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Synthesis, characterization and DNA binding studies of two mixed ligand complexes of ruthenium(II)

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Abstract

Two mixed ligand complexes $[Ru(bpy)_2(DMHBT)]Cl_2$(**1**) and $[Ru(phen)_2(DMHBT)]Cl_2$ (**2**) (where DMHBT is 11,13-dimethyl-13H-4,5,9,11,14-hexaaza-benzo[b]triphenylene-10,12-dione) have been synthesized and characterized by electrospray ionization (ESI) mass, ^1H-^1H correlation spectroscopy (COSY), electronic spectroscopy, fluorescence spectroscopy and cyclic voltammetry. Spectroscopic titration and viscosity changes of calf thymus (CT)-DNA in the presence of incremental amount of complexes **1** and **2** clearly demonstrate that both these complexes bind intercalatively to DNA, with binding constant $2.87 \pm 0.20 \times 10^4 M^{-1}$ and $1.01 \pm 0.20 \times 10^5 M^{-1}$ for complexes **1** and **2**, respectively. All the experimental evidences suggest that the ancillary ligand 2,2′-bipyridine (bpy) and 1,10-phenanthroline (phen) influences the intercalative binding of these complexes to DNA.

Keywords: Ruthenium(II) complexes; Mixed ligand complexes; Intercalation; DNA binding

1. Introduction

Nucleic acid binding metal complexes are currently being investigated in many laboratories because of their utility as DNA structural probes, DNA dependent electron transfer probes, DNA foot printing and sequence-specific cleaving agents and potential anticancer drug [1–4]. In this respect ruthenium(II) complexes have attracted a great deal of attention due to their unique spectroscopic and electrochemical signature [5–16]. Despite a considerable amount of the literature on metal complex DNA interaction, the knowledge of the nature of binding of these complexes to DNA and their binding geometries has remained a subject of debate. The binding mode of $[Ru(phen)_3]^{3+}$ remains an issue of rigorous debate [17,18]. On the other hand there is a consensus about intercalative binding of complexes such as $[Ru(bpy)_2(dppz)]^{2+}$ and $[Ru(phen)_2(dppz)]^{2+}$ (dppz = dipyrido[3,2-a:2′,3′-c]-phenazine). In these two complexes dppz ligand intercalates between the base pairs of double helical DNA [19–24]. Ever since the report of the DNA bases mismatch recognition agent, $[Rh(bpy)_2(chrysi)]^{3+}$ (chrysi = 5,6-chrysenequinone diimine), there has been renewed interest in the synthesis of mixed ligand complexes of transition metal ions. The complex $[Rh(bpy)_2(chrysi)]^{3+}$ has been found to bind at the mismatch sites in DNA specifically and upon photoactivation cleaves the DNA backbone neighboring the site [25]. The source of preferential binding has been reported to be the sterically bulky chrysi intercalating ligand, which is too wide to intercalate readily in to B-form DNA, but binds the destabilized regions associated with base mismatches [26]. Recently, two mixed ligand complexes of ruthenium(II) have also been reported, that bind DNA base pair mismatches [27]. In our laboratory, we have initiated a systematic study to understand the role of ancillary ligands in the DNA binding mode of ruthenium(II) complexes of mixed ligand complexes and to develop base mismatch

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recognition agent [28]. In this communication, we describe the synthesis and DNA binding properties of two new ruthenium(II) mixed ligand complexes.

2. Materials and methods

2.1. Materials

Ruthenium trichloride and calf thymus DNA (CT-DNA) were purchased from SRL Chemicals, Mumbai. 1,10-Phenanthroline and 2,2′-bipyridine were purchased from Ranbaxy Chemicals. 5,6-Diamino-1,3-dimethyl uracil hydrate was purchased from Aldrich Chemicals. LiCl was purchased from SD fine Chemicals. All other chemicals used were of analytical reagent grade and were used without purification.

2.2. Physical measurements

Elemental analyses (C, H, N) were carried out with a Heraeus-CHN-Rapid Analyser at Regional Sophisticated Instrumentation Centre, IIT, Chennai. ^{1}NMR spectra were recorded on a JEOL ECA-500 spectrometer with CD_3OD as solvent and $SiMe_4$ as an internal standard. Hewlett–Packard 1100 electrospray ionization (ESI) mass spectrometer was employed for the investigation of charged metal complex species in CH_3OH solvent. UV–Visible spectra were recorded on a Perkin–Elmer Lamda 35 spectrophotometer. Fluorescence measurements were carried out using Cary Eclipse spectrofluorimeter. Oswald's viscometer was employed for viscosity measurements. Cyclic voltammetric (CV) measurements were carried out using an EG and G PAR 173 Potentiostat/Galvanostat Analyser. The CV experiments were performed in a one compartment cell equipped with a glassy carbon working electrode and platinum wire as the auxiliary electrode. A saturated calomel electrode (SCE) was used as the reference electrode.

2.3. DNA binding studies

All the experiments involving the interaction of the complexes with DNA were carried out in Tris buffer (10 mM, pH 7.5). A solution of calf thymus DNA (CT-DNA) in the buffer gave a ratio of UV absorbance at 260 and 280 nm of about 1.8–1.9:1 indicating that the DNA was sufficiently free from protein. The DNA concentration was determined by the absorption spectroscopy using the molar absorption coefficient 6600 $M^{-1}cm^{-1}$ at 260 nm.

Absorption titration experiment was performed by maintaining the metal complex concentration constant (10 μM) and varying the concentration of nucleic acid from 20 to 200 μM. While measuring the absorption spectra, equal amount of DNA was added to both complex solution and the reference solution to eliminate the absorbance of DNA itself. From the absorption data, the intrinsic binding constant K_b was determined using the following equation through a plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ vs [DNA],

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f)$$

where [DNA] is the concentration of DNA, the apparent absorption coefficient ε_a,ε_f and ε_b corresponds to A_{obsd}/[Ru], the extinction coefficient for free ruthenium complex and extinction coefficient for ruthenium complex in the fully bound form, respectively.

Aqueous solutions of the ruthenium(II) complexes **1** and **2** were excited at 440 nm and its emission was recorded in the absence and presence of 20–200 μM CT-DNA.

Viscosity experiment was carried out on an Ostwald's viscometer, immersed in a thermostated water bath maintained at 25 ± 1 °C. DNA concentration was kept constant (100 μM) and the concentration of metal complexes was varied from 0 to 40 μM. Data are presented as $(\eta/\eta_o)^{1/3}$ vs $1/R$, where $R = [DNA]/[Ru]$ and η is the viscosity of DNA in the presence of the ruthenium(II) complex and η_o is the relative viscosity of DNA alone. Relative viscosity values were calculated from the observed flow time of DNA solution (t) and corrected for the flow time of buffer alone (t_o), using the expression $\eta_o = (t - t_o)/t_o$.

2.4. Synthesis of complexes

2.4.1. Synthesis of 11,13-Dimethyl-13H-4,5,9,11,14-hexaaza-benzo[b]triphenylene-10,12-dione (DMHBT)

1,10-Phenanthroline 5,6-dione was prepared according to the reported procedure [29]. 1,10-Phenanthroline 5,6-dione (0.5 g, 2.38 mmol) was dissolved in 50 mL of hot ethanol. To this, ethanolic solution of 0.4 g (2.38 mmol) of 5,6-diamino-1, 3-dimethyl uracilhydrate was added. This mixture was refluxed for 8 h at 80 °C and the precipitate formed was filtered and dried with diethyl ether. It was recrystallalised from hot ethanol–acetonitrile (5:1) mixture. The purity of the product was ascertained through TLC. Yield = 68%.

2.4.2. Synthesis of [Ru(L)$_2$(DMHBT)]Cl$_2$(L = bpy for **1** and phen for 2)

[cis-$(bpy)_2$ $RuCl_2$]·$2H_2O$ and [cis-$(phen)_2$ $RuCl_2$]·$2H_2O$ required for the synthesis of **1** and **2** were prepared according to reported procedure [30]. Synthesis of $[Ru(L)_2(DMHBT)]Cl_2$ (where L = bpy or phen) was as follows. The starting material [cis-$(bpy)_2$ $RuCl_2$]·$2H_2O$ or [cis-$(phen)_2$ $RuCl_2$]·$2H_2O$ (0.152 mmol) and DMHBT (0.52 g, 0.152 mmol) were heated to reflux in 25 mL methanol for 8 h under nitrogen atmosphere. This solution was cooled to room temperature, filtered and the solvent was removed from the filtrate using rotatory evaporator. The dark brown solid isolated was washed with acetone and dried. The product could be satisfactorily purified by chromatography on alumina gel by using CH_3CN—CH_3OH solution (100:1, v/v) as the eluent.

[Ru(bpy)$_2$(DMHBT)]Cl$_2$: Yield 65%. Anal. calc. for $C_{38}H_{28}Cl_2N_{10}O_2Ru$. C, 55.08; H, 3.41; N, 16.90. Found: C, 55.24; H, 3.32; N, 16.82. ^{1}NMR (CD_3OD): 9.6(doublet (d), 1H, $J = 8.45$ Hz), 9.5(d, 1H, $J = 8.4$ Hz), 8.7(multiplet