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Redox behaviour and reactivity of some di-Schiff base copper(II) complexes towards reduced oxygen species †

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Some new di-Schiff base copper(II) complexes, derived from 2,3-butanedione and 2-(2-aminophenyl)benzimidazole, 2-(aminomethyl)benzimidazole or 2-(2-aminoethyl)pyridine, have been prepared and characterised using UV/Vis, IR, and EPR spectroscopy. Their reactivity towards superoxide radicals was studied, in comparison to similar complexes, indicating that these species are usually good mimics of superoxide dismutase. Evidence of tetrahedral distortion around the copper ion, similar to that observed in the native enzyme, was also found through spectroscopic parameters. However, these new complexes can also generate hydroxyl radicals in appreciable quantities in the presence of hydrogen peroxide, as detected by spin-trapping EPR experiments. By using μmol dm^{-3} concentrations of these complexes, considerable lipid peroxidation of liposomes prepared from L-α-phosphatidylcholine was also confirmed, *in vitro*. Comparative studies with similar complexes pointed at a modulation of both antioxidant and pro-oxidant properties by the diimine ligands. Their redox behaviour was studied by CV, with the aim of establishing some correlation between estimated parameters, and observed reactivity *versus* reduced oxygen species (superoxide radical and hydrogen peroxide). Among five compounds, only one was shown to be resistant to molecular oxygen, after being reduced, in the range −1.50 to +0.50 V *vs.* NHE, in dimethylformamide solution containing tetraethylammonium perchlorate (0.100 mol dm^{-3}), although all exhibited substantial reactivity toward hydrogen peroxide. In a wider range of potential, evidence of oxidation of the ligands was also detected. Tentative conclusions regarding the most important parameters affecting their reactivity are made.

Introduction

Copper complexes have extensively been studied as mimics of active centres in different proteins and enzymes dependent on this metal. Among the most investigated ligands, macroacyclic amines and imines deserve prominent attention. Their flexibility, facility of preparation, and capacity of stabilising usual oxidation states of the copper ion can explain their successful performance in mimicking peculiar geometries around the metal, leading to very interesting spectroscopic properties, and varied reactivities.[1] Accordingly, different complexes containing these types of ligand have been described as functional and structural models of several copper proteins, in a wide range of active centres, including superoxidase dismutase Cu_2,Zn_2 SOD,[2] tyrosinase,[3] hemocyanin,[4] and cytochrome c oxidase.[5]

On the other hand, some copper complexes with similar ligands were also described as good generators of radical species, causing cytotoxic effects *in vivo*, especially in the presence of reducing agents such as glutathione (γ-glutamylcysteinylglycine) or ascorbate.[6,7] This reductive activation results from subsequent oxidation by molecular oxygen of the corresponding copper(I) complexes, when reactive oxygen species are formed.

We have been studying different diimine copper(II) complexes with the aim of verifying their antioxidant and pro-oxidant properties,[8] since model compounds in contrast to proteins usually exhibit both types of activity in substantial and comparable magnitude. In this work we describe some new di-Schiff base copper(II) complexes, derived from 2,3-butanedione, and 2-(2-aminoethyl)pyridine or substituted benzimidazoles (Scheme 1), in order to verify the influence of the structural particularities of diimine ligands on their chemical reactivity. These complexes were characterised by spectroscopic techniques, and had their reactivities towards reduced oxygen species investigated. Their SOD activities as well as their potential in causing oxidative damage were then verified, and compared to those of other similar complexes previously described. The results obtained indicate that both activities can be modulated by the diimine ligand. Finally, electrochemical properties of the complexes were verified in order to correlate them to the observed reactivity.

† Dedicated to Professor José Manuel Riveros on the occasion of his 60th birthday, and to Professor Pawel Krumholz (in memoriam).

Experimental

Materials

The reagents used were analytical grade, purchased from different sources. Diacetyl (or 2,3-butanedione), 2-(2-aminoethyl)-pyridine, 2-pyridinecarbaldehyde, and acetylpyrazine (97%) were from Aldrich Chemical Co. (Milwaukee, WI, USA), 2-(2-aminophenyl)benzimidazole (98%), and 2-(aminomethyl)-benzimidazole dihydrochloride (96%) from Lancaster Synthesis Inc. (Windham, NH, USA), and 1,2-diaminoethane and 1,3-diaminopropane from Riedel-de Häen (Seelze-Hannover, GE). Native Cu_2,Zn_2 SOD, from bovine erythrocytes, lecithin L-α-phosphatidylcholine (type IV-S), and nitroblue tetrazolium (NBT) were from Sigma (St. Louis, MO, USA). 2-Pyridinecarbaldehyde was purified by distillation, just before use. Dimethylformamide (from Merck, Darmstadt, GE) was dried according to the recommended procedure, for the electrochemical measurements.

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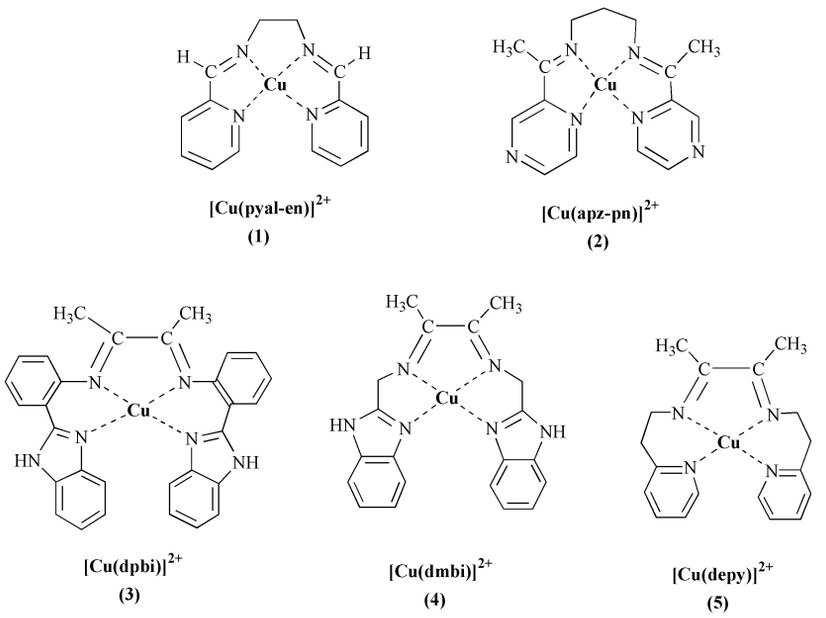

Scheme 1 Di-Schiff base copper(II) complexes prepared.

Syntheses

Two of the studied complexes, [*N*,*N*′-bis(2-pyridyl)methylene-1,2-diaminoethane-*N*,*N*′,*N*″,*N*‴]copper(II), [Cu(pyal-en)][ClO_4]$_2$ **1**, and [*N*,*N*′-bis(2-pyrazinylmethyl)methylene-1,3-diaminopropane-*N*,*N*′,*N*″,*N*‴]copper(II), [Cu(apz-pn)][ClO_4]$_2$·1.5H_2O **2** have been prepared previously and characterised.[8] All the other complexes were prepared as perchlorate salts by reported methods, with suitable minor modifications.[9] **CAUTION**: perchlorate salts of metal complexes with organic ligands are potentially explosive and should be manipulated very carefully, only in small amounts.

Preparations

[2,3-Bis(2-benzimidazol-2-ylphenylimino)butane-*N*,*N*′,*N*″,*N*‴]copper(II) perchlorate, [Cu(dpbi)][ClO_4]$_2$·2H_2O 3. A solution of 2,3-butanedione (2 mmol; 0.18 mL) in 2 mL of ethanol was added, with stirring, to an ethanolic solution of 2-(2-aminophenyl)benzimidazole (4 mmol, or 0.837 g dissolved in 10 mL 80% ethanol v/v) and the resulting solution maintained at 55 °C for 150 min, monitored by the developing brownish yellow colour, indicating the formation of the diimine ligand. After cooling to room temperature, an aqueous solution of copper(II) perchlorate (2 mmol, or 0.741 g dissolved in the minimum volume of water) was then added, dropwise, with continuous stirring. A yellowish brown precipitate was formed after standing of the resulting solution in an ice bath for 30 minutes. The product was collected by filtration, washed with cool ethanol, and dried at vacuum in a desiccator, over silica gel. Yield 75%. Found: C, 46.15; H, 3.32; Cu, 7.90; N, 11.16. Calc. for $C_{30}H_{24}Cl_2CuN_6O_8$·2H_2O: C, 46.98; H, 3.68; Cu, 8.29; N, 10.96%. IR absorptions (KBr disc), $\tilde{\nu}_{max}$/cm^{-1}: 1620s (C=N), 1575vs (C=C), 1445vs (CH_2), 1130vs (C–N), 1090vs and 620s (ClO_4^-), and 240w (Cu–N).

[2,3-Bis(benzimidazol-2-ylmethylimino)butane-*N*,*N*′,*N*″,*N*‴]copper(II) perchlorate, [Cu(dmbi)][ClO_4]$_2$ 4. This complex was similarly prepared by adding, with continuous stirring, an ethanolic solution of 2,3-butanedione (2 mmol, or 0.18 mL dissolved in 15 mL methanol) to an aqueous solution of 2-(aminomethyl)benzimidazole (4 mmol, or 0.880 g dissolved in 3 mL water, adjusting the pH to 8 with concentrated NaOH solution). The solution was then refluxed for approximately 30 minutes, turning orange. Afterwards, 2 mmol (0.741 g) of copper perchlorate dissolved in 3 mL of water were added dropwise, at room temperature. This mixture was then maintained with stirring for approximately 150 minutes, when a green solution resulted. After cooling the final solution in a refrigerator for 15 hours, and then in an ice-bath for 2 hours, greenish brown crystals were obtained, separated by filtration. They were washed with cool water and methanol, and finally dried in vacuum in a desiccator. Yield 27%. Found: C, 40.59; H, 3.38; Cu, 10.11; N, 15.17. Calc. for $C_{20}H_{20}Cl_2CuN_6O_8$: C, 39.58; H, 3.32; Cu, 10.47; N, 14.34%. IR absorptions (KBr disc), $\tilde{\nu}_{max}$/cm^{-1}: 1620w (C=N), 1590w (C=C), 1458s (CH_2), 1130vs (C–N), 1115vs and 620vs (ClO_4^-), and 240w (Cu–N).

{2,3-Bis[2-(2-pyridyl)ethylimino]butane-*N*,*N*′,*N*″,*N*‴}copper(II) perchlorate, [Cu(depy)][ClO_4]$_2$ 5. To 4 mmol (0.489 g) of 2-(2-aminoethyl)pyridine dissolved in 7.5 mL of ethanol were added, with continuous stirring, 2 mmol of 2,3-butanedione (0.18 mL) previously dissolved in 7.5 mL of ethanol. The solution was maintained under reflux for ≈75 minutes, turning deep brown. After cooling to room temperature, an aqueous solution of copper perchlorate (2 mmol, or 0.741 g dissolved in 5 mL water) was added slowly, and the final solution stirred for approximately 2 hours. On standing in an ice-bath for a few hours, grey greenish crystals separated and were washed with cool ethanol and finally dried in vacuum, in a desiccator. Yield 71%. Found: C, 38.49; H, 3.93; Cu, 10.76; N, 9.74%. Calc. for $C_{18}H_{22}Cl_2CuN_4O_8$: C, 38.83; H, 4.08; Cu, 11.41; N, 10.28%. IR absorptions (KBr disc), $\tilde{\nu}_{max}$/cm^{-1}: 1620s (C=N), 1605w (C=C), 1445vs (CH_2), 1100s (C–N), 1075vs and 620vs (ClO_4^-), and 240w (Cu–N).

Characterisation

The complexes prepared were characterised by UV/Vis, infrared, and EPR spectroscopy, in addition to elemental analyses and magnetic susceptibility measurements (data in Table 1). Elemental analyses were performed at the Núcleo de Instrumentação Analítica of our Institution, using a Perkin-Elmer 2400 CHN Elemental Analyser. Copper analysis was carried out by atomic emission spectrometry, in an ICP-AES Spectroflame spectrometer, using 1.2 kW power, argon plasma coolant 12 L min^{-1}, argon auxiliary plasma 1.2 L min^{-1}, and argon aerosol carrier 1.0 L min^{-1}. The sample was introduced at 1.5 mL min^{-1} and copper was monitored at 327, 396 nm, within a detection limit of 4.5 μg L^{-1}. A Beckman DU-70 spectro-

Table 1 Spectroscopic properties of the di-Schiff base copper(II) complexes

Compound	Electronic spectra λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1})	μ_{eff}/μ_B	EPR parameters			
			$g_\perp$	$g_\parallel$	$A_\parallel$/G	$g_\parallel A_\parallel^{-1}$/cm
$[Cu(dpbi)][ClO_4]_2 \cdot 2H_2O$	208 (2.1×10^4); 292 (3.9×10^4); 358 (1.0×10^4); 640 (221)	2.12	2.056	2.248	182	118
$[Cu(dmbi)][ClO_4]_2$	240 (1.5×10^4); 270 (1.3×10^4); 304 (5.7×10^3);	0.692	2.070	2.235	193	111
	356 (4.3×10^3); 710 (101)			2.247	161	133
$[Cu(depy)][ClO_4]_2$	260 (1.1×10^4); 400 (1.2×10^3); 620 (214)	2.02	2.060	2.218	188	114
$[Cu(pyal\text{-}en)][ClO_4]_2$ [8]	230 (2.0×10^4); 275 (7.9×10^3); 285 (7.7×10^3); 630 (106)	2.01	2.058	2.218	184	117
$[Cu(apz\text{-}pn)][ClO_4]_2 \cdot H_2O$ [8]	213 (1.4×10^4); 267 (1.6×10^4); 646 (60)	2.14	2.063	2.229	190	113

photometer, equipped with a thermostat controlled cell compartment, was used for the electronic spectra registration, in ethanol solutions, and for kinetic monitoring. Infrared measurements were performed in a Perkin-Elmer 783 spectrometer, or a BOMEM 3.0 (diffuse reflectance) instrument, in the range 4000 to 200 cm^{-1}, utilising KBr pellets. A Bruker EMX instrument, operating at X-band (frequency = 9.47 GHz, power = 20.17 mW, and modulation frequency = 100 kHz), was used for recording the EPR spectra. The magnetic field was calibrated with 2,2-bis(4-*tert*-octylphenyl)-1-picrylhydrazyl as external standard, for the solid samples, and with $[Cu(edta)]^{2+}$ solutions (1.00 mmol dm^{-3}) for frozen methanol–water (4 : 1 v/v) or acetonitrile solutions of the prepared complexes. In the detection of hydroxyl radicals by the spin-trapping method,[10] 5,5′-dimethyl 3,4-dihydropyrrole *N*-oxide (DMPO) was used as spin scavenger, after purification by recommended methods.[11] Magnetic susceptibilities of the solid samples were measured with a Cahn Faraday balance, model DTL 7500, at room temperature. $Hg[Co(SCN)_4]$ from Aldrich ($\chi = 16.44 \times 10^6$ cm^3 g^{-1} units, at 20 °C) was used for calibration,[12] and corrections were applied for the diamagnetism calculated from Pascal's constants. Effective magnetic moments were calculated by the equation $\mu_{eff} = 2.828(\chi_m T)^{1/2}$, where χ_m is the magnetic susceptibility per formula unit.

SOD activity

This activity was measured by the NBT reduction method, using xanthine/xanthine oxidase as the generator of superoxide radicals.[13] The formation of formazan was monitored at 560 nm, determining the rate of reaction in the presence of different concentrations of each complex studied, and comparing the results with those for the native enzyme. The SOD activity was expressed as IC_{50}, that is the concentration for 50% of reaction inhibition.

Lipid peroxidation

Liposomes were prepared from lecithin L-α-phosphatidylcholine, in 20 mg mL^{-1}, in 100 mmol dm^{-3} sodium phosphate buffer, at pH 7.4. The suspension was sonicated three times during 30 seconds, at 65 W, in an ice-bath, using a Branson 250 soniccator. The freshly obtained liposomes were maintained at 4 °C, protected from light, until used, in no more than one week. The extent of lipid peroxidation, after incubation with hydrogen peroxide for 40 minutes, at 37 °C, was assayed by the TBARS (thiobarbituric acid reactive species) method, monitoring the formation of a red chromophore, similar to that formed with malonaldehyde.[14] In these measurements 500 μL of the sample solution were added to 500 μL of the reagent, containing TBA (0.37% w/v), 250 mmol dm^{-3} HCl, 4 mmol dm^{-3} butylated hydroxytoluene (BHT) and a detergent Triton X-100 (1% w/v). The final mixture was heated to 100 °C, for 15 minutes, and after being cooled was centrifuged at 3000 rpm during 10 minutes. Subsequently, it was purified using a Millipore filter, and its absorbance was determined at 532 nm ($\varepsilon = 1.56 \times 10^5$ dm^3 mol^{-1} cm^{-1}).[15] Measurements were carried out in the absence, and in the presence, of the different copper complexes, in concentrations 50–150 μmol dm^{-3}.

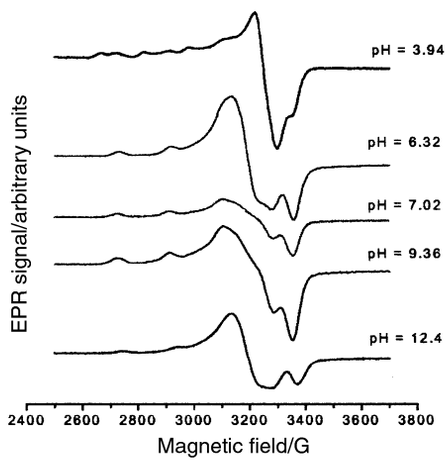

Fig. 1 EPR spectra of frozen methanol–water (4 : 1, v/v) saturated solutions of the complex $[Cu(dmbi)]^{2+}$, at different pH. Power receiver, 20.17 mW; frequency, 9.438 GHz; modulation frequency, 100 kHz; modulation amplitude, 15 G; receiver gain, 1.42×10^4–3.17×10^4.

Electrochemical measurements

Cyclic voltammetry measurements were carried out using a PARC system, from EG&G instrument, consisting of a potentiostat model 283, and a three electrode cell arrangement. The software ECHEM, version 4.30, was used. Solutions of the copper complexes (5 mmol dm^{-3}) were prepared in dimethylformamide, containing 0.1 mol dm^{-3} tetraethylammonium perchlorate. A platinum disc working electrode and a platinum wire auxiliary electrode were employed for the measurements *vs.* an Ag–$AgNO_3$ reference electrode ($E° = 0.503$ V *vs.* NHE).[16] Spectroelectrochemical measurements were carried out using a PARC potentiostat, model 173, in parallel with a Hewlett-Packard diode array spectrophotometer (model 8453). The quartz spectroelectrochemical cell used has a 0.025 cm optic length. A gold minigrid working electrode and a platinum auxiliary microelectrode were employed in these measurements *vs.* an Ag–$AgNO_3$ reference microelectrode. The investigated copper complex was dissolved in acetonitrile (3 mmol dm^{-3}) containing 0.10 mol dm^{-3} NEt_4ClO_4.

Results and discussion

Results of the characterisation of the new prepared complexes are shown in Table 1. All exhibited paramagnetic behaviour, with an effective magnetic moment around 2.0 μ_B, as expected for a mononuclear tetragonal copper(II) complex. However, the compound $[Cu(dmbi)]^{2+}$ displayed a much lower moment ($\mu_{eff} = 0.692\ \mu_B$), indicating some antiferromagnetic interaction in the solid state. This complex also showed two different copper centres ($g_\parallel = 2.235$ and 2.247) in the EPR spectra, in frozen methanol–water solutions. Experiments at different pH indicated that both species are observed at pH ≤ 6. Nevertheless, at pH ≥ 6.3 only one signal was observed, attributed to the characterised species ($g_\perp = 2.070$; $g_\parallel = 2.241$, $A_\parallel = 188.1$ G), as shown in Fig. 1. These two different copper signals

Table 2 Comparative SOD activity and lipophilicity of the new di-Schiff base copper(II) complexes

Compound	IC_{50}/μmol L^{-1} from ref. 8(*b*)	Partition coefficient K_P (octanol–water)
Cu_2, Zn_2 SOD	0.00589	
	0.0143	
	0.0084[2]	
$[Cu(apz\text{-}pn)][ClO_4]_2 \cdot H_2O$	0.0902	0.28
$[Cu(PuPhePy)][ClO_4]_2$	0.270[2]	0.7[22]
$[Cu(pyal\text{-}en)][ClO_4]_2$	0.446	0.22
$[Cu(depy)][ClO_4]_2$	0.447	0.24
$[Cu(PuPy)][ClO_4]_2 \cdot 0.5H_2O$	0.603[2]	0.14[22]
$[Cu(dpbi)][ClO_4]_2 \cdot 2H_2O$	0.617	2.36
$[Cu(dmbi)][ClO_4]_2$	1.58	3.47
$[Cu(Pu\text{-}6\text{-}MePy)(H_2O)][ClO_4]_2$	2.25[2]	

Abbreviations: SOD = superoxide dismutase; PuPy = *N*,*N*′-bis(2-pyridyl)methylene-1,4-butanediamine; PuPhePy = *N*,*N*′-bis(2-pyridylphenyl)methylene-1,4-butanediamine; Pu-6-MePy = *N*,*N*′-bis[2-(6-methyl)pyridyl]methylene-1,4-butanediamine.

observed at lower pH could be explained by a protonation–deprotonation equilibrium involving the imidazole ring, facilitating the interaction between two of the copper species, protonated and deprotonated, in a polymeric chain, similar to structures described in the literature with this type of ligand.[17,18] Evidence of this interaction between the copper centres was provided by magnetic susceptibility measurements in the solid state. The other complexes, on the contrary, exhibited EPR spectra and μ_{eff} values consistent with isolated copper centres.

The electronic spectra of these di-Schiff base copper(II) complexes show very intense absorption bands in the UV region ($\varepsilon \approx 10^4$ dm^3 mol^{-1} cm^{-1}), attributed to ligand transitions ($n \longrightarrow \pi$ and $\pi \longrightarrow \pi^*$), and a characteristic broad band at 600–700 nm due to d–d transitions ($\varepsilon \approx 100$–200 dm^3 mol^{-1} cm^{-1}). Additionally, they showed a band around 350–400 nm, attributed to a LMCT transition, $\pi(imine) \longrightarrow Cu^{II}$, in similar compounds containing the benzimidazole group.[19] Their infrared spectra indicated the usual characteristic bands of diimine complexes: a stretching frequency $\nu_{C=N}$ at 1620 cm^{-1}, ν_{C-N} around 1100–1130 cm^{-1}, and a metal–ligand frequency ν_{Cu-N} around 240 cm^{-1}. The strong band at 620 cm^{-1} and another one around 1075–1115 cm^{-1} are due to ν_{ClO_4}, from non-coordinated perchlorate anion.

Based on these results, a distorted tetragonal structure was verified for these species, similar to that observed in the native SOD enzyme.[20] This tetrahedral distortion can be estimated by the empirical ratio $g_{\|} : A_{\|}$, obtained from the EPR spectra.[2] In addition, all the studied complexes have exhibited pronounced SOD activity,[8] as compared to prior studied compounds, shown in Table 2, although the best functional model, complex $[Cu(apz\text{-}pn)]^{2+}$ **2**, does not exhibit the closest structure to the enzyme.

In spite of showing very good antioxidant properties, these complexes can also generate reactive oxygen species, in aqueous solutions (40 μmol dm^{-3}), at pH 7.4 (phosphate buffer 50 mmol dm^{-3}) and 25 °C, in the presence of hydrogen peroxide (1.52 mmol dm^{-3}). Using the spin-trapping EPR method, the generation of hydroxyl radicals in concentrations 0.20–2.5 μmol dm^{-3} was detected (see Fig. 2).

By adding dimethyl sulfoxide to the reaction solution, the corresponding DMPO–methyl radical adduct was also observed ($a_H = 22.9$ and $a_N = 15.5$ G), corroborating the formation of hydroxyl radicals through redox steps between the copper complexes and hydrogen peroxide, in a Fenton-type reactivity,[21] eqns. (1)–(3). Step (3) is usually used as evidence

$$[CuL]^{2+} + H_2O_2 \longrightarrow [CuL]^{+} + O_2^{\bullet -} + 2\,H^{+} \quad (1)$$

$$[CuL]^{+} + H_2O_2 \longrightarrow [CuL]^{2+} + {}^{\bullet}OH + HO^{-} \quad (2)$$

$${}^{\bullet}OH + (H_3C)_2S{=}O \longrightarrow CH_3S(=O)OH + {}^{\bullet}CH_3 \quad (3)$$

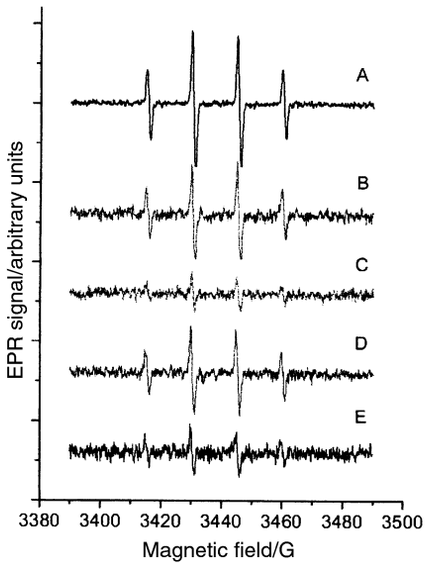

Fig. 2 EPR spectra of the DMPO–OH adduct obtained in solutions of copper(II) complexes (40 μmol dm^{-3}), $[H_2O_2] = 1.52$ mmol dm^{-3}, and [DMPO] = 0.10 mol dm^{-3}, in phosphate buffer (50 mmol dm^{-3}, pH 7.4). Power receiver, 20.17 mW; frequency, 9.69 GHz; modulation frequency, 100 kHz; modulation amplitude, 1 G. A $[Cu(apz\text{-}pn)]^{2+}$ (Gain = 1.12×10^6; [DMPO–OH] = 2.48 μmol dm^{-3}), B $[Cu(dmbi)]^{2+}$ (Gain = 2.52×10^6; [DMPO–OH] = 0.255 μmol dm^{-3}), C $[Cu(dpbi)]^{2+}$ (Gain = 2.52×10^6; [DMPO–OH] = 0.610 μmol dm^{-3}), D $[Cu(depy)]^{2+}$ (Gain = 2.52×10^6; [DMPO–OH] = 0.758 μmol dm^{-3}) and E $[Cu(pyal\text{-}en)]^{2+}$ (Gain = 3.99×10^6; [DMPO–OH] = 0.206 μmol dm^{-3}).

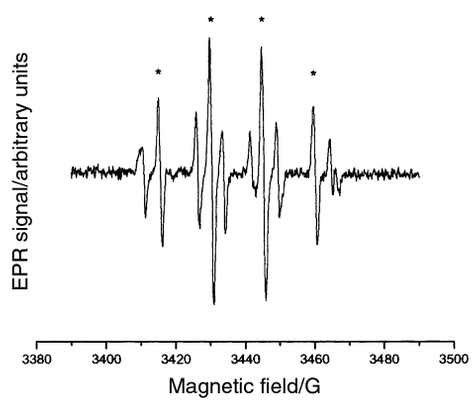

Fig. 3 EPR spectrum of the DMPO–OH (asterisked) and DMPO-methyl adducts obtained in a solution of $[Cu(apz\text{-}pn)]^{2+}$ (47 μmol dm^{-3}), $[H_2O_2] = 1.50$ mmol dm^{-3}, [DMPO] = 0.10 mol dm^{-3}, and [DMSO] = 0.028 mol dm^{-3}, in phosphate buffer (50 mmol dm^{-3}, pH 7.5). Power receiver, 20.17 mW; frequency, 9.70 GHz; modulation frequency, 100 kHz; modulation amplitude, 1 G.

that the detected hydroxyl radicals are formed solely by step (2) in a Fenton-like system. For competitive spin trapping of the free hydroxyl radicals generated, a value of 1.6 : 1 is expected for the ratio $k_{DMSO} : k_{DMPO}$, where k_{DMSO} and k_{DMPO} are the rate constants for the reaction of hydroxyl radical with DMSO and DMPO, respectively.[21] Our results, using the studied copper complexes, indicated values of 1.2–1.3 : 1 for this ratio, calculated by eqn. (4) where [DMPO] and [DMSO] are

$$k_{DMSO}/k_{DMPO} = [DMPO]\,([DMPO\text{–}OH]_0 - [DMPO\text{–}OH])/[DMSO][DMPO\text{–}OH] \quad (4)$$

the concentrations of the spin scavenger DMPO and DMSO, and $[DMPO\text{–}OH]_0$ and [DMPO–OH] are those of the adduct DMPO–OH detected in the absence and in the presence of DMSO, respectively. In Fig. 3 is shown the spectrum obtained for the most active complex $[Cu(apz\text{-}pn)]^{2+}$, in the presence of hydrogen peroxide and DMPO, when DMSO was also added.

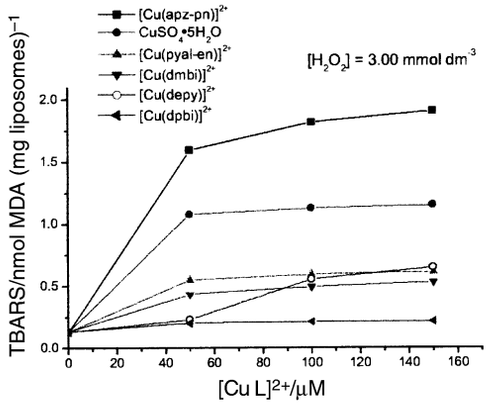

Fig. 4 Lipid peroxidation of L-α-phosphatidylcholine liposomes observed in the presence of different copper(II) complexes, and $[H_2O_2] = 3.00$ mmol dm^{-3}, after 40 min of incubation, at 37 °C, in phosphate buffer (100 mmol dm^{-3}, pH 7.4); MDA = malondialdehyde.

These data are consistent with the formation of hydroxyl radicals mainly by step (2), in a Fenton reaction mechanism.

Additionally, these copper complexes also catalysed the lipid peroxidation of liposomes, obtained from L-α-phosphatidylcholine, in the presence of hydrogen peroxide (3.00 mmol dm^{-3}). Incubation of the prepared liposomes in phosphate buffer (50 mmol dm^{-3}, pH 7.4) with the complexes (50–150 μmol dm^{-3}), in the presence of the oxidant, at 37 °C, for 40 minutes, leads to substantial formation of oxidised products, analysed spectrophotometrically by the TBARS method.[14,15] In these experiments a modulation of the catalytic activity by the ligand was observed, as shown in Fig. 4.

The most active complex in the lipid peroxidation, as well as in the generation of oxygen free radicals, was $[Cu(apz\text{-}pn)]^{2+}$ **2**, which had been also the best functional SOD model, showing a very low value of IC_{50}, as in Table 2. The lipophilicity of these complexes, quantified by measurements of octanol–water partition coefficients,[22] after stirring the solutions for more than 5 hours, at 25 °C, indicated that this is not a determining factor in their SOD or peroxidation activities (see Table 2). However, the best mimics of SOD tend to display the lowest lipophilicities.

Since the cytotoxicity exhibited by some copper complexes seem to be activated by reducing agents,[6,7] such as glutathione or ascorbate, that promote the reduction of copper, subsequently reoxidised by molecular oxygen, we studied the redox properties of the new di-Schiff base complexes. Electrochemical experiments were carried out, by cyclic voltammetry, with the aim of correlating their redox behaviour with their catalytic activities, particularly that of generating reactive oxygen species. Very intriguing results were obtained. One of the studied complexes, $[Cu(depy)]^{2+}$ **5**, showed reversible behaviour, with $E°$ (Cu^{II}–Cu^{I}) = +0.03 V *vs.* NHE (see Fig. 5a) in DMF solution containing tetraethylammonium perchlorate (0.10 mol dm^{-3}). Similar results have been reported for $[Cu(dmg)_2]$,[23] where dmg = dimethylglyoximate, and other copper(II) complexes.[24,25]

The reduction of Cu^{II} to Cu^{I} at more positive potential (+0.03 V) when compared to that of $[Cu(dmg)_2]$, and $[Cu(salacopd)]Cl_2$ (salacopd is also a diimine ligand 2,4,9,11-tetramethyl-6,7,13,14-dibenzo-1,5,8,12-tetraaza-3,10-bis(salicylidene)cyclotetradeca-1,4,8,11-tetraene), −0.86 and −0.78 V, respectively, is consistent with a co-ordination geometry intermediate between square planar and tetrahedral around the copper ion. Besides, an unsaturated nitrogen as the donor atom in the ligand stabilises more a low oxidation state, such as Cu^{I}, than a saturated one, by π-back bonding between the metal and the nitrogen atoms.[24]

Furthermore, an interesting behaviour was observed under a dioxygen atmosphere (see Fig. 5b). In spite of copper(I) complexes usually being good reducing agents, this $[Cu(depy)]^{+}$ complex in the reduced form does not react with molecular oxygen. However, when the scanning was under aerobic conditions at more negative potential a strong cathodic peak at −0.87 V was observed, and further the anodic peak assigned to copper(II) formation disappeared (E_{pa} = +0.06 V). This peak at negative potential is consistent with the reduction of oxygen in DMF to superoxide radicals ($O_2^{\bullet -}$),[26] that subsequently are dismutated by the copper complexes, mimics of SOD, eqns. (5) and (6).

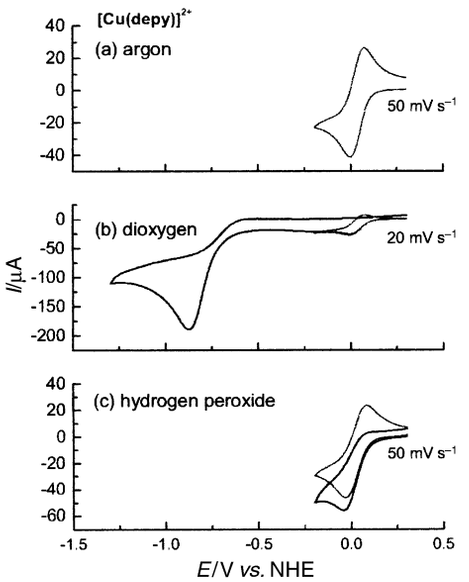

Fig. 5 Cyclic voltammograms of dimethylformamide solutions of the complex $[Cu(depy)]^{2+}$ (5 mmol dm^{-3}), containing 0.10 mol dm^{-3} NEt_4ClO_4: (a) under an argon atmosphere; (b) in the presence of dioxygen; (c) with addition of hydrogen peroxide.

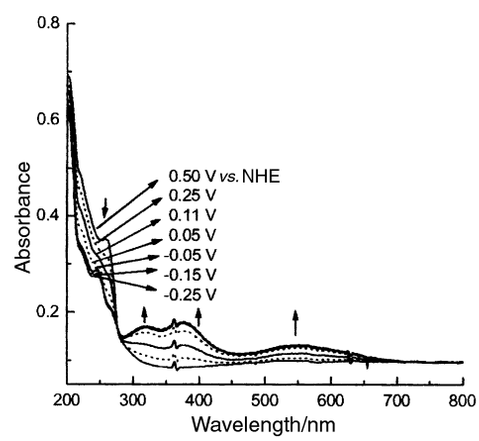

Fig. 6 UV-Vis Spectroelectrochemistry of the complex $[Cu(depy)]^{2+}$ (3 mmol dm^{-3}), in acetonitrile solution, containing 0.10 mol dm^{-3} NEt_4ClO_4, in the range −0.25 to +0.50 V *vs.* NHE.

$$[CuL]^{2+} + O_2^{\bullet -} \longrightarrow [CuL]^{+} + O_2 \quad (5)$$

$$[CuL]^{+} + O_2^{\bullet -} + 2\,H^{+} \longrightarrow [CuL]^{2+} + H_2O_2 \quad (6)$$

Spectroelectrochemical measurements of solutions of this complex, in the range +0.50 to −0.25 V, showed the decay of the band at 260 nm, ascribed to the macroacyclic ligand transition π→π*, and the growth of two new bands at 317 and 380 nm, assigned to MLCT transitions, Cu^{I}→py, and Cu^{I}→imine respectively,[27] as shown in Fig. 6. Another band observed at 558 nm can be also attributed to a CT transition, Cu^{I}→imine, previously observed for similar compounds.[28]

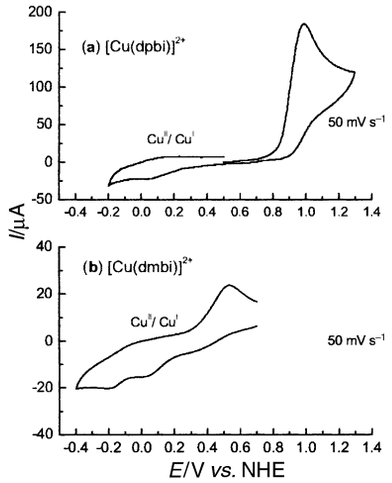

Fig. 7 Cyclic voltammograms of dimethylformamide solutions of the complexes $[Cu(dpbi)]^{2+}$ and $[Cu(dmbi)]^{2+}$ (5 mmol dm^{-3}), containing 0.10 mol dm^{-3} NEt_4ClO_4, under an argon atmosphere.

The $[Cu^{I}(depy)]^{+}$ complex also reacts with hydrogen peroxide, added to the solution, under anaerobic conditions (see Fig. 5c). Therefore, the reduced form of this complex was stable in the presence of molecular oxygen, albeit being quickly oxidised by superoxide radicals or hydrogen peroxide, as shown in Fig. 5.

On the contrary, all the other compounds studied exhibited irreversible waves at more positive potentials, in the absence as well as in the presence of oxygen. The complexes $[Cu(dmbi)]^{2+}$ **4** and $[Cu(dpbi)]^{2+}$ **3** showed a quasi-reversible wave around 0 V, attributed to the Cu^{II}–Cu^{I} reduction (see Fig. 7). While the former presents two successive processes, a quasi-reversible wave ($\Delta E_p = 100$ mV) at $E = -0.13$ V and another irreversible wave at +0.04 V, the $[Cu(dpbi)]^{2+}$ complex shows only a quasi-reversible process at +0.07 V ($\Delta E_p = 70$ mV). This behaviour was expected, since the $[Cu(dmbi)]^{2+}$ complex also exhibited two copper signals in the EPR spectra, suggesting an oligomeric structure containing species where the imidazole moiety appears protonated and deprotonated.[17,18] An additional irreversible wave was also observed for both complexes, at +0.53 and +0.98 V, respectively, assigned to the irreversible oxidation of benzimidazole rings in the ligands.

Although conflicting reports have been published about the redox potential of the bovine erythrocyte Cu_2,Zn_2 SOD, a midpoint potential $E = +0.120$ V *vs.* NHE was determined in an EPR-monitored redox titration of the enzyme, at pH 7.5, 22 °C and $I = 0.2$ mol dm^{-3}.[29] This reduction was shown to be reversible, since the characteristic EPR spectrum of the enzyme has been recovered after its reoxidation with ferricyanide.

Previous authors reported values in the range 280–420 mV *vs.* NHE.[30,31] Spectroelectrochemical measurements under anaerobic conditions, using a gold electrode, and ferricyanide as the mediator, pointed to an $E° = 403$ mV *vs.* NHE, at pH 7.0, when the maximum absorption at 680 nm was monitored, after establishing the equilibrium between the oxidised and reduced forms of the protein at each applied potential.[32] However, more than 4 hours were required to reduce the enzyme with the $[Ru(NH_3)_5(py)]Cl_2$ complex which has an $E° = 260$ mV *vs.* NHE.

In the case of the complexes $[Cu(pyal\text{-}en)]^{2+}$ **1** and $[Cu(apz\text{-}pn)]^{2+}$ **2**, an even more complex redox behaviour was observed. Depending on the direction of the potential scanning, different irreversible waves were observed, with coupled chemical reactions, attesting to their high reactivity. Therefore, the interpretation of these data requires more meticulous electrochemical studies, complemented by spectroscopic measurements in order to identify possible products formed.

Conclusion

Based on spectroscopic and reactivity studies, these di-Schiff base copper(II) complexes behaved as good mimics of the enzyme Cu_2,Zn_2 SOD, showing structural features as well as functional aspects of the copper ion incorporated in the active centre of the enzyme. The estimated IC_{50} values were comparable to those of very good models previously reported in the literature.[2]

Furthermore, their electrochemical behaviour indicated that their pro-oxidant and antioxidant properties depend on many factors, not only on their metal reduction potential. The species derived from diacetyl, complexes **5**, **4** and **3**, exhibited a reversible or quasi-reversible wave at potentials +0.03, +0.04, and +0.07 V *vs.* NHE, respectively, assigned to the Cu^{II}–Cu^{I} reduction, which are not very far from the +0.120 V verified for the native SOD enzyme.[29] The observed order is consistent with the idea that more electronically delocalised ligands lead to more positive potentials, due to higher stabilisation of the reduced metal form.

The influence of their lipophilicities seemed to be unimportant, although the best SOD mimics showed also lower partition coefficients (K_P, octanol–water).

Finally, a modulation of all those properties by the diimine ligand was observed, allowing us to obtain very reactive species, such as $[Cu^{II}(apz\text{-}pn)]^{2+}$, that generates hydroxyl radicals in appreciable concentration and leads to considerable lipid peroxidation, and, on the other hand, to obtain a surprisingly oxygen-resistant species, such as $[Cu^{I}(depy)]^{+}$.

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