Polyhedron 27 (2008) 3443–3450

Contents lists available at ScienceDirect

Polyhedron

journal homepage: www.elsevier.com/locate/poly

# Cobalt complexes of terpyridine ligands: Crystal structure and nuclease activity

Ramasamy Indumathy [a], Mookandi Kanthimathi [a], T. Weyhermuller [b], Balachandran Unni Nair [a,*]

[a] *Chemical Laboratory, Central Leather Research Institute (Council of Scientific and Industrial Research), Sardhar Patel Road, Adyar, Chennai 600 020, India*
[b] *Max-Planck Institut Fur Bioanorganische Chemie, D-45413 Mulheim an der Ruhr, Germany*

ARTICLE INFO

*Article history:*
Received 29 May 2008
Accepted 15 August 2008
Available online 23 September 2008

*Keywords:*
Cobalt complexes
Crystal structures
DNA binding
Oxidative cleavage

ABSTRACT

Cobalt(II) (**1**) and cobalt(III) (**2**) complexes of tridentate ligand, imidazole terpyridine (Itpy), have been synthesized and characterized by both spectroscopic and electrochemical techniques. Single crystal X-ray diffraction studies of complexes **1** and **2** shows that the complexes belong to monoclinic crystal system, with the two Itpy ligands coordinated to the central metal ion. The binding behavior of both the cobalt complexes to calf thymus DNA has been investigated by UV–Vis, fluorescence spectroscopy, viscosity and electrochemical measurements. The results suggest that complexes **1** and **2** bind to DNA through intercalation. The intrinsic DNA binding constant values obtained from absorption spectral titration studies were found to be $(5.07 \pm 0.12) \times 10^3$ $M^{-1}$ and $(7.46 \pm 0.16) \times 10^3$ $M^{-1}$, respectively, for complexes **1** and **2**. Gel electrophoresis studies with the cobalt complexes show that while complex **1** cleaves DNA in the presence of hydrogen peroxide, complex **2** cleaves DNA in the presence of ascorbic acid and hydrogen peroxide**.**

## 1. Introduction

Design of small molecules that are capable of binding and cleaving DNA at specific sites has been an area of considerable interest. Small molecules that bind to DNA have been used as diagnostic probes for both structural and functional aspects of nucleic acid and in the development of new therapeutic agents [1–5]. Towards this end, for the past 25 years, increasing attention has been towards transition metal complexes. These metal complexes are substitutionally inert, possess rich photophysical and electrochemical properties which render them as useful candidates for applications in the fields of molecular biology, biotechnology and medicine [6–10].

Transition metal complexes bind to DNA by both covalent and non-covalent interactions. Covalent binding involves the coordination of the nitrogenous base or the phosphate moiety of the DNA to the central metal ion and is possible in complexes where the metal is coordinatively unsaturated or is coordinated to substitutionally labile ligands. The three different non-covalent binding modes are intercalation, which involves the stacking of the molecule between the base pairs of DNA, groove binding, which comprises the insertion of the molecule into the major or minor grooves of DNA and the electrostatic or external surface binding. Upon binding to DNA, these small molecules are stabilized through a series of weak interactions such as π-stacking interactions of aromatic heterocyclic groups between the base pairs (intercalation), hydrogen bonding and van der Waals interactions of functional groups bound along the groove of the DNA helix [11]. Various factors that govern the binding of metal complexes with DNA are shape and size of the ligand, hydrophobicity, spin state, redox potential and hydrogen bonding ability on the complexes [12,13]. The binding studies of these small molecules with DNA are important in the design and development of new and efficient drugs targeted to DNA.

Earlier studies have focused on interactions of metal ions with DNA to assess how binding of metal ions stabilize or destabilize the DNA duplex and their binding strengths [14]. With the successful application of drugs such as *cis*-platin, auranofin and cardiolyte in the treatment of tumors, rheumatoid arthritis and as imaging agents for the diagnosis of diseases, studies on several transition metal complexes gained prominence [15,16]. In the last decade, DNA interaction studies on polypyridyl metal complexes have been the focus of several bio-inorganic research groups. Polypyridyl complexes of ruthenium and rhodium exhibit interesting spectroscopic and luminescence properties on binding to DNA [17,18]. Barton et al., have shown that the $[Ru(phen)_2(dppz)]^{2+}$ complex acts as a powerful molecular light switch for the detection of DNA [19]. Similarly, $[Rh(bpy)_2(phi)]^{3+}$, $[Rh(phen)_2(phi)]^{3+}$ and $[Rh(phi)_2(bpy)]^{3+}$ have been shown to be efficient agents for photoactivated DNA cleavage [20,21]. But cobalt polypyridyl complexes which have similar photophysical properties and DNA binding properties have not been as widely explored as that of ruthenium and rhodium. Some of the previous reports on cobalt(III) complexes show that hexaammine cobalt complexes induce DNA condensation [22,23] and can be used to probe RNA hairpins [24] and cobalt–phenanthroline and 3,4-diarylsalen complexes have been

---

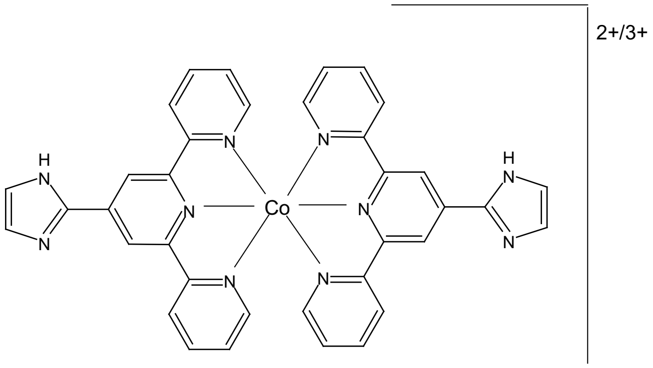
* Corresponding author. Tel.: +91 44 2441 1630; fax: +91 44 2491 1589.
*E-mail address:* bunair@clri.res.in (B.U. Nair).

0277-5387/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.poly.2008.08.003

used in tumor therapeutics [25]. Cobalt(III) nitrogen mustard complex has also been shown to be potential hypoxia-activated prodrug [26,27]. Some of the cobalt(II) complexes, such as [Co(tfa)$_2$(happ)], are known to function as specific probe for DNA bulges due to its ability to cleave DNA specifically [28].

Our research group currently focuses on metallo-intercalators of various metals incorporating terpyridine ligands [29–31]. Terpyridine ligands are known for intercalation and due to the presence of conjugated aromatic core, exhibit several interesting luminescence properties. Like the excited state of the ruthenium(II) complex, excited state of cobalt(II) and cobalt(III) complexes too can bring about cleavage of macromolecules like protein and DNA. Schiff base ligand in the presence of cobaltous chloride has been shown to induce site-specific photocleavage of protein [32]. However, cobalt(II) and cobalt(III) complexes have not been exploited for photodynamic therapy. We have shown that some of the cobalt complexes with tridentate ligands like bzimpy and ptpy are good candidates for photoactivated DNA strand cleavage [33,34]. Towards achieving our goal of developing cobalt complexes of tridentate ligands, which may find therapeutic applications due to their oxidative cleavage or photocleavage of DNA, we report here the syntheses, characterization, DNA binding and cleavage studies of two new cobalt complexes, namely [Co(Itpy)]$^{2+}$ **1** and [Co(Itpy)]$^{3+}$ **2** derived from tridentate imidazole terpyridine ligand (Scheme 1).

A host of physical methods like absorption, fluorescence, cyclic voltammetry and viscosity measurements have been used to probe the interactions of the cobalt complexes with CT-DNA.



**Scheme 1.** Complex **1** is dipositive and **2** is tripositive complex.

## 2. Experimental

### 2.1. Materials

Chemicals such as 2-acetyl pyridine and imidazole-2-carboxaldehyde used for ligand synthesis were purchased from Aldrich Chemicals and used as received. All other chemicals used in this study like ammonium acetate, potassium permanganate, sodium perchlorate and solvents like acetonitrile, dimethyl sulfoxide, diethyl ether, methanol, perchloric acid and hydrochloric acid, purchased from Rankem, were of analytical grade and used as received without further purification. The ligand imidazole terpyridine (Itpy) was prepared using published procedures [35]. Calf thymus DNA was purchased from SRL (India). Agarose (molecular biology grade) and Ethidium bromide were procured from Sigma chemicals. Buffers were prepared using deionised and sonicated triple distilled water. Tris (hydroxymethyl) aminomethane–HCl (Tris–HCl) buffer (pH, 7.2) was used for all DNA binding and cleavage studies. Concentrated stock solutions of CT-DNA (7 mM) were prepared in Tris buffer. A solution of CT-DNA in the buffer gave the UV absorbance ratio of ~1.8–1.9:1, at 260 and 280 nm indicating that the DNA was sufficiently free of protein [36]. DNA concentration, more precisely nucleotide phosphate (NP) concentration, was determined by UV absorbance at 260 nm. The extinction coefficient was taken to be 6600 mol$^{-1}$ cm$^{-1}$ [37]. Stock solutions of DNA were stored at 4 °C and used within 4 days.

### 2.2. Physical measurements

UV–Vis spectra of the complexes and absorption titration studies were conducted using Perkin–Elmer Lambda 35 double beam spectrophotometer at 25 °C. Elemental Analyses were done using Heraeus CHN–O– Rapid Analyser. The infrared spectra of the complexes were recorded on Perkin–Elmer FT-IR spectrometer. Positive ion electrospray ionization mass spectra of the complexes were obtained by using Thermo Finnigan LCQ 6000 advantage max ion trap mass spectrometer. The emission spectra were recorded in varian spectrofluorimeter. Cyclic Voltammetric experiments were performed on CH instrument (USA) model CH-620 B, electrochemical analyzer. The three electrode configuration, a glassy carbon as working electrode, saturated calomel electrode as reference electrode and platinum electrode as auxillary electrode was used. Deoxygenated, anhydrous acetonitrile solutions containing tetrabutyl ammonium perchlorate (TBAP) as supporting electrolyte were used for the electrochemical measurements. All measurements were carried out in 10 mM Tris buffer, 50 mM NaCl (pH 7.2). $^1$H NMR was recorded in DMSO-$d_6$ solvent using JEOL-500 MHz spectrometer. Chemical shifts ($\delta$) are given in ppm.

*Caution*! Perchlorate salts are potentially explosives and should be handled only in small quantities with care.

### 2.3. Synthesis of bis(imidazole-terpyridine) cobalt(II) perchlorate, [Co(Itpy)$_2$](ClO$_4$)$_2$, (**1**)

The ligand imidazole terpyridine (Itpy) was prepared by slight modification of the reported procedure and the authenticity confirmed from ESI-MS, $m/z = 300$ [Itpy+H$^+$]. The cobalt(II) complex **1** was prepared directly by the addition of cobaltous chloride and the ligand imidazole terpyridine (Itpy) in 1:2 mole ratio, using methanol as reaction medium. The ligand Itpy (0.60 g, 2 mmol) in methanolic solution was added to the boiling methanolic solution of cobaltous chloride (0.23 g, 1 mmol). The solution immediately turned dark red. An aqueous solution of sodium perchlorate was added. Upon slow cooling, the dark red precipitate settled down. The crude precipitate was recrystallised from acetonitrile/methanol mixture (1:1 v/v) and the recrystallised product was filtered, washed with diethyl ether and then dried under vacuum. Yield: 85%. Elemental *Anal.* Calc. for [Co(C$_{18}$H$_{13}$N$_5$)$_2$](ClO$_4$)$_2$: C, 50.5; H, 3.04; N, 16.37; Co, 6.90. Found: C, 50.43; H, 3.7; N, 16.81; Co, 6.80%. ESI-MS *m/z*, 328.8, [Co(Itpy)$_2$]$^{2+}$. IR (KBr phase, cm$^{-1}$): 3461 br, 1629 m, 1473 w, 1114 m, 629 w (s, strong; w, weak; m, medium; br, broad). Single crystals of the complex were grown by ether diffusion method and the dark red crystals obtained were used for XRD studies.

### 2.4. Synthesis of bis(imidazole-terpyridine) cobalt(III) perchlorate, [Co(Itpy)$_2$](ClO$_4$)$_3$, (**2**)

Complex **2** was prepared from complex **1**. Cobalt(II) complex **1** prepared above was dissolved in methanol and few drops of dilute perchloric acid was added. To this solution 2.5 ml of hydrogen peroxide (30% w/v) was added and slightly warmed. Slowly, the colour of the solution turned deep yellow indicating the oxidation of cobalt(II) to cobalt(III). On standing, the solution yielded a yellow microcrystalline solid of the complex which was filtered, washed