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Effect of Cobalt-hydroxybenzoate Complex on Genomic DNA

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In this study, the interaction between $[Co(H_2O)_6]$ (*p*-HO-$C_6H_4COO)_2 \cdot 2H_2O$ complex and genomic DNA were investigated. DNA samples have been isolated from cattle leucocytes and the investigation carried out by incubating samples with the complex at 37ºC. The effect of the metal complex on genomic DNA was ascertained by agarose gel electrophoresis at 50 V for 2 h. The intensity of the bands on the gel depends on the concentrations of the complex. The mobility of the bands decreased as the concentration of complex increased, indicating the occurrence of increased covalent binding of the metal complex with DNA. The damage effect of the added ascorbate into the medium might be depend on the free radicals produced from oxidation of ascorbate by molecular oxygen and this damage was reduced by the metal complex.

Key Words: DNA damage, Gel glectrophoresis, Genomic DNA, Cobalt, Complex.

INTRODUCTION

During the past decades, tremendous interest has been aroused to explore the potential applications of metal complexes as non-radioactive probes of nucleic acid structure and as DNA cleaving agents[1-8]. In these complexes, the metal or ligands may be varied in an easily controlled way to facilitate the individual application. Transition metal complexes can interact non-covalently with DNA by intercalation, groove binding, or external electrostatic binding. Among the factors governing the binding modes, it appears that the molecular geometry of the metal complex is the molecular shape. The complexes which are best target against the DNA helical structure display the highest binding affinity. Many useful applications of these complexes require that the complex binds the DNA through an intercalative mode with the ligand into the adjacent base pairs of DNA.

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However, the vast majority of such studies have been focused on complexes of Ru(II) but much literature is not available for other metal complexes. In fact, some of these complexes also exhibit interesting properties upon binding to DNA[9]. Further studies with other metals and ligands to evaluate and understand the effect factors on the DNA binding and of the mechanism leading cleavage are necessary[9,10].

Elucidation of the chemical mechanisms leading to DNA damage is critical for understanding the molecular basis of cancer and aging and will contribute to the development of new therapeutic strategies[11-17]. Studies pertaining to DNA cleavage by synthetic reagents are of considerable interest because of their utility as tools in molecular biology. This has resulted in the development of both sequence specific DNA cleavers[17-19] and DNA foot printing agents[20-23]. In most of the cases, the cleavage of DNA was carried out by metal complexes or organic dyes. In recent years, there has been substantial interest to investigate the binding properties of metal complexes, particularly polypyridyl complexes of ruthenium, with biomolecules like DNA[24-30]. It has been shown that the complexes can bind to DNA by different modes such as intercalation in the major groove or electrostatic interaction[31]. Some chiral complexes have the ability to display enantioselective DNA binding, discriminating between the right- and left-handed DNA[31]. The observations indicate that modification in mobility is believed to be attributed to the binding of complex to the DNA and/or the conformational changes of DNA cleaving properties. Ruthenium(II) complexes having extended aromatic planar bidentate ligands have been extensively studied. On the other hand, only a few studies on cobalt complexes have been reported[31].

In this paper, we concentrate our work on complex of $[Co(H_2O)_6]$ (p-HO-$C_6H_4COO)_2\cdot 2H_2O$ [Cobalt(II)(hexaaqua)]*bis*(*p*-hydroxybenzoate) dihydrate) which possess the same interesting characteristics and DNA cleaving properties. The aim of the present study was to determine whether in presence of $[Co(H_2O)_6]$(p-HO-$C_6H_4COO)_2\cdot 2H_2O$ complex, there was an increased damage to DNA. The binding and cleavage covered a wide range studies have been carried out for the complex. We investigated the interaction between the metal complex and DNA by gel electrophoresis.

EXPERIMENTAL

DNA isolation

Peripheral blood samples (6-9 mL) were collected into EDTA-tubes from cattle. DNA samples were isolated from the leukocytes by 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 process. All common chemicals and solvents were purchased from Aldrich and Sigma.

Synthesis: $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$,[Cobalt(II)(hexa-aqua)]*bis*(*p*-hydroxybenzoiate)dihydrate) complex were sythesized by the reported method[32].

$[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ Genomic DNA binding: The complex solution was prepared in MiliQ water and was sterilized by passing through Milipore filter. The pH of the solution was adjusted to 7.4 by adding slowly NaOH solution. Solution of genomic DNA in the buffer consisting of 1 mM *tris*-HCI at pH 7.5, 1 mM NaCI and 1 mM EDTA was used. The 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 100 mM while that of DNA remained unchanged in terms of nucleotide. The mixtures were incubated for 12 h and then the reaction was quenched by. At the end of incubation, 6 μL of loading dye (0.25 % bromo phenol blue in 40 % sucrose solution) was added to the mixtures[31,33].

Gel electrophoresis: Agarose gel (1.5% w/v) in TBE buffer (45 mM Tris, 45 mM boric acid and 1 mM EDTA, pH 8.0) containing 0.5 μmL^{-1} of ethidium bromide was prepared. Then, 15 μL of each of the incubated the complex-DNA mixtures was loaded on the gel and electrophoresis was carried out under TBE buffer system at 50 V for 1 h. At the end of electrophoresis the gel was visualized under UV light using a Bio-Rad *trans* illuminator. The illuminated gel was photographed by using a polaroid camera (a red fitler and Polaroid type of film was used)[31,33].

Interaction between $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ complex and genomic DNA: Genomic DNA was allowed to interact with the metal complex. In order to compare the effect of interaction of the metal complex between genomic DNA, two sets of electrophoretic assay were carried out. The mixtures were incubate for 3 and 12 h following which the reaction was stopped by rapid cooling to 0ºC. At the end of incubation the mixtures were subjected to electrophoresis as described earlier. Another set of experiments were carried out in presence of ascorbate. The mixtures were incubated for 3 and 12 h and quenched at 0ºC, at the end of which the mixtures were subjected to electrophoresis. Solutions of the metal complex and ascorbate were mixed and were adjusted to pH 7.4. The volumes of the mixture were added to 1 μL of genomic DNA so that the concentration of the complex was varied from 0.1, 1 and 10 mM.

RESULTS AND DISCUSSION

Interaction between $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ complex and genomic DNA: The binding of $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$

with genomic DNA was examined. Fig. 1 shows the interaction of the complex with genomic DNA. When genomic DNA was allowed to interact with $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ at pH 7.4, it was found that the unreacted DNA band was not very bright. There was a pronounced increase in intensity of the band for most of the concentrations of $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$. The actual changes in intensity of the bands with the increase in concentrations of $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ were as follows. First it was found that (as in the case of unreacted DNA), the band at 0.1 mM $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ concentration was bright (Fig. 1a). The other two bands at next higher concentrations 1 and 10 mM were very bright and they had almost the same brightness and intensities (Fig. 1a). The electrophoretic mobility of the band was found to decrease slightly as the concentrations of $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ was increased from 0.1, 1 to10 mM (Fig. 1). We examined effect of long term incubating time period on the interaction between the complex and DNA. In the same way, the mixtures were incubate for 12 h following which the reaction was stopped by rapid cooling to 0ºC. It was found that unterated DNA band was a little bright. there was a pronounced decrease in intensity of the band for most of the concentrations of $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$. The band at 0.1 mM $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ concentration was very faint than untreated DNA (Fig. 1c). The other band at next higher concentration 1 mM was also very faint, but the band at next higher concentration 10 mM had a little bright band compared to first two lower concentration (Fig. 1c). The electrophoretic mobility of the band was found to increase slightly as the concentrations of $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$[9].

When genomic DNA was allowed to interact with mixtures of $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ in presence of ascorbate, the intensity of the band was found to decrease slightly as the concentrations of the complex was increased (Fig. 1b). As the concentration of the mixture was increase, the mobility of the band increased slightly over the concentration range 0.1,1 to 10 mM. The decrease in intensity and the increase in electrophoretic mobility suggest a reduction in the size of the DNA molecule due to its partial cleavage for short term incubation time period and except untreated DNA, all the bands of the mixture were dispeared for long term incubation period 12 h (Fig. 1d). The results show that mixtures of $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ in precence of ascorbate are somewhat more damage to genomic DNA than ascorbate alone[9].

Effect of binding of the complex to DNA on absorption spectra: Absorption titration can monitor the interaction of a metal complex and DNA. In general, complex bound to DNA through intercalation usually results in hypochromism and red shift (bathochromism), due to the strong stacking interaction between aromatic chromophore of the complex and the base pairs of DNA[30]. The absorption spectra of the complex in the

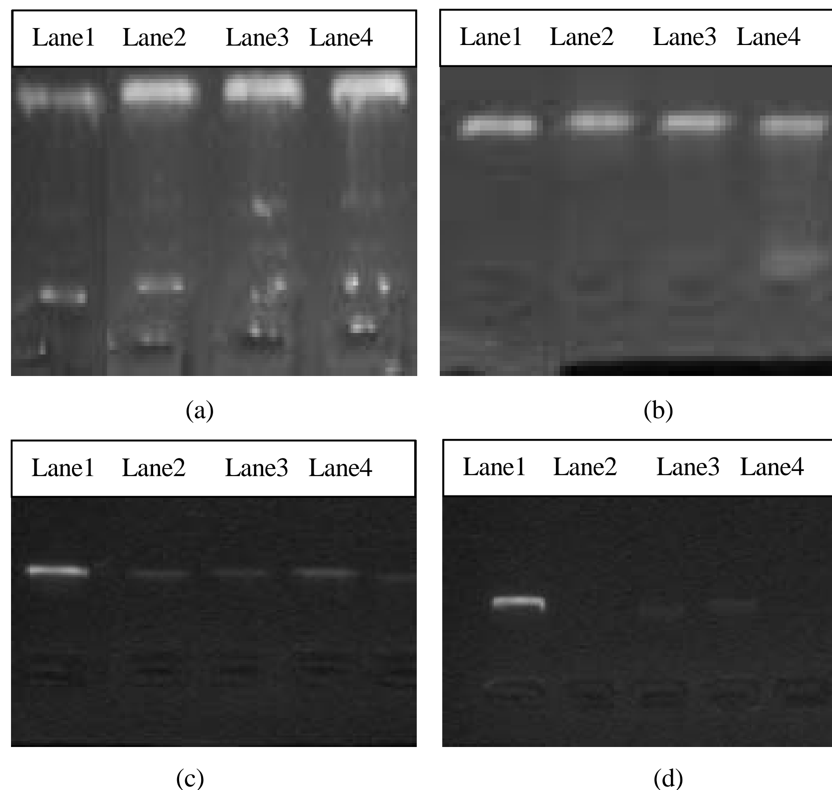

Fig. 1. (a) Interaction between $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2{\cdot}2H_2O$ and genomic DNA in TAE buffer at pH 7.4 in air and incubating for 3 h. Lane 1: untreated genomic DNA; lanes 2-4: DNA + $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2{\cdot}2H_2O$ with 0.1,1 to 10 mM, respectively. (b) Interaction between $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2{\cdot}2H_2O$ and genomic DNA in presence of ascobate in TAE buffer at pH 7.4 in air and incubating for 3 h. Lane 1: untreated genomic DNA; lanes 2-4: DNA + $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2{\cdot}2H_2O$ with 0.1,1 to 10 mM, respectively. (c) Interaction between $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2{\cdot}2H_2O$ and genomic DNA in TAE buffer at pH 7.4 in air and incubating for 12 h . Lane 1: untreated genomic DNA; lanes 2-4: DNA + $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2{\cdot}2H_2O$ with 0.1, 1 to 10 mM, respectively. (d) Interaction between $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2{\cdot}2H_2O$ and genomic DNA in presence of ascobate in TAE buffer at pH 7.4 in air and incubating for 12 h. Lane 1: untreated genomic DNA; lanes 2-4: DNA + $[Co(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2{\cdot}2H_2O$ with 0.1,1 to 10 mM, respectively

absence and presence of genomic DNA are illustrated in Fig. 2. In the UV region, the intense absorption bands observed in the cobalt complex is attributed to intraligand p-p* transition of the coordinated groups. With increasing genomic DNA concentration, the hypochromism increases and is accompanied by a red shift in the UV band of the complex. In order to compare quantitatively the binding strength of the two complexes, the intrinsic binding constants K_b of them with genomic DNA were obtained by monitoring the changes in absorbance with increasing concentration of DNA.

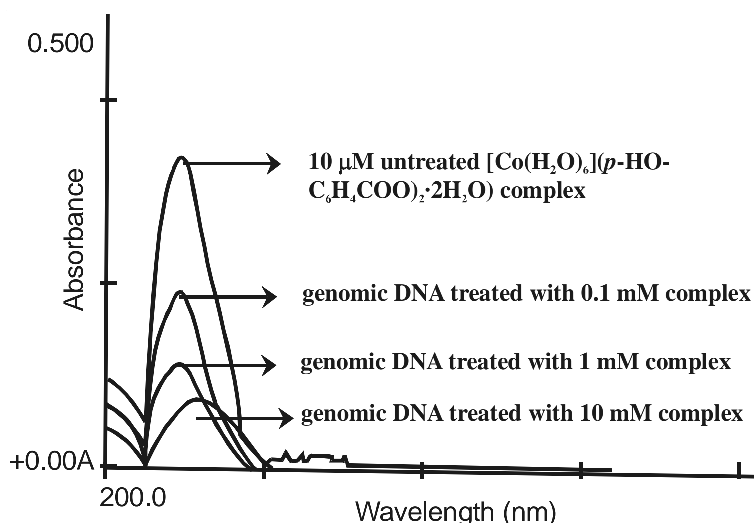

Fig. 2. Absorption spectra of $[Co(H_2O)_6]$(*p*-HO-$C_6H_4COO)_2 \cdot 2H_2O$) complex (0.1, 1 and 10 mM) in the absence (top) and presence of genomic DNA. Arrows show that the absorbance changes upon increasing complex concentrations

Conclusion

When genomic DNA was allowed to interact with $[Co(H_2O)_6]$(*p*-HO-$C_6H_4COO)_2 \cdot 2H_2O$ complex, it was found that mixtures of the metal complex caused damage to DNA. The results suggest that covalent binding of the metal complex caused a change in the conformation of genomic DNA such that more of intercalated and hence an increase in intensity of the band was generally observed[18]. The decrease in intensity of the band is believed to one or both of the following two reasons: (i) a change in conformation of the DNA due to its binding with the metal complex such that less etidium bromide can intercalate within DNA and (ii) some damage to DNA brought about by its covalent binding with the metal complex. The authors suggest that the strong binding was to N7 positions of guanine whereas the weak binding was due to the cooperativity of the transition of DNA to a new double-helical conformation. The results described in this study show that changing the ligand environment can modulate the binding property of the complex with DNA[31].

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