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Characterization and biological activities of two copper(II) complexes with diethylenetriamine and 2,2′-bipyridine or 1,10-phenanthroline as ligands

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Abstract

Two new mixed ligand copper(II) complexes with diethylenetriamine, 2,2′-bipyridine and 1,10-phenanthroline have been synthesized. The crystal and molecular structures of $[Cu(dien)(phen)](ClO_4)_2$ and $[Cu(dien)(bipy)](BF_4)_2$ (dien = diethylenetriamine, phen = 1,10-phenanthroline, bipy = 2,2′-bipyridine) were determined by X-ray crystallography from single crystal data. These two complexes have similar structures. The EPR spectral data also suggest that these complexes have distorted square pyramidal geometry about copper(II). Anti-microbial and superoxide dismutase activities of these complexes have also been measured. They show the higher SOD activity than the corresponding simple Cu(II)–dien/Cu(II)–PMDT (PMDT = *N*,*N*,*N*′,*N*′,*N*″-pentamethyldiethylenetriamine) complexes because of a strong axial bond of one of the nitrogen atoms of the α-diimine. Both the complexes have been found to cleave plasmid DNA in the presence of co-reductants such as ascorbic acid and glutathione.

Keywords: Copper(II) complexes; 2,2′-Bipyridine (bipy); 1,10-Phenanthroline (phen); Superoxide dismutase (SOD); DNA cleavage; Crystal structure

1. Introduction

Transition metal compounds containing diethylenetriamine have been of interest for many years [1–5]. Diethylenetriamine(dien) is a tridentate nitrogen donor ligand with donor groups suitably placed for forming 2 five-membered chelate rings. Copper(II) complexes have found possible medical uses in treatment of many diseases including cancer [6,7]. It has been known that the anti-cancer activity of some copper(II) complexes may be based on their ability to inhibit DNA synthesis [6]. 2,2′-Bipyridine (bipy) and 1,10-phenanthroline (phen) chelators also act as potential anti-tumor agents [8,9]. They can be of better anti-tumor activity if their hydrophilic groups are masked by copper ions to form water-soluble neutral complexes. These neutral complexes are expected to be more permeable through the cell membrane [10,11]. In this paper we report synthesis, single crystal X-ray structure, EPR electronic spectroscopic investigation and magnetic susceptibility of two new complexes $[Cu(dien)(bipy)](BF_4)_2$ and $[Cu(dien)(phen)](ClO_4)_2$ with diethylenetriamine and 2,2′-bipyridine or 1,10-phenanthroline. Biological studies (antibacterial and superoxide dismutase) studies of these complexes are also discussed.

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2. Experimental

2.1. Materials and methods

2,2′-Bipyridine, 1,10-phenanthroline, copper chloride dihydrate (all S.D. Fine Chemicals) and copper perchlorate hexahydrate (Aldrich) were used as supplied. Other chemicals were used as obtained without further purification. Solvents were purified by standard methods before use. Elemental analyses were performed on Elementar Vario EL III Carlo Erba 1108 analyzer. Copper was gravimetrically estimated as CuSCN. FAB mass spectra were recorded on a JEOL SX 102/DA 6000 mass spectrometer using Xenon (6 kV, 10 mA) as the FAB gas. The accelerating voltage was 10 kV and the spectra were recorded at room temperature (r.t.) with *m*-nitrobenzyl alcohol as the matrix.

The magnetic susceptibility data were recorded on a Gouy balance at room temperature using mercury(II) tetrathiocynato cobaltate(II) ($\chi_g = 16.44 \times 10^{-6}$ c.g.s. unit) as calibrant.

E.P.R. spectra were recorded at RT and also LNT (77 K) on a Varian E-line Century Series E.p.r. Spectrometer operating at 9.4 GHz with TCNE as a Calibrant. UV–vis spectra were recorded on a Shimadzu UV–vis 160 spectrometer with quartz cells.

2.2. Bioactivity

Anti-microbial (anti-bacterial) and superoxide dismutase (SOD) activities were evaluated using the following methods.

2.2.1. Anti-microbial activity

The in vitro anti-microbial (anti-bacterial) activities of these complexes were tested using paper disc diffusion method [12]. The chosen strains were G(+) *Staphylococcus* and G(−) *Proteus vulgaris*, *Salmonella*, *Pseudomonas* sp. and *Escherichia coli*. The liquid medium containing the bacterial subcultures was autoclaved for 20 min at 121 °C and at 15 lb pressure before inoculation. The bacteria were then cultured for 24 h at 36 °C in an incubator. Nutrient agar was poured onto a plate and allowed to solidify. The test compounds (DMSO solutions) were added dropwise to a 10 mm diameter filter paper disc placed at the centre of each agar plate. The plates were then kept at 5 °C for 1 h then transferred to an incubator maintained at 36 °C. The width of the growth inhibition zone around the disc was measured after 24 h incubation. Four replicas were made for each treatment.

2.2.2. SOD activity

In vitro SOD activity was measured using alkaline DMSO as a source of superoxide radical (O_2^-) and nitrobluetetrazolium (NBT) as O_2^- scavenger [13,14]. In general, 400 μL sample to be assayed was added to a solution containing 2.1 mL of 0.2 M potassium phosphate buffer (pH 8.6) and 1 mL of 56 μM NBT. The tubes were kept in ice for 15 min and then 1.5 mL of alkaline DMSO solution was added while stirring. The absorbance was then monitored at 540 nm against a sample prepared under similar condition except NaOH was absent in DMSO. A unit of superoxide dismutase [SOD] activity is the concentration of complex or enzyme, which causes 50% inhibition of alkaline dimethylsulphoxide (DMSO) mediated reduction of nitroblue tetrazolium chloride (NBT).

2.2.3. DNA cleavage

Electrophoresis experiments were performed with pBR322 DNA. The cleavage of pBR322 DNA by $[Cu(dien)(bipy)]^{2+}$ (**1**) and $[Cu(dien)(phen)]^{2+}$ (**2**) were accomplished by mixing 2 μL of pBR322 DNA (100 ng/μL), 3 μL of 50 mM Tris (pH 8.0, 5 mM NaCl) buffer, varying concentrations of Cu complexes (30, 50 μL) and co-reductants. After mixing, the DNA solutions were incubated for 1 h at 37 °C. The reactions were quenched by the addition of loading buffer (40% sucrose, 0.25% bromophenol blue and 1 mM EDTA). The reaction mixtures were analyzed by agarose gel electrophoresis (Tris–Boric acid–EDTA buffer, pH 7.5) at 50 V for 4 h. The gel was stained with 0.5 μg/mL ethidium bromide and photographed under UV illuminator.

2.3. Synthesis of the complexes

2.3.1. $[Cu(dien)(phen)](ClO_4)_2$

Methanol–acetonitrile (5:1 v/v) solution of $Cu(ClO_4)_2\cdot 6H_2O$ (0.740 g, 2.0 mmol), dien (0.263 g, 2.0 mmol) and phenanthroline (0.396 g, 2.0 mmol) were mixed, well stirred and set aside overnight. The blue crystals of $[Cu(dien)(phen)](ClO_4)_2$ which were obtained were washed with ethanol and dried in vacuo, yield 70%. This give the satisfactory elemental analysis: found C 35.39, H 4.06, N 12.06 and Cu 12.03%; calc. C 35.19, H 3.84, N 12.83 and Cu 11.64%. FAB mass spectra of this complex show the base peak at *m*/*z* 446 (M^+).

2.4. $[Cu(dien)(bipy)](BPh_4)_2$

Methanol–acetonitrile (5:1 v.v.) solution of $CuCl_2\cdot 2H_2O$ (0.341 g, 2.0 mmol), dien (0.263 g, 2.0 mmol) and bipyridine (0.312 g, 2.0 mmol) were mixed and well stirred. Addition of aqueous solution of $NaBPh_4$ gave the dark blue desired product. The product was washed, re-crystallized in acetonitrile and dried in vacuo, yield 69%. This gives the satisfactory elemental analysis: found C, 34.36; H, 4.34; N, 13.90; and Cu, 12.37%; calc. C, 33.80; H, 4.22; N, 14.08; and Cu, 12.78%. FAB mass spectra of this complex show the base peak at *m*/*z* 410 (M^+).

2.5. Crystal structure determination, solution and refinement

A blue prismatic crystal of $[Cu(dien)(phen)](ClO_4)_2$ and $[Cu(dien)(bipy)](BF_4)_2$ were mounted on a glass fiber and used for data collection. Cell constants and an orientation