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Synthesis, structure and properties of an imidazolate-bridged dicopper complex of a novel macrocycle with two alcohol-pendants as an active site model of Cu,Zn-SOD

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Abstract

The imidazolate-bridged dicopper complex of a macrocyclic ligand with two hydroxyethyl pendants L, L = 3,6,9,17,20,23-hexaza-6,20-bis(2-hydroxyethyl)-tricyclo[23.3.1.1.^{11,15}]triaconta-1(29),11(30),12,14,25,27-hexaene, has been synthesized as a possible model for Cu,Zn-SOD. Single-crystal X-ray analysis revealed the geometry of the imidazolate-bridged Cu–Cu center, and the distance between the two Cu(II) ions is 5.8135(9) Å. The two metals are each pentacoordinated with geometries between trigonal-bipyramid (TBP) and tetragonal-pyramid (TP). Magnetic measurement of the imidazolate-bridged dicopper complex exhibited the presence of an antiferromagnetic exchange interaction with a coupling constant of $-2J = 72.8$ cm^{-1}. Investigation on the pH-dependent ESR of the complex in frozen 50% aqueous DMSO solution at 110 K revealed the existence of the imidazolate-bridged dicopper structure in solution mainly in the range $5.5 \leq \text{pH} \leq 12$. The SOD activity of the complex has been evaluated by the nitro blue tetrazolium assay and the complex catalyzed the dismutation of superoxide at pH 7.8 with an IC$_{50}$ value of 0.36 μmol dm^{-3}.

Keywords: Cu,Zn-SOD model; Crystal structures; ESR magnetic susceptibility; SOD-like activity

1. Introduction

Copper, zinc-superoxide dismutase (Cu, Zn-SOD) is believed to protect the cell against oxidative damage and inflammation due to toxic oxygen intermediates by the catalytic dismutation of superoxide radicals to oxygen molecules and hydrogen peroxides [1]. X-ray crystal structure analysis shows that the protein from bovine erythrocyte consists of two identical subunits held together almost entirely by hydrophobic interactions. Each unit contains an imidazolate-bridged bimetallic active site incorporating one zinc(II) and one copper(II) atom with a separation of 6.3 Å. The copper ion is coordinated to four imidazolate N atoms of histidine residues and one water molecule in a distorted square-pyramidal geometry, while the zinc ion is coordinated to one carboxylate O atom from an aspartic acid residue and three imidazole N atoms of histidine residues in a distorted tetrahedral structure [2,3]. The copper ion is essential for the SOD activity and if the zinc ion is replaced by another copper ion, the dicopper derivative has the same activity as that of the native enzyme [3]. For this reason, the design and synthesis of imidazolate-bridged dicopper model complexes have intrigued chemists' everlasting interest.

In order to obtain good model complexes with similar structural features as well as biological properties in common with the active site of native SOD, a series of imidazolate-bridged bimetallic complexes have been synthesized and investigated [4–13]. Some examples of imidazolate bridging two mononuclear complexes were reported previously, but the imidazolate bridges in aqueous solution are stable only in a very narrow pH range [5,6]. To improve the stability, some imidazolate-bridged dicopper complexes of macrocycles or cryptands have also been synthesized, and complex **1** shown in Scheme 1 is undoubtedly a successful one [8–13]. In this model complex, the expected stability is achieved

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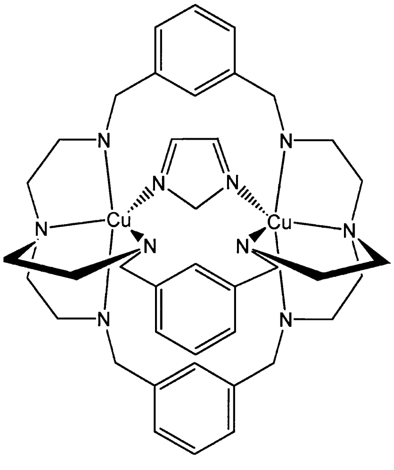

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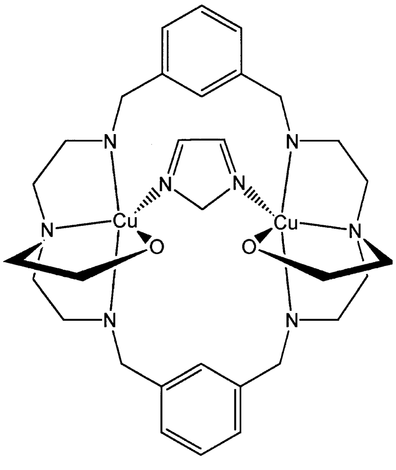

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Scheme 1.

and the imidazolate-bridge in the title compound is stable in the range pH 4.5–12. We thought that the disbanding of one chain in the cryptand ligand in complex **1** by removing a phenyl ring would improve the flexibility of the ligand, and the corresponding model complex is still expected to have a high catalytic activity. Moreover, considering the coordination environment of four N and one O around copper ions in the native enzyme, it is reasonable to replace two N atoms with O atoms. For these reasons, a novel macrocyclic ligand 3,6,9,17,20,23-hexaza-6,20-bis(2-hydroxyethyl)-tricyclo [23.3.1.1.^{11,15}]-triaconta-1(29),11(30),12,14,25, 27-hexaene (L) was synthesized for SOD model complex investigation. The synthesis, X-ray crystal structure determination, magnetic susceptibility measurement, pH-dependent ESR investigation, and the SOD-like activity were studied and reported herein.

2. Experimental

2.1. Physical measurements

Element analysis was performed on a Perkin–Elmer 240C. ES MS spectra were obtained from a Finnigan MAT LCQ ES Mass spectrometer with a mass to charge (*m/s*) range of 1000 and MeOH was used as mobile phase. The polycrystalline powder ESR spectra at room temperature (r.t.) and the 110 K ESR spectra in frozen 50% aq. Me_2SO solutions as a function of pH were recorded with a Bruker 200 D-SRC spectrometer employing X-band radiation and a cylindrical cavity with 100 kHz magnetic field modulation. Calibrations of the microwave frequency were performed with an EIP845A microwave frequency counter. The variable temperature magnetic susceptibility data were obtained on 28.6 mg of polycrystalline sample from 2 to 300 K in a magnetic field of 10 T after zero-field cooling using a SQUID magnetomer. Diamagnetic corrections were estimated from Pascal's constants.

2.2. Materials

2-[bis(2-aminoethyl)amino]Ethanol was prepared as a colorless sticky liquid according to the literature method [14]. All other chemicals used in the experiment were of analytical grade from commercial sources and used without further purification.

2.2.1. Synthesis of ligand L

The hexa hydrobromide salt of L was prepared according to the procedure that we recently described [15]. *Anal*. Calc. for L·6HBr: C, 34.24; H, 5.13; N, 8.56. Found: C, 34.03; H, 5.29; N, 8.29%. The deprotonated hexaaza macrocyclic ligand was obtained as follows: L·6HBr and excessive NaOH were dissolved in a small volume of water, and CH_2Cl_2 was added to extract the product. A colorless viscous oil was obtained when the organic phase was evaporated to dryness.

2.2.2. $[LCu_2(im)](ClO_4)_3(H_2O)_{2.5}(CH_3CN)_{0.5}$

To an aqueous solution (5 ml) of $Cu(ClO_4)_2 \cdot 6H_2O$ (0.125 g, 0.338 mmol), an aqueous solution of imidazole (0.012 g, 0.169 mmol) was added dropwise over 10 min. After the addition of 10 ml aqueous solution of L (0.066 g, 0.169 mmol), an equivalent amount of acetonitrile was added. The solution was then stirred for another 0.5 h at 30 °C to give a dark blue solution. Blue block-like crystals suitable for X-ray analysis were formed 2 days later. *Anal*. Calc. for $[LCu_2(im)](ClO_4)_3(H_2O)_{2.5}(CH_3CN)_{0.5}$: C, 36.34; H, 5.25; N, 11.26. Found: C, 36.66; H, 5.23; N, 11.66%.

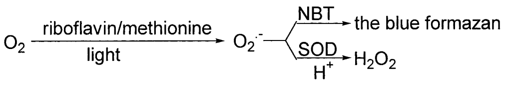
Caution: perchlorate salts of metal complexes with organic ligands are potentially explosive. Only small amounts of these materials should be prepared and they should be handled with great caution.

2.3. X-ray crystallographic study of $[LCu_2(im)](ClO_4)_3(H_2O)_{2.5}(CH_3CN)_{0.5}$

Diffraction data were collected on a Siemens SMART CCD system equipped with monochromated Mo Kα radiation ($\lambda = 0.71073$ Å) at r.t. The data processing and empirical absorption corrections were carried out by SAINT [16] and SADABS [17] programs. The structure was solved by direct methods and refined on F^2 by full-matrix least-squares techniques using the SHELXTL software [18]. Except for the solvent molecules and disordered oxygen atoms in perchlorate anions, the non-hydrogen atoms were refined anisotropically. The hydrogen atoms were placed in their calculated positions and allowed to ride on their respective parent atoms.

2.4. SOD activity determination

The SOD activity was evaluated using the nitro blue tetrazolium (NBT) assay according to the following scheme [19].



Superoxide anions are produced from the riboflavin/methionine system. The indicator utilized in this case is NBT, which reacts with $O_2^{\bullet -}$ to form blue formazane. When the SOD model complex is added into the solution, it inhibits the reaction by reacting with superoxide directly. The value of inhibition can thus be used to measure activity of the SOD model complex. Each time, a cell containing 3.3×10^{-8} M riboflavin, 0.01 M methionine, 4.6×10^{-5}M NBT, 0.05 M phosphate buffer at pH 7.8, 25 °C, and 10^{-7}–10^{-6} M model complex or 2×10^{-9}–2×10^{-8} M native Cu,Zn-SOD, was illuminated for 300 s, and the reduction of NBT under illumination during the 300 s was measured in terms of increased absorbance at 560 nm on a UV-3100 spectrophotometer. The concentration of the complex required to attain 50% inhibition of the reduction (defined as IC_{50}) was determined as an indication of SOD-like activity. The reaction rate in a blank sample was also measured to eliminate effects coming from other impurities. The macrocycle ligand showed no SOD-like activity and the complex did not affect the generation of superoxide anions by directly interacting with the riboflavin/methionine system.

3. Results and discussion

3.1. Crystal structure of $[LCu_2(im)](ClO_4)_3(H_2O)_{2.5}(CH_3CN)_{0.5}$

An ORTEP view of the complex is shown in Fig. 1. Crystallographic data are summarized in Table 1. Selected bond distances and angles are given in Table 2.

The structure consists of a packing of discrete $[LCu_2(im)]^{3+}$ cations, perchlorate anions, acetonitrile molecules and water molecules. In the cation, two Cu(II) ions are bridged by an imidazolate anion with a separation distance of 5.8135(9) Å, which is shorter than that in Cu,Zn-SOD (Cu···Zn = 6.3 Å) and other similar complexes [10,12]. Each Cu(II) ion is penta-coordinated with a coordination geometry between trigonal-bipyramid (TBP) and tetragonal-pyramid (TP). The distortion (Δ) of the coordination polyhedron from a regular TP ($\Delta = 1$) toward a regular TBP ($\Delta = 0$) has been calculated according to the procedure of Mutterties and Galy [20,21]. The calculation results reveal $\Delta = 0.54$ for Cu1 and $\Delta = 0.43$ for Cu2, indicating that the geometry around Cu1 is distorted toward TP and the geometry around Cu2 is distorted toward TBP. The apical position of Cu1 is occupied by O1 appended to an alcohol pendant with bond length Cu1–O1 = 2.233(5) Å, and Cu1 deviates 0.158(2) Å from the basal plane determined by the two secondary amine nitrogen atoms N2, N3, one tertiary amine nitrogen atom N1, and one bridged imidazolate nitrogen atom N7 with bond lengths Cu1–N1 = 2.018(5) Å, Cu1–N2 = 2.076(5) Å, Cu1–N3 = 2.089(4) Å, Cu1–N7 = 1.916(4) Å. The axial positions of Cu2 are occupied by the tertiary amine nitrogen atom N4 and the bridged imidazolate nitrogen atom N8 with the angle N64–Cu2–N8 = 176.0(2)°, and bond lengths of Cu2–N4 and Cu2–N8 are 2.018(4) and 1.920(4) Å, respectively. The Cu2 atom deviates 0.172(2)

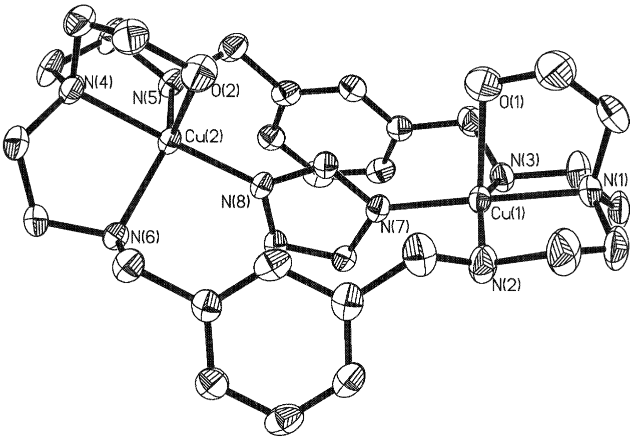


Fig. 1. An ORTEP drawing (30% probability ellipsoids) of $[LCu_2(im)](ClO_4)_3(H_2O)_{2.5}(CH_3CN)_{0.5}$. All hydrogen atoms, perchloride anions and solvent molecules are omitted for clarity.

Table 1
Crystallographic data for $[LCu_2(im)](ClO_4)_3(H_2O)_{2.5}(CH_3CN)_{0.5}$

Formula	$C_{32}H_{55.50}N_{8.50}O_{16.50}Cl_3Cu_2$
Formula weight	1056.78
Crystal system	monoclinic
Space group	$P2(1)/n$
T (K)	293(2)
λ (Å)	0.71073
Color of crystal, habit	dark blue, prism
a (Å)	13.814(1)
b (Å)	23.048(2)
c (Å)	14.095(1)
$\alpha = \gamma$ (°)	90.00
β (°)	102.357(2)
V (Å^3)	4383.5(6)
Z	4
ρ_{calc} (g cm^{-3})	1.601
μ (mm^{-1})	1.232
θ Range (°)	1.72–25.00
Reflections collected	21 544
Independent reflections	7713 [$R_{int} = 0.0456$]
Data/restraints/parameters	7713/0/537
GOF on F^2	1.001
R_1 [a] [$I > 2\sigma(I)$]	0.0666
wR_2 [$I > 2\sigma(I)$]	0.1780

[a] $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, $wR_2 = |\Sigma w(|F_o|^2 - |F_c|^2)|/\Sigma|w(F_o)^2|]^{1/2}$, $w = 1/[(F_o^2)^2 + (0.1103P)^2]$, where $P = (F_o^2 + 2F_c^2)/3$.

Table 2
Selected bond lengths (Å) and angles (°) for $[LCu_2(im)](ClO_4)_3(H_2O)_{2.5}(CH_3CN)_{0.5}$

Bond lengths			
Cu(1)–N(7)	1.916(4)	Cu(2)–N(8)	1.920(4)
Cu(1)–N(1)	2.018(5)	Cu(2)–N(4)	2.018(4)
Cu(1)–N(2)	2.076(5)	Cu(2)–N(5)	2.077(5)
Cu(1)–N(3)	2.089(4)	Cu(2)–N(6)	2.093(4)
Cu(1)–O(1)	2.233(5)	Cu(2)–O(2)	2.233(4)
Bond angles			
N(7)–Cu(1)–N(1)	179.2(2)	N(8)–Cu(2)–N(4)	176.0(2)
N(7)–Cu(1)–N(2)	95.0(2)	N(8)–Cu(2)–N(5)	96.7(2)
N(1)–Cu(1)–N(2)	84.3(2)	N(4)–Cu(2)–N(5)	85.6(2)
N(7)–Cu(1)–N(3)	95.9(2)	N(8)–Cu(2)–N(6)	95.5(2)
N(1)–Cu(1)–N(3)	84.7(2)	N(4)–Cu(2)–N(6)	85.0(2)
N(2)–Cu(1)–N(3)	155.7(2)	N(5)–Cu(2)–N(6)	137.2(2)
N(7)–Cu(1)–O(1)	99.0(2)	N(8)–Cu(2)–O(2)	96.7(2)
N(1)–Cu(1)–O(1)	81.4(2)	N(4)–Cu(2)–O(2)	79.3(2)
N(2)–Cu(1)–O(1)	100.1(2)	N(5)–Cu(2)–O(2)	113.8(2)
N(3)–Cu(1)–O(1)	99.6(2)	N(6)–Cu(2)–O(2)	105.3(2)

Å from the mean plane determined by O2 appended to the other alcohol pendant and two secondary amine nitrogen atoms N5, N6, with bond lengths Cu2–O2 = 2.233(4) Å, Cu2–N5 = 2.077(5) Å, Cu2–N6 = 2.093(4) Å.

Good flexibility is manifested by the macrocycle: the distance between the two tertiary amine nitrogen atoms N4 and N6 is 9.562 Å, which accommodates the imidazolate-bridged dicopper structure well. Each hydroxyethyl pendant affords the fifth coordination atom for each Cu(II) ion at the same side of the plane defined by the four secondary amine nitrogen atoms of the macrocycle, and none of the two hydroxyethyl pendants is deprotonated. The bridged imidazolate ring is sandwiched between the two phenyl rings with dihedral angles of 13.9(3)° to the C8–C13 phenyl plane and 25.3(2)° to the C22–C27 phenyl plane. The distance between the centers of imidazolate ring and phenyl rings are 3.338 Å for the C8–C13 phenyl plane and 3.584 Å for the C22–C27 phenyl plane.

3.2. Magnetic susceptibility

The temperature dependence of the magnetic susceptibility for the title compound was measured between 2 and 300 K. The magnetic behavior is depicted in Fig. 2 showing variation of the magnetic molar susceptibility (χ_M) and the effective magnetic moment (μ_{eff}) versus T. When the temperature decreases, χ_M increases continuously and reaches a maximum around 65 K, then falls to a minimum at 15 K. The μ_{eff} decreases from 2.5μ_B at 300 K to 0.25μ_B at 3.5 K, smaller than the value of 2.83μ_B for an uncoupled copper complex ($S = 1/2$). Such behaviors of χ_M and μ_{eff} are characteristics of an antiferromagnetic exchange interaction between two Cu(II) ions [22]. The abrupt rise of χ_M below 15 K is due to a small amount of paramagnetic impurities, mainly Cu^{2+} ($S = 1/2$). The susceptibility data were analyzed by a modified Bleaney–Bowers equation, where $-2J$ is the magnetic exchange parameter, g is the landé factor and p is the molar fraction of paramagnetic impurity [23].

$$\chi_M = 2Ng^2\beta^2/3kT[1 + 1/3\exp(-2j/KT)]^{-1}(1-p)$$
$$+ p2Ng^2\beta^2S(S+1)/3KT + \chi_{TIP}$$

The experiment data fit the equation well to give $g = 2.09$, $-2J = 72.8$ cm^{-1} and $p = 0.006$. Compared with

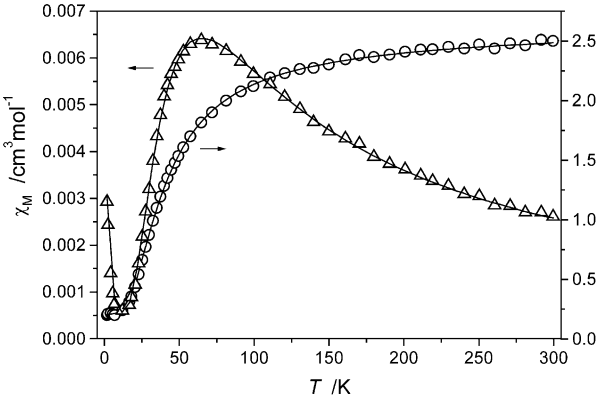


Fig. 2. Temperature dependence of the molar magnetic susceptibility (χ_M) and effective magnetic moment (μ_{eff}) of a powder sample of $[LCu_2(im)](ClO_4)_3(H_2O)_{2.5}(CH_3CN)_{0.5}$. The solid lines result from a least-squares fit according to the Bleaney–Bowers equation.

$-2J$ of other imidazolate-bridged dicopper complexes [10,12] the value of this complex is closer to the value of -52 cm^{-1} in the modified bovine erythrocyte SOD (Cu,Cu-SOD) [3].

3.3. ESR studies and the behaviors of the imidazolate bridge in solution

The X-band ESR spectrum of the title complex was measured as a polycrystalline powder at room temperature and is shown in Fig. 3.

The spectrum exhibits the expected features of an antiferromagnetic spin–spin interaction: (i) The $\Delta M_S = \pm 1$ zero-field splitting region shows a fairly broad signal centered at $g = 2.104$. (ii) The $\Delta M_S = \pm 2$ half-field signal is observed at about 1500 G, which again suggests a magnetic interaction between two copper(II) ions through the imidazolate bridge.

The 110 K ESR spectra of the title complex in frozen 50% aq. DMSO solution as a function of pH are displayed in Fig. 4.

The spectra of the complex, in the range $5.5 \leq \text{pH} \leq 12$, exhibit the expected characteristics of an antiferromagnetic dinuclear species. The $\Delta M_S = \pm 1$ region shows two broad signals at about 2800 G ($H_{(XY)1}$) and 3700 G ($H_{(XY)2}$), and two flattened signals at 2650 G ($H_{(Z)1}$) and 3700 G ($H_{(Z)2}$) (overlapped by ($H_{(XY)2}$) at 3700 G), indicating zero-field splitting effects on $g_\perp$ and $g_\parallel$ signals, respectively. The spectral data between $5.5 \leq \text{pH} \leq 12$ are directly readable and the D, $g_\perp$ and $g_\parallel$ values for these spectra were calculated by the method in the literature [24–26]. The parallel and perpendicular components of the separations $d_\perp$ and $d_\parallel$ derive from

$$d_\parallel = 2|D|/(g_\parallel \beta) \quad d_\perp = |D|/(g_\perp \beta)$$

where D is the zero-field splitting parameter. The two broad signals of 2800 G ($H_{(XY)1}$) and 3700 G ($H_{(XY)2}$) assigned to the split $g_\perp$ signals allow calculation of D.

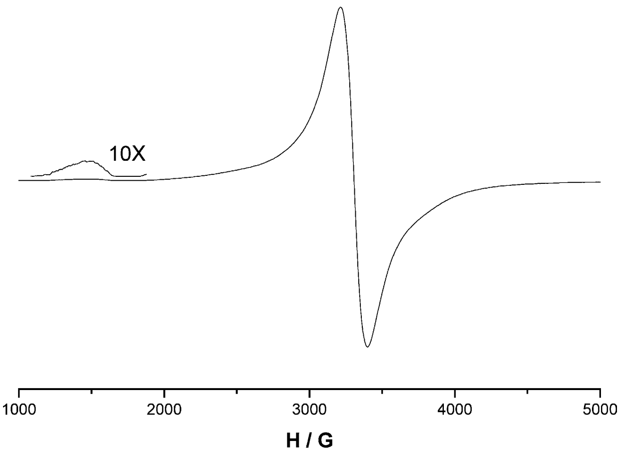


Fig. 3. Polycrystalline power ESR spectrum at room temperature of the complex $[LCu_2(im)](ClO_4)_3(H_2O)_{2.5}(CH_3CN)_{0.5}$.

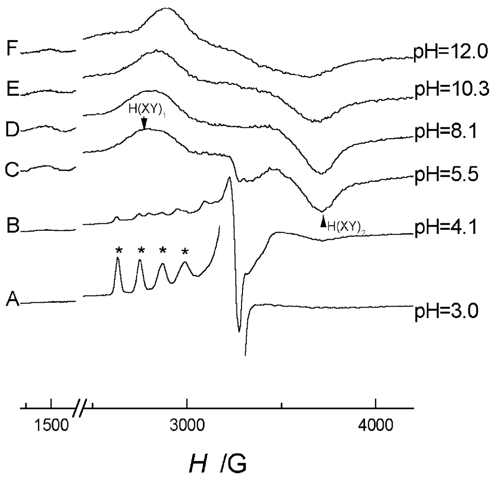


Fig. 4. The ESR spectra of $[LCu_2(im)](ClO_4)_3(H_2O)_{2.5}(CH_3CN)_{0.5}$ (0.001 mol l^{-1}) in frozen water/DMSO solution (1:1, v/v) at 110 K as a function of pH.

As for $g_\parallel$, since the signal G ($H_{(Z)2}$) is overlapped by the $g_\perp$ signal at 3700 G, the value of $g_\parallel$ was determined by D. Analysis of the spectra gave the following values: $|D| = 0.088$, $g_\parallel = 2.17$ and $g_\perp = 2.08$. These values are consistent with other imidazolate bridged dinuclear Cu(II) complexes [10,12].

The characteristic spin–spin interaction signal appearing in spectra C–F in Fig. 4 indicates that the imidazolate bridge is stable at $5.5 \leq \text{pH} \leq 12$. Decreasing the pH below 5.5 will cause the disruption of the imidazolate bridge, the successive protonation of the macrocycle and the gradual release of free copper(II) ions. The ES MS spectrum of the complex at pH 5.0 proves the co-existence of $[(LCu_2(im))ClO_4]^{2+}$ and the corresponding imidazolate bridge disrupted species $[H_{-2}LCu_2]^{2+}$ and $[(H_{-1}LCu_2)ClO_4]^{2+}$ (shown in Fig. 5(a)), where $H_{-2}L$ represents the ligand with two deprotonated hydroxyethyl pendants and $H_{-1}L$ represents the ligand with one deprotonated hydroxyethyl pendant. The signal of free Cu(II) ions appears in spectrum B (pH 4.1) in Fig. 4, and spectrum A shows that they become dominant in solution at pH 3.0, which indicates the complete disruption of the imidazolate bridge. So the imidazolate bridge begins to disrupt in the range $4.1 \leq \text{pH} \leq 5.5$. If the pH is allowed to rise to around 12, competition by OH$^-$ which can coordinate copper(II) to substitute the imidazolate nitrogen atoms will happen. In spectrum F (pH 12) in Fig. 4, although the weak half-field signal is still discernable, the ES MS spectrum of the complex in aqueous solution pH 12 shown in Fig. 5(c) reveals the existence of $[H_{-2}LCu_2(OH)(H_2O)]^+$. Since the imidazolate bridge in native Cu,Cu-SOD is stable over the range $4.5 \leq \text{pH} \leq 11$, the model complex mimics more closely the behavior of imidazolate-bridged copper(II) ions in aqueous solution [3].

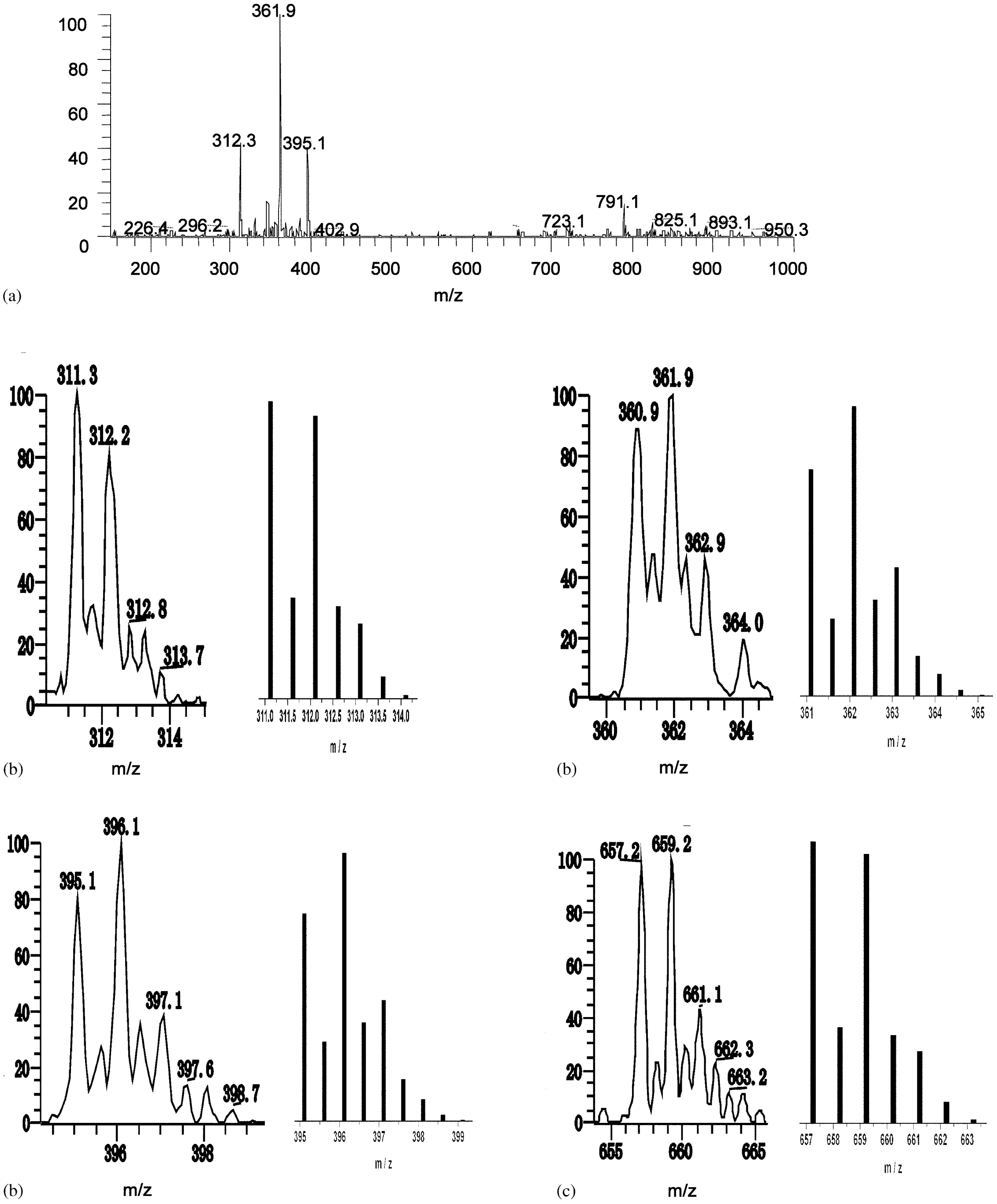


Fig. 5. (a) ES MS spectrum of an aqueous solution of the complex at pH 5.0. The peak $m/z = 312.3$ is assigned to $[H_{-2}LCu_2]^{2+}$, $m/z = 361.9$ is assigned to $[(H_{-1}LCu_2)ClO_4]^{2+}$, $m/z = 395.1$ is assigned to $[(LCu_2(im))ClO_4]^{2+}$. (b) Comparison of the observed (traces) and calculated (bars) isotopic distributions for the peaks at $m/z = 312.3$, 361.9 and 395.1. (c) Comparison of the observed (traces) and calculated (bars) isotopic distributions for the peaks at $m/z = 657.2$ appearing in the spectrum at pH 12.0, which is assigned to $[(H_{-2}LCu_2)(OH)(H_2O)]^{+}$.

3.4. Superoxide dismutase activities

The imidazolate-bridged dicopper macrocyclic complex was synthesized as a model for the active site of Cu,Zn-SOD. The SOD-like activity of the complex was investigated by NBT assay and catalytic activity toward the dismutation of superoxide anion was measured. The macrocycle provides a stable environment similar to that in the active site in the native enzyme, ensuring the stable existence of the complex at the investigated pH value in aqueous solution.

In this work, the SOD-like activity was measured at pH 7.8. The relationship between the inhibition (%) and initial concentration of the complex is shown in Fig. 6.

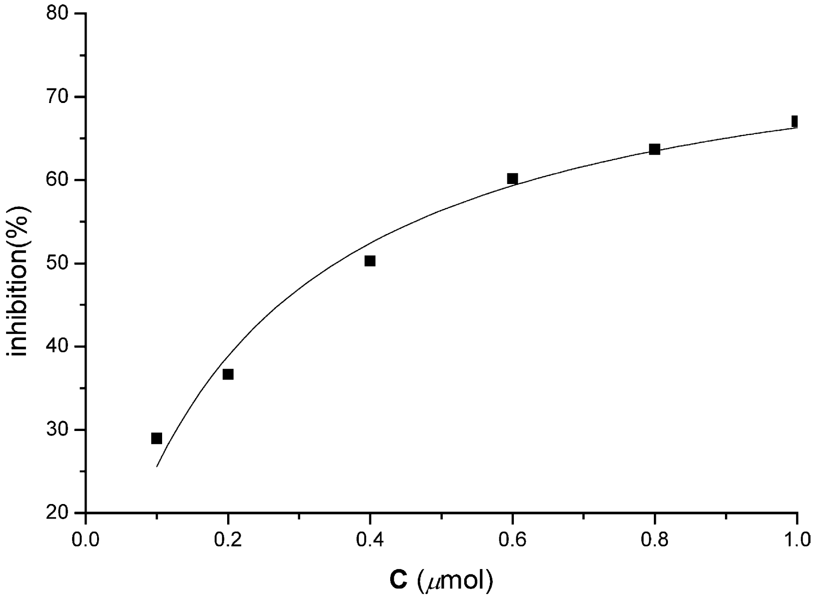


Fig. 6. The relationship between the inhibition (%) and initial concentration of the title complex.

The chromophore concentration required to yield 50% inhibition of the reduction of NBT (IC_{50}) was determined by following the literature method [19]. The IC_{50} value of 0.36 μmol dm^{-3} of the title complex is nine times higher than the value exhibited by the native enzyme ($IC_{50} = 0.04$ μmol dm^{-3}), an activity of 11% of native enzyme. The activity is in the same order of magnitude as the best SOD model complexes [4,10,12]. The result is reasonable because the macrocycle is flexible and the imidazolate-bridged dicopper center is accessible to $O_2^{\bullet -}$.

4. Conclusions

The complex appears to be a good model for the physical properties of the active site in Cu,Cu-SOD. The ligand incorporating two alcohol pendants offers similar N4O coordination atoms for each Cu(II) ion, different from other models in which five nitrogen atoms act as coordination atoms. Both TP and TBP coordination geometry are adopted by the Cu(II) ions. The title complex also well mimics the behavior of the native enzyme. The magnetic coupling constant of $-2J = 72.8$ cm^{-1} is observed and $[LCu_2(im)]^{3+}$ survives in a broader pH range $5.5 \leq pH \leq 12$. The SOD-like activity evaluation reveals that the complex is among the list of models with the highest activity.

5. Supplementary material

Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Center, CCDC No. 189319. Copies of the data are available from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk on request, quoting the deposition number.

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