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DNA interaction, SOD, peroxidase and nuclease activity studies of iron complex having ligand with carboxamido nitrogen donors

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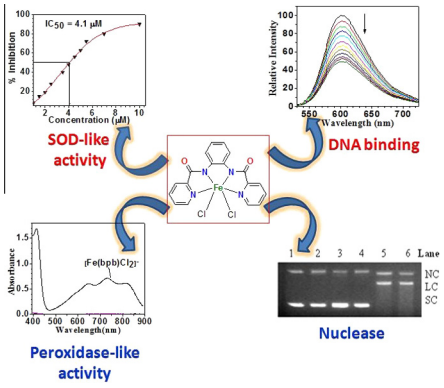
HIGHLIGHTS

- Superoxide dismutase, peroxidase like activity of $(Et_3HN)[Fe^{III}(bpb)Cl_2]$ complex.
- DNA interaction as well as nuclease study of small molecule SOD mimic.
- Mechanism of nuclease suggest possible participation of reactive oxygen species (ROS).

GRAPHICAL ABSTRACT



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ABSTRACT

Complex $(Et_3HN)[Fe^{III}(bpb)Cl_2]$, **1** {where H_2bpb: N,N′-(1,2-phenylene)bis(pyridine-2-carboxamide)} was synthesized and characterized by reported procedure (Yang et al., 1991). Complex **1** was found to be effective in superoxide scavenging activity and an IC_{50} value of 4.1 μM was obtained in xanthine–xanthine oxidase nitro blue tetrazolium assay. Peroxidase-like activity of this complex was determined by the oxidation of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). DNA interaction studies of complex **1** showed binding of DNA through external or groove binding. Complex **1** exhibited chemical nuclease activity in the presence of hydrogen peroxide and cleaved supercoiled *pBR322* DNA to its linear and nicked circular form at physiological pH. Mechanistic studies indicated possible role of hydroxyl radical (·OH) species in DNA cleavage activity via hydroperoxo intermediate: $[Fe^{III}—OOH^-]^{2+} \rightarrow [Fe^{IV}=O]^{2+} + {}^{\cdot}OH$.

Introduction

In recent years, studies on interaction of redox active transition metal complexes with DNA received considerable interest due to their plural applications in metallopharmaceutical research for the development of novel drugs [1] and bio-molecular probes [2]. In this regard, first row transition metals namely manganese, iron and copper complexes are preferred because they are biologically relevant and less toxic [1,3]. Our quest originated from our interest to study the interaction of DNA with metal complexes derived from the ligand containing carboxamido nitrogen donor(s). Carboxamido nitrogen (N_{am}) stabilizes higher oxidation states of metal ions and metal complexes having N_{am} donor(s) were found to be important for the activity of nitrile hydratase enzyme [4], metal binding in prion protein [5,6], photolabile nitrosyl complex synthesis [7,8], research in green chemistry [9]. Bleomycin in presence of iron showed nuclease activity [10,11] where carboxamido nitrogen was ligated to metal centre. We have recently communicated the role of carboxamido nitrogen in superoxide scavenging

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activity as well as nuclease activity for iron and manganese complexes [12–14]. Moreover, it has been documented in literature that native SOD as well as small molecule SOD mimics exhibit nuclease activity [15,16]. This prompted us to examine the superoxide scavenging activity and DNA interaction studies of iron complex ligated to a tetradentate ligand, H_2bpb which possesses two N_{am} donors (shown in Scheme 1). We have also reported similar results of a series of manganese complexes derived from tridentate ligands having phenolato donor(s) [17]. Interestingly, N_{am} and phenolato oxygen both prefer to stabilize higher oxidation states of metal [14,17].

It is well known that redox active manganese and iron possess similar chemistry [18] and their metal complexes could participate in Fenton type reactions. Manganese and iron complexes derived from H_2bpb ligand were used for several purposes. First, complexes of iron were used for synthesis of photolabile nitrosyl complex [19]. Second, oxo-transfer reaction was studied for iron complex derived from H_2bpb ligand [20,21]. Third, manganese complex derived from H_2bpb ligand was used for SOD activity studies [22], however to the best of our knowledge its iron complex was never been used for SOD activity and DNA interaction studies. It is also reported in the literature that this planar tetradentate ligand got certain similarities with ligands like salen and porphyrin [2,19] and could be used for peroxidase activity. However, DNA interaction studies with salen complexes of manganese and iron was studied [23,24]. Manganese and iron porphyrin complexes and their DNA interaction studies were also reported in the literature [25,26]. Herein, we report the superoxide dismutase and peroxidase like activity of complex $(Et_3NH)[Fe(bpb)Cl_2]$, **1** and examined their DNA interaction studies by ethidium bromide displacement assay, circular dichroism (CD) spectral studies. Nuclease activity of this small molecule SOD mimic was examined in this report. We also investigated the mechanism of nuclease activity.

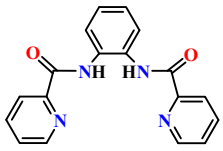
Experimental

Materials

The supercoiled *pBR322* DNA and CT-DNA were purchased from Bangalore Genei (India). Tris(hydroxymethyl)aminomethane–HCl (Tris–HCl) buffer was prepared in deionised water. Xanthine and NBT purchased from Himedia (India), Xanthine oxidase and ABTS (Fluka), Ethidium bromide (EB) were purchased from Sigma Aldrich (Germany).

Apparatus

Electronic absorption spectra were recorded in methanol, dimethylformamide solvents with an Evolution 600, Thermo Scientific UV-visible spectrophotometer. Emission quenching titrations were carried out on Varian fluorescence spectrophotometer. Circular dichroic spectra of DNA were obtained by using a Chirascan circular dichroism operating at 25 °C. Nuclease activity was performed on Gel Electrophoresis by Genei (Banglore, India). The fragments were visualized and photographed by using a UV illuminator (BIO RAD).



Scheme 1. Diagram of ligand H_2bpb.

Synthesis of complex $(Et_3NH)[Fe(bpb)Cl_2]$

The complex $(Et_3NH)[Fe(bpb)Cl_2]$, **1** was synthesized and characterized by reported literature procedure [20].

Biological studies

Superoxide dismutase activity

The SOD activity of complex **1** was tested by indirect method using xanthine–xanthine oxidase–nitro blue tetrazolium (NBT) assay [27–29]. Sets of assays were performed separately in duplicate in 50 mM potassium phosphate buffer at pH 7.2. These experiments were carried out in phosphate buffer (50 mM) using 0.6 mM xanthine, 0.3 mM NBT, 0.07 U/mL xanthine oxidase and catalase 1000 U/mL (final volume = 750 μL). The reaction was started after adding 0.07 U/mL xanthine oxidase and measurement was started after 3 min for each experiment.

Peroxidase activity

Oxidation of 2,2′-azinobis(3-ethylbenzothiazoline)sulfonic (ABTS) with H_2O_2 was tested in presence of complexes at pH 7. An aqueous solution of ABTS (50 μL; 0.009 M) and a methanolic solution of complex **1** (10 μL; 10^{-3} M) was added to the water (3 mL). The oxidation of ABTS starts immediately after addition of an aqueous solution of H_2O_2 (50 μL, 10 M) [30].

DNA-binding experiments

Fluorescence spectroscopic studies. Fluorescence quenching experiments were carried out by successive addition of 0–12 μM of complex **1** to CT-DNA (25 μM) solution containing 0.5 μM ethidium bromide (EB) in 0.1 M phosphate buffer (pH 7.2) in 5% dimethylformamide (DMF). This sample was excited at 250 nm and emission was observed at 602 nm. EB emits intense fluorescence in presence of DNA due to its strong intercalation between the adjacent DNA base pairs. The emission spectra of EB bound to DNA in absence and presence of the metal complex was given according to Stern–Volmer equation [31],

$$F_o/F = 1 + K_{SV}\,[R]$$

where F_o and F are the fluorescence intensities in the absence and the presence of complex, respectively. K_{SV} is a linear Stern–Volmer quenching constant, $[R]$ is the concentration of quencher. In the quenching plot of F_o/F vs $[R]$, K_{sv} is given by the ratio of the slope to intercept.

Circular dichroism

Circular dichroic spectra of DNA were obtained in the region between 220 and 320 nm and scanned in 1 mm path length cuvette with 10 min incubation time for each sample. Each spectrum was averaged from three successive accumulations and smoothed within permissible limits. Concentration of CT-DNA was 50 μM and 200 μM for complex **1** in 10% methanol in phosphate buffer.

DNA cleavage studies

DNA cleavage activity was measured by the conversion of supercoiled *pBR322* plasmid DNA to nicked circular and linear DNA forms. Supercoiled *pBR322* DNA (200 ng) in Tris–boric acid–EDTA (TBE) buffer (pH 8.2) was treated with complex **1** taken dimethylformamide (10%) in the presence or absence of additives. The oxidative DNA cleavage by complex **1** was studied in the presence of H_2O_2 (1.6 mM, oxidizing agent). The samples were incubated at 37 °C and loading buffer was added (25% bromophenol blue and 30% glycerol). The agarose gel (0.8%) containing 0.4 μg/mL of ethidium bromide (EB) was prepared and electrophoresis