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An unusual oxidation of the imidazolyl ring in a cobalt terpyridyl complex: Crystal structure and photonuclease activity of the transformed cobalt terpyridyl complex

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ABSTRACT

This work describes a serendipitous oxidation of an imidazolyl ring to a carboxylic acid/ester group in a terpyridyl cobalt complex. The ligand transformation depends on both the oxidant and the solvent employed in the reaction and occurs during the oxidation of Co(II) to Co(III). While attempting to oxidize the parent $[Co(Itpy)_2]Cl_2$ (Itpy = imidazole terpyridine) complex using chlorine gas, it was found that both the metal and ligand had undergone oxidation, as was confirmed by single crystal XRD and ESI-MS. Studies using hydrogen peroxide as the oxidant revealed the formation of the expected cobalt(III) Itpy complex with an intact imidazole ring. The crucial role played by the solvent in the ligand transformation is established from the fact that while a methanolic medium transforms the imidazole ligand to a carboxymethyl ester, acetonitrile yields carboxylic acid functionality. It is likely that the chlorine radical generated during the oxidation of Co(II) to Co(III) is responsible for the unusual oxidation of the imidazolyl ring in the terpyridyl cobalt complex. The oxidized complex, $[Co(Etpy)_2](ClO_4)_3$, **1**, (Etpy = carboxymethylester terpyridine) has been characterized spectroscopically and electrochemically, and DNA binding studies were carried out on it. From the absorption spectral studies, it was established that complex **1** binds to DNA in grooves and its intrinsic binding strength has been found to be $(1.51 \pm 0.18) \times 10^4\ M^{-1}$. Since the imidazolyl ring was oxidized to a carboxymethyl ester, complex **1** lacks peripheral planarity and therefore binds in the grooves of the DNA. Further, molecular docking studies also confirm that the complex **1** is a major groove binder and is stabilized through hydrogen bonding interactions. The DNA cleavage experiments using pBR 322 show that the complex **1** exhibit efficient photonuclease activity in the presence of molecular oxygen.

1. Introduction

Transition metal complexes are being explored with great interest in nucleic acid and peptide chemistry since they find use in a wide range of applications, such as diagnostic and therapeutic agents [1–5]. Compounds capable of cleaving DNA upon light irradiation are used in the photodynamic therapy of cancers (PDT), which involves a non-invasive treatment of tumors using photoactive drugs [6,7]. Photoactive pro-drugs capable of releasing a cytotoxic drug only at the irradiated site can increase specificity and thereby minimize toxicity to the surrounding healthy cells. Porphyrin based compounds, like photofrin and their analogues, have found clinical applications in PDT [8,9]. However, due to hepatotoxicity, there is an immediate need to search for new non-porphyrinic organic and inorganic compounds which overcome this defect [10]. Transition metal complexes with polypyridyl ligands are known to exhibit photoinduced DNA cleavage. Some metal polypyridyl complexes, such as $[Ru(phen)_2dppz]^{2+}$, $[Ru(bpy)_2dppz]^{2+}$, $[Ru(bpy)_2dpq]^{2+}$, $[Co(phen)_2H_2biim]^{3+}$ and $[Co(bpy)_2H_2biim]^{3+}$ etc., are known to be photocleavers of DNA [11,12].

The research group of Nair is actively involved in the synthesis of metal complexes containing various substituted terpyridine ligand systems [13–19]. The terpyridine molecule serves as a tridentate, nearly coplanar, N_3 donor ligand, and in some rare cases also as a mono or bidentate ligand. The choice of the terpyridine (tpy) family of ligands over bipyridyl (bpy) ring systems is mainly due to two reasons. Firstly, being a tridentate ligand they form bis complexes with an octahedral geometry, which are thermodynamically more stable than the $M(bpy)_3$ analogues due to the chelate effect [20]. Secondly, the tpy ligands do not pose any complications in chirality as compared to the bipyridyl systems, where $M(bpy)_3$ complexes

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tend to be chiral, giving rise to Δ or Λ enantiomers. Square planar and octahedral metal complexes with tpy ligands have been reported to be of great biological interest. While complexes exhibiting planar structures easily insert themselves in between DNA base pairs, and hence are considered good metallo-intercalators, complexes with non-planar structures have been known to interact with DNA through other modes of binding [21]. Metal tpy complexes, with luminescence properties, are good candidates for photo-activated processes and can be used in DNA photocleavage. Metal ions complexed to tpy ligands, such as $[Ru(Itpy)_2]^{3+}$, $[Ru(bitpy)_2]^{2+}$, $[Co(pytpy)_2]^{3+}$, $Cr(ttpy)_2]^{3+}$ and $Cr(Brphtpy)_2]^{3+}$, have been reported to cleave DNA photolytically [13,17,22].

Cobalt complexes find potential application in hypoxia activated prodrugs [23]. Maiya's group has demonstrated that cobalt(III) complexes containing polypyridyl ligands are efficient photocleavers of DNA [24]. Similar DNA photocleavage by cobalt complexes has been discovered by Ji's group [25]. Cobalt complexes which have similar photophysical and photochemical properties as that of ruthenium and rhodium complexes have not been exploited much for their DNA binding and photonuclease activity. In our earlier work, we reported the synthesis, DNA binding and photo-induced cleavage properties of a cobalt pyridine terpyridine (pytpy) complex [17]. As part of our ongoing interest in designing new substituted tpy metal complexes, we have synthesized an imidazole terpyridine (Itpy) cobalt complex [18]. While attempting to oxidize the cobalt(II) Itpy complex to its cobalt(III) counterpart using chlorine gas, we observed unusual oxidation products. In addition to the metal oxidation, the imidazolyl ring had surprisingly transformed into a carboxylic ester. This could be considered as a metal assisted in-situ ligand transformation. A similar oxidation involving the conversion of a pyridyl ring to COO^- has been brought about by copper under hydrothermal conditions [26].

The substituents at the peripheral or at the 4′ position of the tpy ligand are known to modulate the electronic and the redox properties of cobalt complexes, and therefore its photonuclease activity [27]. In the metal complexes, the ligands play a key role in determining the mode of interaction with DNA and cleavage of DNA. Metal tpy complexes containing substituents at the 4′ position, such as pyridyl, benzimidazolyl, anthracenyl, pyrenyl etc., have been shown to be DNA intercalators [17,28]. Likewise, $[Co(Itpy)_2]^{2+/3+}$ complexes with an imidazolyl substituent at the 4′ position of the tpy unit also show intercalating ability [18]. However, in the present case, the imidazolyl substituent of the ligand has been oxidized to a carboxymethyl ester functionality which lacks planarity. This could probably influence the interaction of the transition metal complex with DNA.

In the present communication, we report the unusual oxidation of the imidazolyl ring of a terpyridine cobalt complex to a carboxylic ester, the effect of solvent on the transformation and its functionalization to amide. The effect of substituents at the 4′ position of cobalt tpy complexes on the spectroscopic, redox and DNA binding and cleavage properties has also been demonstrated.

2. Experimental

2.1. Materials

Chemicals used for the synthesis of the ligand (Itpy), imidazole-2-carboxaldehyde and 2-acetyl pyridine, were purchased from Sigma–Aldrich. The other chemicals, namely ammonium acetate, sodium hydroxide, potassium permanganate and solvents like acetonitrile, dimethyl sulfoxide, methanol, acetic acid and perchloric acid, were procured from SRL India. Calf thymus DNA (CT-DNA) used for the DNA binding studies was also procured from SRL India. The buffers Tris–HCl [Tris (hydroxymethyl) aminomethane–HCl] and TBE (Tris Boric acid EDTA) were prepared using deionised triple distilled water. Stock solutions of CT-DNA were prepared by dissolving it in Tris buffer (pH 7.2) and then dialysing for 24 h. It was then filtered through cellulose acetate filter of pore size 0.2 μm. The DNA solution so obtained gave a ratio of UV absorbance A_{260}/A_{280} of 1.82. This value indicates that the stock CT-DNA solution was sufficiently free from protein [29]. The concentration of DNA stock solution was determined from UV absorbance at 260 nm after suitable dilution in buffer and using a molar extinction coefficient (ε_{260}) value of 6600 $cm^{-1}M^{-1}$ [30]. Supercoiled plasmid pBR 322 DNA was purchased from Bangalore Genei (India) and used for the DNA cleavage experiments.

2.2. Physical techniques

Elemental analyses were performed using Eurovector EA elemental analyzer. UV–Vis absorption spectra were recorded on a Perkin Elmer lambda 35 double beam spectrophotometer using 3 mL quartz cuvettes of 1 cm pathlength. IR spectra of the complexes were obtained from a Perkin Elmer RX-I FT-IR spectrophotometer. The complexes were made into pellets using KBr and scanned between 4000 and 400 cm^{-1}. The ^1H NMR spectra were recorded in DMSO-d_6 solution using a JEOL-500 MHz spectrometer. A Thermo Finnigan LCQ 6000 advantage max ion trap mass spectrometer was employed for electrospray ionization mass spectra analysis (ESI-MS). The carrier solvent used was acetonitrile and the capillary voltage was maintained at 20 kV. Cyclic voltammetry was performed with a CH instrument (USA) model CH-620 B, electrochemical analyzer employing a glassy carbon electrode as the working electrode, a saturated calomel electrode as the reference electrode and a platinum electrode as the counter electrode. Tetrabutyl ammonium perchlorate and NaCl were used as supporting electrolytes for non-aqueous and aqueous medium, and nitrogen gas was purged through the system prior to all the electrochemical work. Time resolved fluorescence measurements for the cobalt(III) complexes were performed using a picosecond laser excited TCSPC (Time Correlated Single Photon Counting) spectrometer. The excitation source was a tunable Ti-Sapphire laser (Tsunami, Spectrophysics, USA) with a pulse width of <2 ps and a repetition rate of 82 MHz. The emission was monitored using a MCP-PMT (Hamamatsu-C 4878) detector. Decay traces were deconvoluted by a nonlinear least squares analysis using IBH software.

2.3. Syntheses

2.3.1. $[Co(Etpy)_2](ClO_4)_3.2.5H_2O$ (**1**)

The starting complex $[Co(Itpy)_2]Cl_2$ was prepared as reported [18]. The complex $[Co(Itpy)_2]Cl_2$ (0.73 g, 1 mmol) was dissolved in methanol and Cl_2 gas was passed through this methanolic solution. The color of the solution changed from dark brown to yellow, which indicated completion of the oxidation. Then a saturated solution of sodium perchlorate was added to precipitate the cobalt(III) complex. The precipitate thus obtained was filtered, washed with ether and dried in vacuum (yield: 79%). The solid complex was then dissolved in an acetonitrile:water (9:1 v/v) solvent mixture and kept for slow evaporation at room temperature. After a week, yellow needle shaped crystals were obtained. *Anal.* Calc. for $C_{34}H_{31}N_6Cl_3O_{18.5}Co$: C, 41.46; H, 3.17; N, 8.53. Found: C, 41.89; H, 3.07; N, 8.81%. ESI-MS *m/z*: 213.87 ($[Co(Etpy)_2]^{3+}$), 369.93 ($[Co(Etpy)_2+ClO_4^-]^{2+}$). ^1H NMR (DMSO-d_6) δ (ppm): 9.76 (s, 2H), 9.21 (d, *J* 7.7 Hz, 2H), 8.32 (t, *J* 7.7 Hz, 2H), 7.52 (d, *J* 6.1 Hz, 2H), 7.45 (t, *J* 6.1 Hz, 2H), 4.2 (s, 3H). IR (KBr pellet, cm^{-1}) 1733, 1427, 1276, 1107, 1087, 626.