Available online at www.sciencedirect.com
ScienceDirect
Journal of Inorganic Biochemistry 101 (2007) 434–443
JOURNAL OF Inorganic Biochemistry
www.elsevier.com/locate/jinorgbio

Cobalt complexes of terpyridine ligand: Crystal structure and photocleavage of DNA

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

[a] *Chemical Laboratory, Central Leather Research Institute, Adyar, Chennai 600 020, India*
[b] *Max-Planck Institut Fur Bioanorganische Chemie, D-45413 Mulheim an der Ruhr, Germany*

Received 7 August 2006; received in revised form 6 November 2006; accepted 6 November 2006
Available online 15 November 2006

Abstract

Two new cobalt complexes, $[Co(pytpy)_2](ClO_4)_2$, **1**, and $[Co(pytpy)_2](ClO_4)_3$, **2** where pytpy = pyridine terpyridine, have been synthesized and characterized. Single-crystal X-ray structure of both the complexes has been resolved. The structure shows the complexes to be a monomeric cobalt(II) and cobalt(III) species with two pytpy ligands coordinated to the metal ion to give a six coordinate complex. Both cobalt(II) and cobalt(III) complexes crystallize in meridional configuration. The interaction of these complexes with calf thymus DNA has been explored by using absorption, emission spectral, electrochemical studies and viscosity measurements. From the experimental results the DNA binding constants of **1** and **2** are found to be $(1.97 \pm 0.15) \times 10^4\ M^{-1}$ and $(2.7 \pm 0.20) \times 10^4\ M^{-1}$ respectively. The ratio of DNA binding constants of **1** and **2** have been estimated to be 0.82 from electrochemical studies, which is in close agreement with the value of 0.73 obtained from spectral studies. The observed changes in viscosity of DNA in the presence of increasing amount of complexes **1** and **2** suggest intercalating binding of these complexes to DNA. Results of DNA cleaving experiments reveal that complex **2** efficiently cleaves DNA under photolytic conditions while complex **1** does not cleave DNA under similar conditions.

Keywords: DNA interaction; Cobalt complexes; Photocleavage

1. Introduction

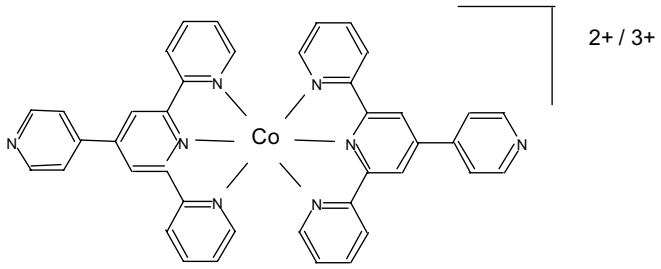
Recent years have seen a growing interest in the binding of small molecules to DNA and DNA cleaving with metal complexes [1–3]. The interaction of transition metal complexes with DNA has been extensively studied in order to develop novel probes of DNA structure [4,5] and DNA mediated electron transfer reactions [6]. Metal ion coordination to nucleic acids is not only required for charge neutralization, it is also essential for the biological function of nucleic acids [7]. Metal ions are used in purifying nucleic acids [8] and in probing the structure and biochemistry of nucleic acids [9]. The precise understanding of the DNA binding properties of metal complexes gains importance because of therapeutic approaches; study of nucleic acid conformations and in the development of new tools for nanotechnology [10–12]. Prominent among the various metal complexes employed by our group so far in the studies of DNA interaction, are those of metallo-intercalators which incorporate either pyridine, phenanthroline or a modified phenanthroline moiety as ligands [13–16]. In these complexes, the ligands or metal ion may be varied in an easily controlled way to facilitate the individual application. Polypyridyl transition metal complexes can bind to DNA by non-covalent interactions such as external surface binding, groove binding for large molecules and intercalation for planar molecules or compounds containing a ring system [17]. All the studies reveal that modification of the metal or ligands would lead to subtle or substantial changes in the binding modes, location of binding and

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

0162-0134/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.jinorgbio.2006.11.002

affinity of DNA binding. This gives valuable information to explore the various site-specific DNA probes and potential chemotherapeutical agents [18]. The interaction of transition metal polypyridyl and Schiff base ligand complexes with DNA has been extensively studied by us [19–21] because, their general photoactivity make them suitable candidates as probes for DNA secondary structure, photocleavers and antitumour drugs [22–24]. Cobalt complexes have gained importance because of their application as potential hypoxia-activated prodrug [25–27]. We chose to concentrate our studies on complexes of cobalt, which have the same interesting characteristics and DNA cleaving properties as ruthenium complexes, but have not received as much attention as the ruthenium(II) systems [28,29]. Among different methodologies adopted for the photocleavage of DNA, the one based on irradiation with light of longer or shorter wavelength has gained importance for their potential use in photodynamic therapy (PDT) of cancer [30–33]. We have designed new cobalt complexes derived from a tridentate ligand, pyridine terpyridine with the aim to involve the metal based d–d and/or charge transfer band(s) in the photoexcitation process.

Substituted pyridines including terpyridines are prominent building blocks in both organic and inorganic supramolecular chemistry [34,35] with their π-stacking ability, directional H-bonding and coordination properties. The coordination chemistry of 2,2′:6′,2″-terpyridine (terpy) and its derivatives has been intensively explored due to the array of interesting electronic, photonic, magnetic, reactive and structural properties shown by the transition metal complexes of this family of ligands [36]. The luminescence properties of pyridine terpyridine ligands stem from the conjugated aromatic cores [37]. The present work is in continuation of our interest in defining and evaluating DNA binding properties of pyridine based ligands, which would help in the design of newer drugs and develop new selective and efficient DNA recognition and cleaving agents. We aim at exploring the design of new metal complexes, which possess more potent DNA binding affinities and DNA cleaving ability. In this paper, we report the synthesis and characterization of two new cobalt complexes derived from terpyridine ligand, as shown below:



1 and **2** (**1** is dipositive and **2** is tripositive complex)

A series of physical methods like absorption, fluorescence, thermal denaturation studies,cyclic voltammetry and viscosity measurements have been used to probe the interactions of the cobalt complexes with CT DNA. The photochemical cleavage of DNA by complex **2** is also demonstrated.

2. Experimental section

2.1. Materials

The materials used in this investigation such as 2-acetyl pyridine, pyridine-4-carboxaldehyde were purchased from Aldrich Chemicals and used as received. Other materials like sodium hydroxide, ammonium acetate, sodium perchlorate, potassium permanganate and solvents like acetonitrile, dimethyl sulphoxide, diethyl ether, perchloric acid and hydrochloric acid were of reagent grade. The ligand, pyridine–terpyridine was prepared using published procedures [38]. The purity of the ligand was examined by measuring the melting point and compared with the literature value. CT DNA was purchased from Bangalore Genie (India). Agarose (molecular biology grade), and ethidium bromide (EB) were from Sigma. Tris (hydroxymethyl) aminomethane–HCl (Tris–HCl) buffer was prepared using deionized and sonicated triple distilled water. All the experiments regarding the binding and cleavage of DNA using complexes **1** and **2** were carried out in Tris buffer (pH, 7.2). A solution of 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 [39]. The DNA concentration per nucleotide was determined by absorption spectroscopy using molar absorption coefficient 6600 M^{-1} cm^{-1} at 260 nm [40].

2.2. Physical measurements

Elemental analyses were performed using a Heraeus CHN-O-rapid analyzer. UV–visible spectra of the complexes were recorded on a Perkin–Elmer Lambda 35 double beam spectrophotometer at 25 °C. The emission spectra were recorded on a Hitachi 650-40 spectrofluorimeter. The electrospray ionization (ESI) mass spectra of the complexes were recorded with a Micro mass Quattro II triple quadrupole mass spectrometer. The infrared spectrum of the complex was recorded on a Perkin–Elmer FTIR spectrometer. Cyclic voltammetry was performed on an EG and G PAR 173 potentiostat/galvanostat analyzer. Tetrabutyl ammonium perchlorate (TBAP) was used as the supporting electrolyte. The sample in dried DMSO was purged with nitrogen prior to measurement. A standard three electrode system comprising of glassy carbon as a working electrode, platinum electrode as an auxillary electrode and a saturated calomel as reference electrode (SCE) was used. Cyclic voltammetric investigations on complexes **1** and **2** have been carried out in 10 mM Tris buffer, 50 mM NaCl (pH 7.2). ^{1}H NMR was recorded in DMSO – d_6 solution using JEOL – 500 MHz spectrometer. Chemical shifts (δ) are given in ppm. (Caution. Perchlorate