Full Research Paper

DNA-Binding Study of Tetraaqua-bis(p-nitrobenzoato)cobalt(II) Dihydrate Complex: \([\text{Co(H}_2\text{O})_4(p-\text{NO}_2\text{C}_6\text{H}_4\text{COO})_2] \cdot 2\text{H}_2\text{O}\)

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Abstract: The interaction of \([\text{Co(H}_2\text{O})_4(p-\text{NO}_2\text{C}_6\text{H}_4\text{COO})_2] \cdot 2\text{H}_2\text{O}\) with sheep genomic DNA has been investigated by spectroscopic studies and electrophoresis measurements. The interaction between cobalt(II) \(p\)-nitrobenzoate and DNA has been followed by gel electrophoresis while the concentration of the complex was increased from 0 to 14 mM. The spectroscopic study and electrophoretic experiments support the fact that the complex binds to DNA by intercalation via \(p\)-nitrobenzoate into the base pairs of DNA. The mobility of the bands decreased as the concentration of complex was increased, indicating that there was increase in interaction between the metal ion and DNA.

Keywords: Cobalt(II) complexes; DNA-binding; \(p\)-nitrobenzoate

1. Introduction

The interaction of transition metal complexes with DNA has been extensively studied in the past few years. The carcinogenicity of cobalt, nickel, cadmium, and arsenic compounds have been known for the long time. Even though only weakly mutagenic, several kinds of cellular damage have been identified, related with their carcinogenic potentials [1]. They were put in the induction of oxidative potential interactions with the removal of different kinds of DNA damage. DNA damage and
epigenetic alterations, like gene silencing, change in DNA methylation patterns as shown for particulate nickel compounds and arsenite [1]. Additionally, there is accumulating evidence that metal ions interfere with distinct steps of diverse DNA repair systems. First indications that metal ions may diminish DNA repair processes were obtained by pronounced comutagenic effects of arsenic(III), cobalt(II), nickel(II) and cadmium(II) in bacteria as well as in mammalian cells. Based on these observations, detailed studies have been conducted to oxidative elucidate potential interactions with the removal of different types of DNA damage [1].

These studies are also important for the development of probes nucleic acid structure [2–5] as well as for determining the mechanism of metal ion toxicity [6–8]. There has been major interest in the rational design of novel transition metal complexes which bind and cleave duplex DNA with high sequence or structure selectivity [9–11]. Indeed, there is already a considerable literature involving the practical use of transition metal complexes as chemical nucleases [12–24]. Many useful applications of these complexes require that the complex bind to DNA through an intercalative mode with the ligand intercalating into the adjacent base pairs of DNA. However, most of these complexes contain only planar aromatic ligands and investigations of such complexes with ligands containing substituents as DNA-binding reagents have been relatively few. In fact, some of these complexes also exhibit interesting properties upon binding to DNA [25–29]. The features common to these complexes are that the molecule has a high affinity for double-stranded DNA, and that the molecule also binds a redox-active metal ion cofactor. The ligands or the metal in these complexes can be varied in an easily controlled manner to facilitate an individual application. All the studies reveal that modification of the metal or ligands would lead to subtle or substantial changes in the binding modes, location and affinity [7,8], giving rise to changes to explore various valuable conformation or site-specific DNA probes and potential chemotherapeutical agents. Currently, much attention has been paid to the complexes of Ru(II) [9–15]. But, the other metal ion complexes have attracted much less attraction than ruthenium(II) complexes.

In this paper, we chose to concentrate our work on the complex tetraaqua-bis(p-nitrobenzoato) cobalt(II) dihydrate, \([\text{Co(H}_2\text{O)}_4(p-\text{NO}_2\text{C}_6\text{H}_4\text{COO})_2\text{]}\text{.}2\text{H}_2\text{O}\), (CoPNB), which has interesting characteristics and DNA binding properties, but which has not received as much attention as the Ru(II) systems [6,16–19]. The binding properties to genomic DNA of this cobalt(II) complex, containing a ligand with nitro substituents at the 5-position of the phenyl group, was studied using absorption spectroscopy and electrophoresis measurements. These studies are necessary for the further comprehension of binding of transition metal complexes to DNA; they can also serve as complementary studies for the corresponding ruthenium complexes.

2. Results and Discussion

The interaction of the complex with DNA was investigated using absorption spectra. The absorption spectra of complex in the absence and presence of genomic DNA (at a constant concentration of the complex) was studied. Figure 1 represents the absorption spectra of CoPNB in the absence and presence of increasing amounts of genomic DNA. In the UV region, the intense absorption bands were seen. The Co(II) complex is believed to be the intraligand transition of the coordinated groups [29]. Addition of increasing amounts of the complex results in hypochromism and
bathochromic shift in the UV spectra of CoPNB [29]. The hypochromism in the intraligand band reaches as high as at around 270 nm with red shift at the ratio of [DNA]/[Co]. These spectral characteristics suggest the complex binds to DNA by intercalation. After intercalating the base pairs of DNA, the π-π* orbital of the intercalated ligand can couple with the π orbital of the base pairs, due to the decrease the π-π* transition energy which results in the bathochromism [29].

![Figure 1](image1.png)

**Figure 1.** Absorption spectra of CoPNB (14 mM) in the absence and presence upon addition of genomic DNA (0-10 mM). Arrow represents the absorbance changing when concentration of genomic DNA is increased.

The interaction of tetraaqua-bis(p-nitrobenzoato)cobalt(II) dihydrate complex, \([\text{Co}(\text{H}_2\text{O})_4(p\text{-NO}_2\text{C}_6\text{H}_4\text{COO})_2]\) \(\cdot\) \(2\)\(\text{H}_2\text{O}\), (CoPNB) with DNA was studied by electrophoresis. Figure 2 shows that the complex interacts with DNA.

![Figure 2](image2.png)

**Figure 2.** Interaction between cobalt(II) \(p\)-nitrobenzoate and genomic DNA in presence of TAE buffer at pH 7.3 in air, and 24 h incubation. Lane 1-3: DNA + cobalt(II) \(p\)-nitrobenzoate with 0.14, 1.4 to 14 mM, respectively; lane 4: untreated genomic DNA in TAE buffer at pH 7.3 in air, and incubation for 24 h.

When genomic DNA was allowed to interact with the complex at pH 7.3, it was seen that although the untreated DNA band appeared, there was no change in the brightness of untreated DNA. There was a substantial increase in intensities of the bands for the concentrations of CoPNB. The real changes in intensity of the bands with the increase in concentrations of the complex were as follows. First of all, in the case of untreated DNA, it was found that the band (lane 1) at 0.14 mM CoPNB concentration had a clearer apperance than that of untreated DNA (Figure 2). In the next 1.4 mM concentration, the
band (lane 2) was brighter than that of first band and untreated DNA, and in case of third next highest concentration at 14 mM, it was found that the band (lane 3) was the brightest of first two bands and the band of untreated DNA. They had almost the same brightness and intensities (Figure 2). The electrophoretic mobilities of the bands were seen to decrease as the concentrations of the complex were increased from 0.14, 1.4 to 14 mM (Figure 2).

The results suggest that the covalent binding of the CoPNB complex caused a change in the conformation of the DNA such as more of ethiumdium bromide intercalated, and thus an increase in the intensity of the bands were generally observed.

3. Experimental

3.1 Chemicals

All common solvents and reagents were purchased commercially from Aldrich or Sigma and used without further purification. All experiments involving the interaction of the complex with DNA, were conducted in distilled buffer (Tris–HCl, NaCl, pH 7.4). A solution of genomic DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm, showing that the DNA was sufficiently free of protein [13].

3.2. DNA Isolation

Blood samples (6-9 mL) were gathered into EDTA-tubes from cattle, and DNA samples were separated from the leukocytes with a commercial kit (MBI Fermantas®-Genomic DNA Purification Kit #K0512, USA) using the salting out DNA extraction method. Isolated DNA concentrations were measured spectrophotometrically (Spectramax® Plus 384, Molecular Devices, USA), and DNA samples were concentrated at 100 ng/µL prior to processing.

3. 3. Measurements

UV–Vis spectra were recorded on a Heyλios Range UV-Visible Spectrophotometer. For the absorption spectra, an equal solution of DNA was added to both complex solution and reference solution to eliminate the absorbance of DNA itself. For the gel electrophoresis experiments, genomic DNA was treated with Co(II) complex in 30 mM Tris–HCl, 15 mM NaCl buffer, pH 7.3 Agarose gel (1.5 % w/v) in TBE buffer (45 mM Tris, 45 mM boric acid, and 1 mM EDTA, pH 7.3), containing 0.5 µml-1 of ethidium bromide, was prepared. Then, 15 µL of each of the incubated complex-DNA mixtures was loaded on the gel, and electrophoresis, was carried out under TBE buffer system at 50 V for 24 h. At the end of electrophoresis, the gel was visualised under UV light using a Bio-Rad Trans illuminator. The illuminated gel was photographed by using a Polaroid camera (a red filter and Polaroid film were used) [30].

3.4 Synthesis

The complex, \([\text{Co(H}_2\text{O)}_4(\text{p}-\text{NO}_2\text{C}_6\text{H}_4\text{COO})_2]\_2\text{H}_2\text{O}, \) tetraaquabis(p-nitrobenzoato)cobalt(II) dihydrate, was synthesize (Figure 3) [31]. The title compound was prepared from the reaction
CoSO₄·7H₂O (0.01 mol) with sodium p-nitrobenzoate solution (0.02 mol). The mixture was filtered and set aside to be crystallized at ambient temperature for a few days. Pink crystals were obtained (Figure 4) [31], and Figure 5 shows octahedral environment of the cobalt atom [31].

![Chemical structure of the complex (CoSO₄·7H₂O with sodium p-nitrobenzoate)](image)

**Figure 3.** Chemical structure of the complex (\([Co(p-O2NC₆H₄COO)₂(H₂O)₄]·2H₂O\)).

### 3. 5 Methods

#### 3.5.1 Cobalt(II) p-nitrobenzoate (CoPNB) complex- Genomic DNA Binding Experiment

The DNA was allowed to interact with the metal complex. The DNA-binding experiments were performed at the room temperature. For these series of experiments, cobalt(II) p-nitrobenzoate complex was used as the source of reactive. The solutions of complex were prepared in MiliQ water and sterilized by passing through Milipore filter. The pH of the solutions was fixed to 7.3 by adding slowly NaOH solution. The solution of genomic DNA in the buffer, consisting of 1 mM Tris-HCl at pH 7.3, 1 mM NaCl and 1 mM EDTA, was used [30]. Appropriate volume of the complex was added to 5 µl of genomic DNA, and the total volume was made up to 100 µl by adding MilliQ water, so that the concentration of the complex ranged from 0 to 14 mM while that of DNA remained unchanged in terms of nucleotide. The mixtures were incubated for 24 h, and then the reaction was stopped by rapid cooling at 0 °C. At the end of incubation, 6µl of loading dye (0.25 % bromophenol blue in 40 % sucrose solution) was added to the mixtures [30]. In order to compare to the effect of interaction of the metal complex between DNA and the complex, the sets of electrophoretic assay were performed.
Conclusions

Complex [cobalt(II) $p$-nitrobenzoate] was synthesized and characterized. The results from optical experiments together with electrophoretic measurement support complex binds to DNA via electrostatic interaction as well as to partial intercalation via the ring into the base pairs of DNA. The results suggest that the covalent binding of the metal complex caused a change in the conformation of genomic DNA such as more of intercalated and thus an increase in intensity of the band was generally observed. The results described in this study show that changing the ligand environment can modulate the binding property of the complex with DNA [30].
Abbreviations

[Co(H₂O)₄ (p- NO₂C₆H₄COO)]₂H₂O  tetraaqua-bis(p-nitrobenzoato)cobalt(II) Dihydrate
Tris  tris(hydroxymethyl)aminomethane
UV/VIS  UV-visible
Cobalt(II) p-nitrobenzoate  CoPNB

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