Asian Journal of Chemistry Vol. 21, No. 8 (2009), 5888-5896

Investigation of DNA-Binding Ability Between Di(*p*-aminobenzoato)tetraaquacobalt(II) Complex and Cat Genomic DNA

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The di(*p*-aminobenzoato)tetraaquacobalt(II) complex [Co(PAB), where PAB is *p*-$NH_2C_6H_5CO_2$, has been obtained by the condensation of *p*-aminobenzoate in the presence of cobalt(II) sulfate. The interaction of complex of Co(PAB) with the cat genomic DNA was investigated by spectroscopic, gel electrophoresis, fluoresence spectroscopy and viscosity measurements. The experimental data indicate that the complex may bind to DNA *via* an intercalative manner. The viscosity measurements and electrophoresis with spectroscopic studies support that complex, interacting with the cat genomic DNA by an intercalation mode through the base pairs of DNA.

Key Words: Co(II) complexes, DNA-binding, DNA, *p*-Amino benzoate.

INTRODUCTION

The DNA-binding of metal complexes have been comprehensively investigated as DNA structural examines during the past 10 years[1-3]. In these kind of metal complexes, metal and ligands may be different in a smoothly controlled manner to simplify the individual applications. There are several types of sites in the DNA molecule where binding of metal complexes can occur among base pairs, groove binding and helix[4]. The plenty of biological studies have proven that DNA is the major intracellular target of some anticancer drugs because of the interaction between small molecules and DNA that may create DNA damage in cancer cells, stopping the division of cancer cells and ending in cell death[5-8]. The studies of transition metal complexes are very important to discover the possible new generation drugs. A number of useful applications of transition metal complexes require that the complex bind to DNA *via* an intercalative mode with the ligand intercalation into the next to base pairs of DNA. Because of the unusual binding properties and

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general photoactivity, these coordination compounds were workable candidates as DNA secondary structure probe, photocleavers and antitumor drugs[8,9]. Cobalt was recognized as an essential metal element widely distributed in the biological system such as cells and body and the interaction of cobalt complex with DNA has attracted much attention[10-14]. The ligands or the metal in these complexes can be varied in an easily controlled manner to facilitate an individual application. The change in metal ion or ligand lead to changes in the binding mode and affinity[15-17]. Differention of substitutive group in the intercalative ligand can produce some interesting differences in the space configuration and the electron density distribution of transition metal complexes[18-20], which may cause some differences in spectral properties and the DNA binding behaviours of metal complexes and can be useful to solve the binding mechanism of transition metal complexes to DNA[18-20]. However, these types of studies essentially focus on the interaction of Ru(II) complexes with DNA and other metal complexes have got less attention form scientists[21-29]. In this study, we have synthesized cobalt(II) *p*-aminobenzoate complex, which interact with DNA *via* an intercalative manner with base pairs of cat genomic DNA[6,14,29]. In order to more clearly assesment and understanding of the factors on the DNA binding mechanism.

In this paper, we studied di(*p*-aminobenzoato)tetraaquacobalt(II) complex and its binding ability to cat genomic DNA. The DNA-binding properties have been investigated by spectroscopic, gel electrophoresis, fluoresence spectroscopy and viscosity measurements.

EXPERIMENTAL

All chemicals were purchased and used without further purification. The compound of CoPAB Co(II)(*p*-aminobenzoate) was prepared by the procedures given below. The structure of the complex is shown in Fig. 1. All the tests involving the interaction of the complex with DNA were conducted in a buffer (5 mM *Tris*-HCl, 50 mM NaCl, pH 7.1). A solution of the cat 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[30]. The DNA concentration per nucleotide was determined[31] by absorption spectroscopy using the molar absorption coefficient at 260 nm.

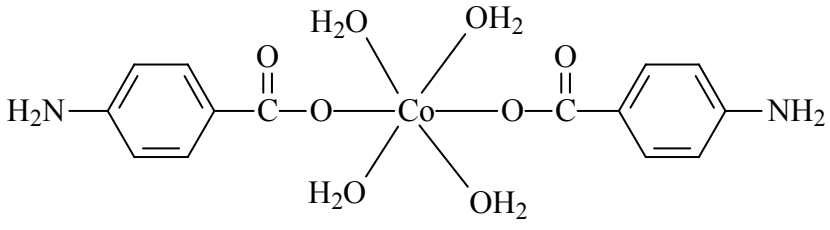

Fig. 1. Structure of $[(H_2NC_6H_4CO_2)_2Co(II)\cdot 4H_2O]$ complex

Genomic DNA obtained from the blood sample was used as well[32]. All experiments involving the interaction of the complex with DNA were conducted in Milli-Q water and all solutions were prepared with Milli-Q water. The spectroscopic test was performed in the buffer (10 mM NaCl 50mM *Tris*-HCl, pH 7.1) at the room temperature.

Synthesis of di(*p*-aminobenzoato)tetraaquacobalt(II) (CoPAB) complex: The crystals of di(*p*-aminobenzoato)tetraaquacobalt(II), $[(H_2NC_6H_4CO_2)_2Co(II)\cdot 4H_2O]$, were synthesized by the reaction of an aqueous solution of cobalt(II)sulfate with sodium *p*-aminobenzoate in the ratio of 1:2 (Fig. 1). The mixture, which had been heated to boiling. The solution was filtered and set aside for crystallization at ambient temperature for 24 h. Light-brown crystals crystals were obtained[33].

Physical measurements: Viscosity experiments were conducted using an Ubbelohde viscometer at a constant temperature of 29.0 ± 0.1 ºC in a thermostatic water bath. A digital stopwatch was used to measure 6 times the flow time and each sample was measured 6 times and an average flow time was calculated. Genomic DNA samples of *ca.* 200 b.p. of average length were prepared by sonicating in order to minimize complexities arising from DNA flexibility[34]. Data were presented as $(\eta/\eta_0)^{1/3}$ *versus* the concentration of the Co(II) complex, where η represents the viscosity of DNA in the presence of the complex, η_0 represents the viscosity of DNA alone.

Absorption spectra were recorded on a Shimadzu UV-Vis spectrophotometer using cuvettes of 1 cm path length. Absorption spectral measurements were performed using DNA stock solutions treated with the cobalt complex. For the gel electrophoresis experiments, the cat genomic DNA was interacted with the Co(II) complex in 45 mM *Tris*-HCl, 20 mM NaCl buffer, pH 7.1 and the solutions were incubated for 2 h in the dark at room temperature. The samples were analyzed by electrophoresis for 3 h at 50 V on a 0.8 % agarose gel in *tris*-acetic acid EDTA buffer; pH 7.1 The gel was stained with 1 lg/mL ethidium bromide and then photographed under UV light[35].

DNA-binding experiments: The DNA-binding experiments were performed at the room temperature. CoPAB, $(H_2NC_6H_4CO_2)_2Co(II)\cdot 4H_2O$, complex was used as the main reactive. The solutions of complex were prepared by using MiliQ water. The pH of the solutions of the complex was fixed to 7.1 by adding slowly NaOH solution. The solution of the cat genomic DNA in the buffer, consisting of 1 mM *Tris*-HCI at pH 7.1, 1 mM NaCI and 1 mM EDTA, was used[29]. DNA was permitted to interact with the metal complex. By comparing the effects of interaction of CoPAB complex with that DNA and CoPAB-DNA complex, solutions were left for incubation for 24 h before the absorption spectra were carried out. For the gel electrophoresis experiments, genomic DNA was interacted with CoPAB in 50 mM *Tris*-HCl, 20 mM NaCl buffer, pH 7.1. The samples were electrophoresed for 3 h at 50 V on a 0.8 % agarose gel in *tris*-acetic acid-EDTA buffer. The gel was stained with ethidium bromide and then photographed under UV light[36].

RESULTS AND DISCUSSION

Absorption spectral studies: The electronic absorption spectroscopy is useful technique for DNA binding studies[17,37]. The absorption spectrum of CoPAB complex in 20 mM NaCl/50 mM *Tris*-HCl buffer is illustrated in Fig. 2. The intense band around 260 nm arises from the complex-DNA interaction. The binding of complex with DNA *via* intercalation usually produces hypochromism and bathochromism because of the intercalative mode comprising a strong stacking interaction between an aromatic chromophore and the base pairs of DNA[27,36,38,39]. It has been proved to bind to DNA *via* intercalation of the extended the ligands in between the DNA base pairs. The electronic absorption spectra of CoPAB complex, in the presence and absence of the cat genomic DNA, is shown in Fig. 2. As the concentration of DNA is increased, it yields hypochromism and bathochromic change in the UV spectra band of CoPAB. Fig. 2 represents the shift in absorption spectra of CoPAB complex on addition of DNA. The spectral data may prove a mode of binding that includes interaction between the complex and the base pairs of DNA. Using the [DNA]/($\varepsilon_a - \varepsilon_f$) *vs.* [DNA] plots, the binding constants, K_b, for the complex was found 2.1 ($\pm$ 0.2) $\times 10^5$ M^{-1} (Fig. 3.). The binding mechanism of the complex with DNA was found that the binding was intercalation.

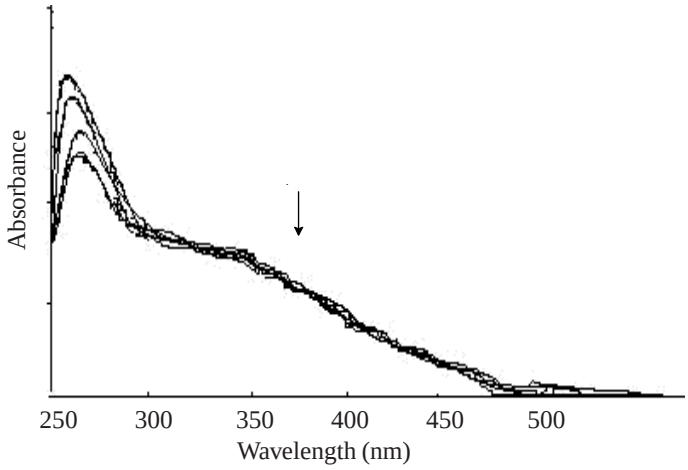

Fig. 2. Absorption spectra of $(H_2NC_6H_4CO_2)_2Co(II)\cdot 4H_2O$ in the absence and presence of DNA in *Tris*-HCl buffer. The change of absorbance occurs upon increasing the genomic DNA concentration, [DNA] = 0.5 mM. The arrow illustrates the intensity change on increasing the DNA concentration

Emission studies: The interaction of the complex with DNA was studied using fluorescence spectroscopy method. Ethidium bromide yields intense fluorescence light in the presence of DNA because of strong intercalation between the DNA base pairs. It was previously reported that the enhanced fluorescence can be quenched

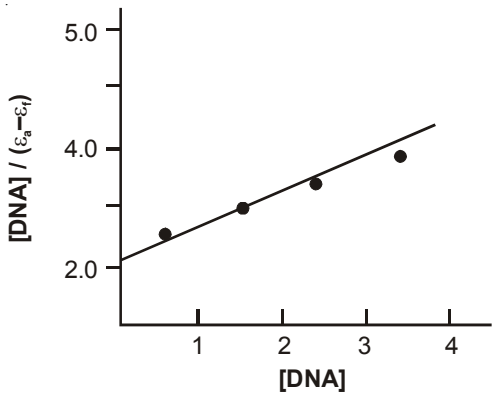

Fig. 3. Plots of [DNA]/($\varepsilon_a - \varepsilon_f$) *vs.* [DNA] for the titration of DNA with $(H_2NC_6H_4CO_2)_2Co(II)\cdot 4H_2O$

by the addition of a second molecule[27]. The emission spectra of ethidium bromide-DNA in the absence and presence of CoPAB complex is shown in Fig. 4. The complex of $[(H_2NC_6H_4CO_2)_2Co(II)\cdot 4H_2O]$ releases emission between at 485 and 545 nm in the buffer (pH 7.1) at room temperature, when CoPAB complex was added to DNA. The complex caused reduction in emission intensity[26]. CoPAB complex is faster than ethidium bromide to bind to DNA. The result shows that the complex mainly binds to the double helix of DNA with intercalation mode. It is observed that the complex binds to DNA when the fluorescence intensity is increased. The emission intensity difference between absence of genomic DNA and presence of genomic DNA is great for CoPAB as shown in Fig. 4.

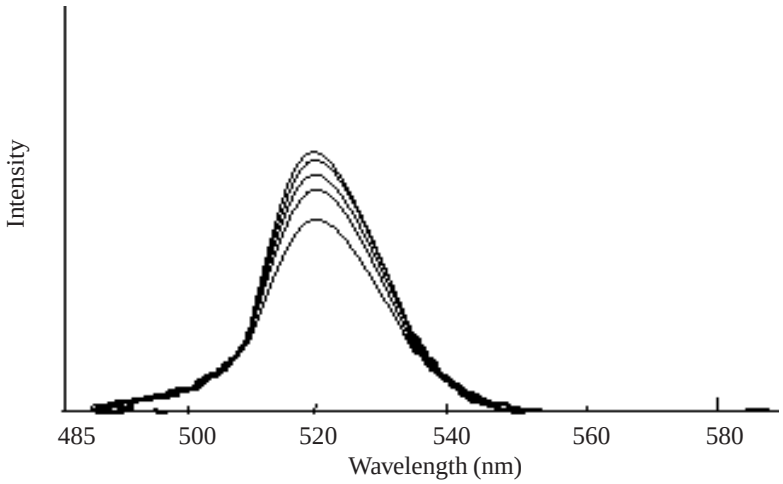

Fig. 4. Emission spectra of complex of $(H_2NC_6H_4CO_2)_2Co(II)\cdot 4H_2O$ in buffer, pH 7.1 at 25 ºC in the presence of DNA. The arrow shows the intensity change on increasing the DNA concentration

The cat genomic DNA with CoPAB complex was treated at different concentrations and agarose gel electrophoresis was carried out. The gel images are shown in Fig. 5. In this work, the effect of increasing concentration of CoPAB, at pH 7.1 on the cat genomic DNA was investigated. The changes in intensity, mobility and other small fragments were observed by agarose gel electrophoresis. The cat genomic DNA originally appeared as bright band (lane C) at pH 7.1 (Fig. 5). When the cat genomic DNA was allowed to interact with CoPAB at pH 7.1, it was found that even though the unreacted DNA band was very bright and changes occurred at the band, there were significant increases in intensities of the bands for most of the concentrations of CoPAB. The concentrations of the metal complex were changed from 10, 1 to 0.l mM. The actual changes in intensity of the bands with the increase in concentration of CoPAB were as follows. First, it was found that (as in the case of unreacted DNA, lane C), the band (lane 1 at 10 mM CoPAB concentration was less bright than untreated DNA and the band formed the smear (Fig. 5). Another band (lane 2) at next lower concentration of 1 mM had much more brightness compared with the higher concentration (lane 1) and untreated DNA (lane C) (Fig. 5). As for the band (lane 3) at next, the lowest concentration of 0.l mM had almost the same brightness as compared with lane 2 and lane C and it had much more brightness compared with lane 1. The electrophoretic mobilities of the bands were observed to decrease slightly as the concentrations of CoPAB were increased from 0.1 to 10 mM (Fig. 5). The change in mobility might be due to a change in conformation of the DNA. While free DNA moves in the electric field toward the anode, making slow the mobility of band in the case of CoPAB complex. This work makes clear

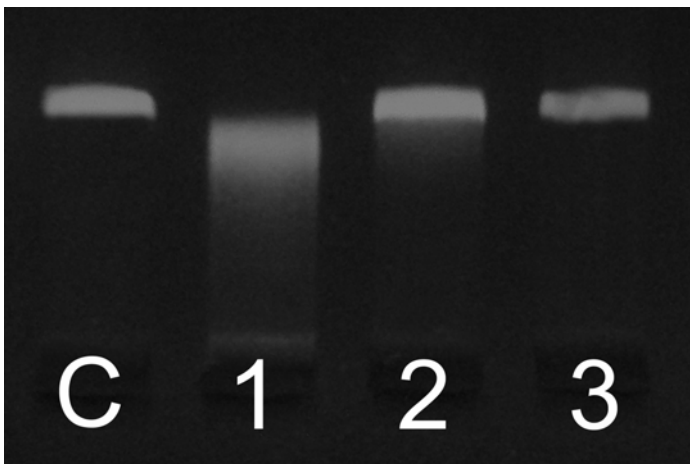

Fig. 5. Effect of concentration of the complex on cat genomic DNA and agarose gel electrophoresis diagram of cat genomic DNA by $(H_2NC_6H_4CO_2)_2Co(II)\cdot 4H_2O$. Lane C: untreated cat genomic DNA, Lanes 1-3: DNA + cobalt complex in the concentration of 10, 1, 0.1 mM

that the complex interacts with the cat genomic DNA, thereby resulting in the formation of stable complex. The binding between DNA and CoPAB is considered to take place mainly through intercalation the participating species. The CoPAB-DNA complex is produced spontaneously resulting from the formation of ion pairs between amino groups of *para*-aminobenzoate and the phosphate groups of DNA. This clearly proves that the CoPAB complex, making neutralizing the negative charges of DNA, could be made easy further because of the increase of *p*-aminobenzoate groups of CoPAB complex[40].

Viscosity studies: The viscosity studies yield a significant result for intercalation[27,34,35,41,42]. Viscosity experiments were performed for the complex at constant temperature (29.0 ± 0.1 ºC) on an Ubbelohde viscometer in a thermostatic water bath. DNA samples around 200 base pairs in average length were prepared[34,35]. The binding form of the complex to DNA was tried to explain with viscosity measurements. For complex of CoPAB, the viscosity of DNA increases when concentration of CoPAB complex increases. CoPAB complex changes the relative viscosity of cat genomic DNA with binding by the intercalation mode shown in Fig. 6. The results show that the presence of CoPAB has an effect on the viscosity of the DNA. The specific viscosity of the DNA sample increases clearly with the addition of CoPAB. The viscosity increase of DNA indicates the intercalative binding mode of the complex because this result might result in the effective length of DNA[27]. Thus, it is considered that the viscosity increase of the DNA might cause by the addition of CoPAB complex. As a result, the complex binds to DNA *via* the intercalation form.

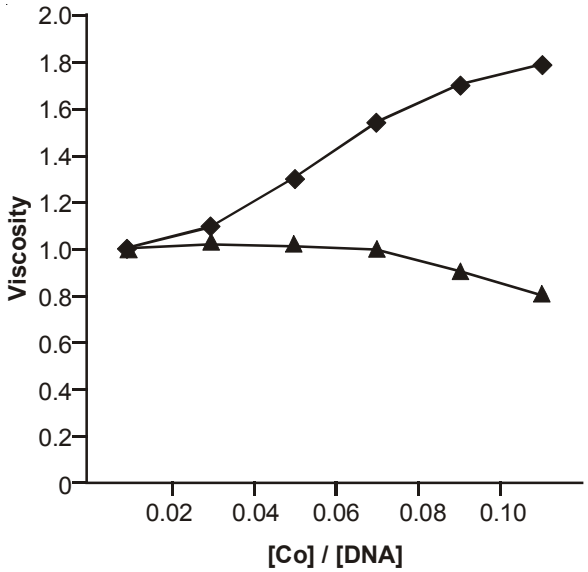

Fig. 6. Effect of increasing the amount of CoPAB (◆) on the relative (▲) viscosity of cat genomic DNA at 29 ± 0.1 ºC

Conclusion

The binding behaviour of complex $(H_2NC_6H_4CO_2)_2Co(II)\cdot 4H_2O$ with cat genomic DNA had been investigated by gel electrophoresis, viscosity, absorption, fluorescence measurement techniques. It is found that $(H_2NC_6H_4CO_2)_2Co(II)\cdot 4H_2O$ complex interacts with the cat genomic DNA. The results show that the complex interacts by intercalation with the cat genomic DNA.

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(*Received*: 29 May 2008; *Accepted*: 19 May 2009) AJC-7554