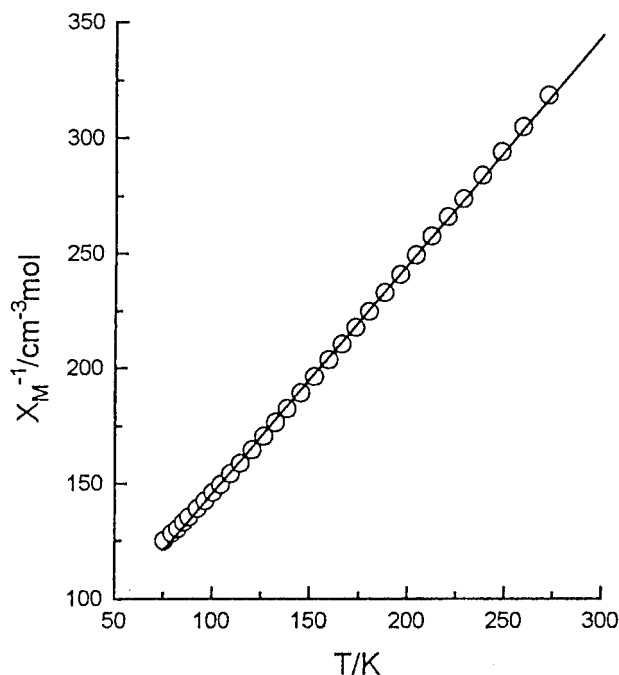


Figure 2. Temperature dependence of the molar susceptibility for $[\text{LCu}_2(\text{Im})](\text{ClO}_4)_3(\text{H}_2\text{O}) \cdot 1/2(\text{MeCN})$.

compares well with that in $[\text{LCu}_2(\text{Im})(\text{DMSO})_2](\text{ClO}_4)_3$ (DMSO), ($J = -26.94 \text{ cm}^{-1}$)⁽¹⁰⁾ and in $\text{Cu}_2\text{Cu}_2\text{SOD}$ ($J = -26.9 \text{ cm}^{-1}$)⁽²⁾, which can be explained by the structural similarity of these compounds. The two models contain tridentate amine ligands with imidazolate as bridging ligand. The unpaired electron of copper(II) occupies the $d_{x^2-y^2}$ magnetic orbital. Taking into account the dihedral angles between the imidazole ring and copper coordination planes (α_1 and α_2) which are 66.2° and 55.8° in the title compound, the observed antiferromagnetic coupling should be predominately mediated through the σ exchange pathway⁽¹⁰⁾.

The ϕ values are 131.8° and 130.6° , which are close to those in $[\text{LCu}_2(\text{Im})(\text{DMSO})_2](\text{ClO}_4)_3(\text{DMSO})$ ⁽¹⁰⁾. Consequently, a moderate antiferromagnetic interaction is propagated through the imidazolite bridge as expected.

Superoxide dismutase activities

The relationship between the inhibition (%) and initial concentration of the compound is shown in Figure 3. The chromophore concentration required to yield 50% inhibition of the reduction of NBT (IC_{50}) was determined by the literature method⁽⁸⁾, and the IC_{50} value of $0.187 \mu\text{mol} \cdot \text{dm}^{-3}$ of the title complex is 17 times higher

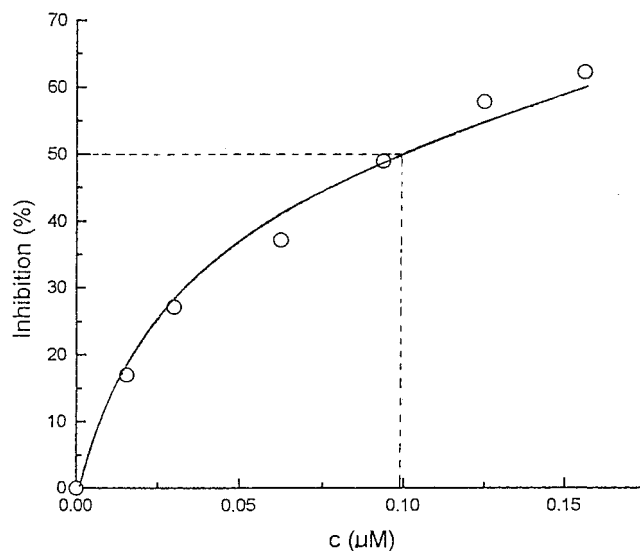


Figure 3. The relationship between the inhibition (%) and initial concentration of the title complex.

than the value exhibited by the native enzyme ($\text{IC}_{50} = 0.011 \mu\text{mol} \cdot \text{dm}^{-3}$), that is, the superoxide dismutase activity of this compound is *ca.* 5.9% of that of the protein.

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