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An Investigation on the Effects of Nickel(II) *p*-Hydroxybenzoate on Genomic DNA

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In this study, an investigation was performed to determine the interaction between genomic DNA and Ni(II) *p*-hydroxybenzoate complex. DNA samples have been isolated from the cattle leucocytes and the investigation was carried out by incubating the samples with the Ni complex at 37ºC for varying times. The effect of the Ni(II) complex on genomic DNA was carried out by agarose gel electrophoresis at 50 V for 2 h. The intensity of the bands on the gel changed due to varied concentrations of the complex. The mobility of the bands declined as the concentrations 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 dependent on the free radicals produced from oxidation of ascorbate by molecular oxygen and this damage is considered to reduced by the metal complex.

Key Words: DNA damage, Sheep genomic DNA, Nickel complex, Gel electrophoresis, UV spectra.

INTRODUCTION

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 most significant 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 bind to DNA through an intercalative mode with the ligand intercalating into the adjacent base pairs of DNA. During the several years, tremendous interest has been aroused to explore the potential applications of metal complexes as non-radioactive probes of nucleic acid structure and as possible DNA cleaving agents[1-8]. In these complexes, the metal or ligands may be varied in an easily controlled

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way to facilitate the individual application. However, the vast majority of such studies have been focused on complexes of Ru(II) and, to a far lesser extent, on other metal complexes. In fact, some of these complexes also exhibit interesting properties upon binding to DNA. Further studies using various metals and ligands to evaluate and understand the effect factors on the DNA binding and cleavage mechanism are necessary[9].

Explaining 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 in understanding 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 of ligand enviroment would lead to changes in binding affinities, photophysical properties and DNA cleaving properties of the metal complex. So far, ruthenium(II) complexes having extended aromatic planar bidentate ligands have been extensively studied. On the other hand, only a few studies on nickle, cobalt and zinc complexes having non-planar bidentate ligands have been reported[31].

In this study, we concentrate our work on complex of $[Ni(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ [hexaaqua*bis*(*p*-hydroxybenzoato)nickel(II)] dihydrate which possess the same interesting characteristics and DNA cleaving properties, but have not received as much attention as the Ru(II) systems[15-31]. In present studies, the interaction between the nickel(II) complex and DNA (genomic DNA) by gel electrophoresis and UV have been reported.

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: [Nickel(II) (hexaaqua)] *bis*(*p*-hydroxybenzoate)dihydrate $[Ni(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$, has been synthesized by known method[32]. *p*-Hydroxybenzoic acid (0.02 mol) was added into hot solution of sodium bicarbonate:

$$2\ p\text{-}HO\text{-}C_6H_4COOH + 2NaHCO_3 \rightarrow 2\ p\text{-}HO\text{-}C_6H_4COONa + 2CO_2 + 2H_2O$$

Nickel(II) sulphate solution (0.01 mol) was added on before prepared sodium *p*-hydroxybenzoate for synthesizing of [nickel(II)(hexaaqua)]*bis*(*p*-hydroxybenzoate)dihydrate:

$$NiSO_4 + 2\ p\text{-}HO\text{-}C_6H_4COONa + 6H_2O \rightarrow [Ni(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O + Na_2SO_4$$

Then the solution was left at room temperature, until crystallazation was completed. After several days, green-coloured crystals were obtained, then produced crystalls were left for drying at room temperature[32].

$[Ni(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ genomic DNA binding: In present experiments, $[Ni(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ was used as the source of reactive. The complex solutions were prepared in MiliQ water and was sterilized by passing through Milipore filter. The pH of the solutions were adjusted to 7.4 by adding slowly NaOH solution. Solution of genomic DNA in the buffer consisting of 1 mM *tris*-HCl at pH 7.5, 1 mM NaCl and 1 mM EDTA was used. 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 complexes ranged from 0 to 100 mM while that of DNA remained unchanged in terms of nucleotide. The mixtures were then incubated at 37ºC for 3 h and 12 h and the reaction was quenched by rapid cooling to 0ºC. 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 $[Ni(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ 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. Appropriate 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. 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 electrophorsed as described earlier. Other 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 electrophorsed as described earlier[33].

RESULTS AND DISCUSSION

Interaction between $[Ni(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ complex and genomic DNA: The effect of increasing concentration of $[Ni(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ was examined at pH 7.4 on genomic DNA. The changes in both intensity, mobility and other small fragments were monitored by agarose gel electrophoresis. Genomic DNA originally appeared as a bright striking band at pH 7.4 (Fig. 1), indicating that the molecular mass of the DNA covered a wide range of values. When it was allowed to interact with $[Ni(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ at pH 7.4, it was found that although the unreacted DNA band was not very bright, but there was a pronounced increase in intensity of the bands for most of the concentrations of $[Ni(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$. Gel electrophoresis of unreacted genomic DNA at pH 7.4 gave two bands correspoding to supercoiled Form **I** and singly-nicked Form **II** (Fig. 1a) with the Form **I** band being not more prominent. However, electrophoretic mobility of the bands was found to decrease slightly with the increase in concentration of $[Ni(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$. The decrease in mobility is believed due to the binding of the complexe to DNA. The decrease in mobility could also be due to a change in conformation of the DNA. The concentrations of the metal complex were varied from 0.1, 1 to 10 mM. The actual changes in intensity of the bands with the increase in concentrations of $[Ni(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ were as follows. First it was found that (as in the case of unreacted DNA), the band at 0.1 mM $[Ni(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ concentration was brighter than untreated DNA (Fig. 1). The other two bands at next higher concentrations 1 and 10 mM had much more brightness compared to the lower concentration (Fig. 1). The electrophoretic mobility of the band was found to decrease slightly as the concentrations of $[Ni(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ was increased from 0.1, 1 to 10 mM (Fig. 1)[7]. We examined the effect of 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 untreated DNA band was bright. There

was a pronounced decrease in intensity of the band for most of the concentration of $[Ni(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$. The band at 0.1 mM $[Ni(H_2O)_6](p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ concentration was faint brighter than untreated DNA (Fig. 1c). The other two bands at next higher concentrations 1 and 10 mM had also faint bands compared to the lower concentration (Fig. 1c). The electrophoretic mobility of the band was found to decrease slightly as the concentrations of $[Ni(H_2O)_6](p\text{-}HO\ C_6H_4COO)_2\cdot 2H_2O$.

When genomic DNA was allowed to interact with mixtures of $[Ni(H_2O)_6]$ $(p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ and in presence of ascorbate, the intensity of the band was found to decrease slightly as the concentration 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

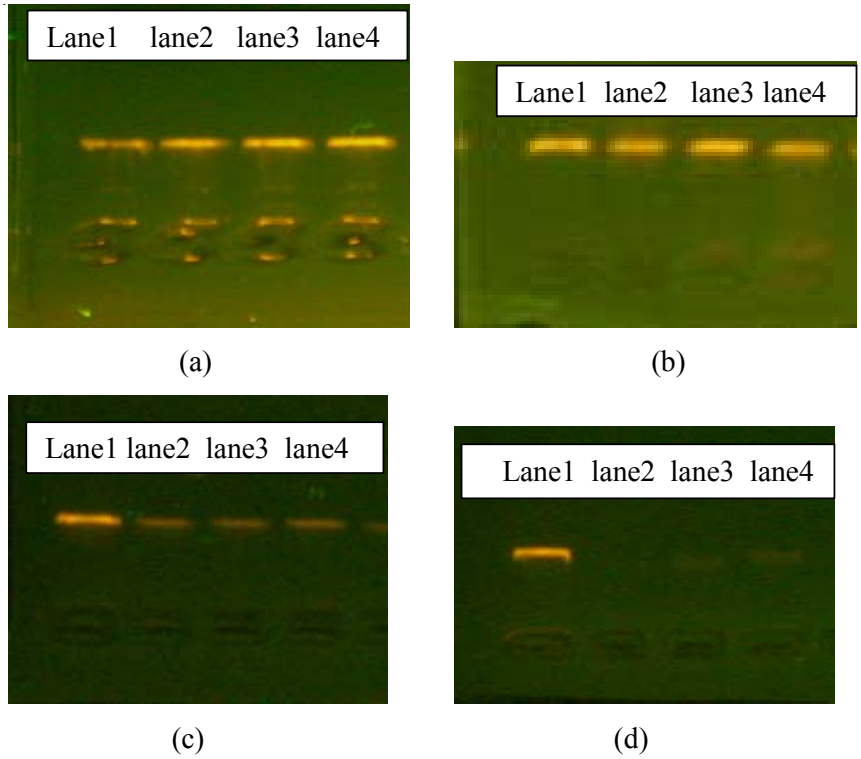

Fig. 1. (a) Interaction between Ni(II)-*p*-hydroxybenzoate complex and genomic DNA at pH 7.4 (incubated for 3 h). Lane 1: untreated genomic DNA; lanes 2-4: DNA + Ni(II)-*p*-hydroxybenzoate complex with 10, 1 and 0.1 mM, respectively. (b) Interaction between Ni(II)-*p*-hydroxybenzoate complex and genomic DNA in presence of ascobate at pH 7.4 (incubated for 3 h). Lane 1: untreated genomic DNA; lanes 2-4: DNA + $[Ni(H_2O)_6]$ $(p\text{-}HO\text{-}C_6H_4COO)_2\cdot 2H_2O$ with 10, 1, 0.1 mM, respectively

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 disappeared for long term incubation 12 h (Fig. 1d). The results show that mixtures of $[Ni(H_2O)_6]$ $(p\text{-}HO\text{-}C_6H_4COO)_2 \cdot 2H_2O$ and ascorbate damage more to genomic DNA.

Effect of binding of complexes to DNA on absorption spectra: Absorption titration can monitor the interaction of a metal complexes 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 complexes in the absence and presence of genomic DNA are given in Fig. 2. The electronic absorption spectra of the two complexes are similar in shape to that of $[Ni(H_2O)_6]$ $(p\text{-}HO\text{-}C_6H_4COO)_2 \cdot 2H_2O$. In the UV region, the intense absorption bands observed in the Ni(II) and complex are attributed to intraligand *p*–*p** transition of the coordinated groups[30]. 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 complex, the intrinsic binding constants K_b of them with genomic DNA were obtained by monitoring the changes in absorbance with increasing concentration of DNA.

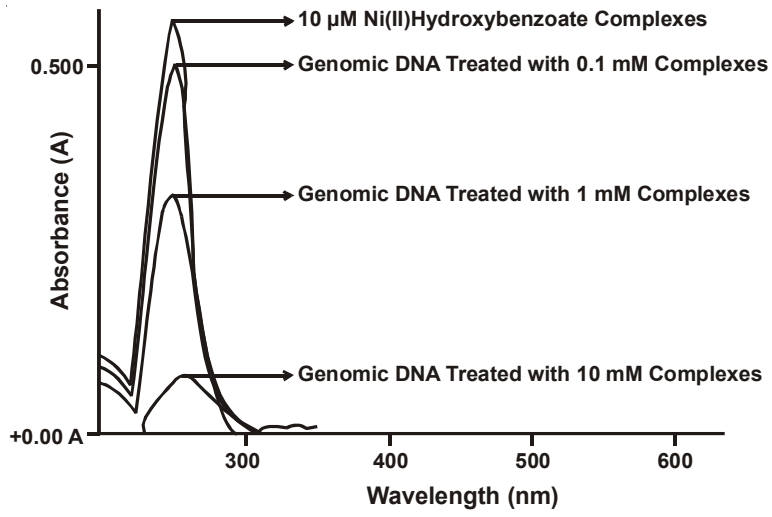

Fig. 2. Absorption spectra of Ni(II)*p*-hydroxybenzoate complex (10 mM) in the absence (top) and presence of genomic DNA (0.1-10 mM). Arrows show the absorbance changes due to complex concentrations

Conclusion

The results described in this study show that changing the ligand environment can change the binding property of the complex with 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. The decrease in intensity of the band is a change in conformation of the DNA due to its binding with the metal complexes such that less etidium bromide can intercalate within DNA and some damage to DNA brought about by its covalent binding with the metal complex. The results show that mixtures of Ni(II)-*p*-hydroxybenzoate complex and ascorbate are somewhat more damage to genomic DNA.

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REFERENCES

1. A. Sigel, in ed.: H. Sigel, Metal Ions in Biological Systems, Marcel Dekker, New York Vol. 33 (1996).
2. D.M. Perrin, A. Mazumder and D.S. Sigman, in eds.: W. Cohn and K. Moldave, Progress in Nucleic Acid Chemistry and Molecular Biology, 52, Academic Press, New York/ Orlando, p. 123 (1996).
3. G. Pratviel, J. Bernadou and B. Meunier, *Adv. Inorg. Chem.*, **45**, 251 (1998).
4. K.E. Erkkila, D.T. Odom and J.K. Barton, *Chem. Rev.*, **99**, 2777 (1999).
5. I. Ortmans, C. Moucheron and A. Kirsch-De Mesmaeker, *Coord. Chem. Rev.*, **168**, 233 (1998).
6. J.A. Cowan, *Chem. Biol.*, **5**, 634 (2001)
7. L.-N. Ji, X.-H. Zou and J.-G. Liu, *Coord. Chem. Rev.*, **216**, 513 (2001).
8. L.-N. Ji, Q.-L. Zhang and H. Chao, *Chin. Sci. Bull.*, **46**, 1332 (2001).
9. X-L. Wang, H. Chao, H. Li, X-L. Hong, Y-J. Liu, L-F. Tan and L-N. Ji, *J. Inorg. Biochem.*, **98**, 1143 (2004).
10. B. Ames, *Science*, **221**, 1256 (1983).
11. B. Epe, in eds.: B. Halliwell and O.J. Aruoma, DNA and Free Radicals, p. 41 (1993).
12. J. Piette, *J. Photochem. Photobiol.*, **11B**, 241 (1991).
13. H. Wiseman and B. Halliwel, *Biochem. J.*, **313**, 17 (1996).
14. M.I. Simon and H.V. Vunakis, *J. Mol. Biol.*, **4**, 48 (1962).
15. P.B. Dervan, *Science*, **232**, 464 (1986).
16. A.S. Bouterin, V. Vlassov, S.A. Kazakov, I.V. Kutiavin, M.A. Podyminogin, *FEBS Lett.*, **172**, 43 (1984).
17. C. OhUigin, D.J. McConell, J.M. Kelly and W.J.M. Van Der Putten, *Nucleic Acids Res.*, **15**, 7411 (1987).
18. P.G. Schultz and P.B. Dervan, *Proc. Natl. Acad. Sci. USA*, **80**, 6834 (1983).
19. M.W. Van Dyke and P.B. Dervan, *Nucleic Acids Res.*, **11**, 5555 (1983).
20. I.L. Cartwright and S.C.K. Elgin, *EMBO J.*, **3**, 3103 (1984).
21. D.S. Sigma, *Acc. Chem. Res.*, **19**, 180 (1986).

22. A. Spassky and D.S. Sigma, *Biochemistry*, **24**, 8050 (1985).
23. A. Pyle and J.K. Barton, in ed.: S.J. Lippard, Progress in Inorganic Chemistry, Wiley, New York, Vol. 38, p. 413 (1990).
24. K.E. Erkkila, D.T. Odom and J.K. Barton, *Chem. Rev.*, **99**, 2777 (1999).
25. C. Moucheron, A.K.D. Mesmaeker and J.M. Kelly, in ed.: M.J. Clarke, Structure and Bonding, Springer, Berlin, Vol. 92, p. 163 (1998).
26. Y. Xiong and L.N. Ji, *Coord. Chem. Rev.*, **185**, 711 (1999).
27. X.H. Zou, B.H. Ye, H. Li, J.G. Liu, Y. Xiong and L.N. Ji, *J. Chem. Soc. Dalton Trans.*, 1423 (1999).
28. Q.X. Zhen, Q.L. Zhang, J.G. Liu, B.H. Ye, L.N. Ji and L. Wang, *J. Inorg. Biochem.*, **78**, 293 (2000).
29. J.G. Liu, B.H. Ye, H. Li, Q.H. Zhen, L.N. Ji and Y.H. Fu, *J. Inorg. Biochem.*, **76**, 265 (1999).
30. V.G. Vaidyanathan and B.U. Nair, *J. Inorg. Biochem.*, **91**, 405 (2002).
31. Z. Hossain and F. Huq, *J. Inorg. Biochem.*, **91**, 398 (2002).
32. A.N. Shnulin, G.N. Nadzhatov, I.R. Amiraslanov, B.T. Usubaliyev and K.S. Mamedov, *Koordinasionnaya Khimiya*, **7**, 1409 (1981).
33. A. Arslantas, A.K. Devrim, N. Kaya and H. Necefoglu, *Int. J. Mol. Sci.*, **7**, 111 (2006).

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