Asian Journal of Chemistry Vol. 20, No. 8 (2008), 5827-5833

An Investigation on the Effects of a Zinc Complex on Goose Genomic DNA

A. KADIR DEVRIM†, ALI ARSLANTAS*, NECATI KAYA‡ and HACALI NECEFOGLU
Department of Chemistry, Faculty of Arts and Sciences
Kafkas University, 36300 Kars, Turkey
Fax: (90)(474)2122706; Tel: (90)(474)2120201
E-mail: arsoz33@gmail.com; arslant30@hotmail.com

A zinc complex with *p*-aminobenzoate [$Zn(p\text{-}H_2NC_6H_4COO)_2(H_2O)_4]_n \cdot 1.5nH_2O$ has been synthesized. The interaction of the complex with genomic DNA was investigated by viscosity measurements and spectrometric methods. The experimental results indicated that complex bound to DNA by intercalative mode *via* the ligand. In this study, DNA breaking activity of the compound have also been examined through an agarose gel electrophoresis experiment using genomic DNA. The result showed that no DNA breakings took place. The electrophoretic mobility of the bands was observed to decrease slightly as the concentrations of ZnPAB were increased from 0.065 to 0.65 and 65 mM. The change in mobility could be due to a change in conformation of the DNA.

Key Words: DNA-Metal interaction, Goose genomic DNA, Zinc complex, Agarose gel electrophoresis.

INTRODUCTION

The many biological experiments conducted so far recommends that DNA is the primary intracellular target of anticancer drugs because the interaction between DNA and small molecules can cause DNA damage in cancer cells, preventing the division of cancer cells and resulting in cell death[1-3]. These studies, the interaction of transition metal complexes, having multidentate aromatic ligands, with DNA has gained much attention, because of their possible applications as new therapeutic drugs and their properties of photochemical that make them potential research of DNA structure and conformation[4-9]. It is necessary to understand the binding properties in developing new potential DNA targeting antitumor drugs. Basically, metal complexes interact with double helix DNA to make non-

†Department of Biochemistry, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey.
‡School of Health Sciences, Celal Bayar University, Manisa, Turkey.

covalent or covalent way. There are three binding modes, such as intercalation, groove binding and external static electronic effects. DNA binding modes are one of the most significant among these interactions, intercalation. It was noted that the intercalating ability appeared to increase with the planarity of ligands[10,11]. Additionally, the coordination geometry and ligand donor atom type also play key roles in determining the binding extent of complexes to DNA[12,13]. The metal ion type and its flexible valence, which are responsible for the geometry of complexes, also affect the intercalating ability of metal complexes to DNA[14,15].

The role of zinc in a wide range of cellular processes, including cell proliferation, reproduction, immune function and defense against free radicals, has been well established[16,17]. Zinc is thought the most plenty trace intracellular element and there exists increasing evidence that zinc plays an important role in both genetic stability and function[18]. *in vitro*, significant amounts of zinc are incorporated in the nuclei[19]. It is clear that mechanistically, zinc has a considerable impact on DNA as component of DNA replication and transcription and DNA repair[20-22]. Recently, the ligand based on *p*-aminobenzoate and its metal complex has been synthesized. However, to our best knowledge, there are no reports on its biological activities and interactions with DNA. In this paper, the binding behaviours of the complex $[Zn(p\text{-}H_2NC_6H_4COO)_2(H_2O)_4]_n \cdot 1.5nH_2O$, *bis*(*p*-aminobenzoato)zinc (ZnPAB), with goose genomic DNA were studied by viscosity measurements, spectrophotometric and electrophoresis measurements.

EXPERIMENTAL

Zinc sulfate and *p*-aminobenzoate (PAB) were commercially available and used without further purification. Goose genomic DNA was obtained from Department of Biochemistry, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey. *Tris*[hydroxymethyl]aminomethane (TRIS) and TAE buffers, ethidium bromide and agarose were purchased from Aldrich and Sigma.

Viscosity experiments were conducted using an Ubbelodhe viscometer maintained at a constant temperature at of 29 °C in a thermostatic bath[23]. A digital stopwatch was used to measure six times the flow time and each sample was measured six times and an average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ *versus* binding ratio[24], where η is the viscosity of DNA in the presence of complex and η_0 is the viscosity of DNA alone[25]. UV-Vis spectra of the complex was studied in a Heyλios Range of UV-Visible spectrophotometer. For the absorption spectra, an equal solution of DNA was added to both complex solution and standard solution to prevent the absorbance of DNA itself. UV measurements were conducted on a Heyλios UV/Vis spectrometer with quartz cuvettes.

For the gel electrophoresis experiments, goose genomic DNA was reacted with Zn(II) complex in 50 mM *Tris*-HCl, 20 mM NaCl buffer, pH 7.2. Agarose gel (1.5 % w/v) in TBE buffer (45 mM *Tris*, 45 mM boric acid and 1 mM EDTA, pH 7.2) containing 0.5 μ mL^{-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 24 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)[26].

Synthesis of $[Zn(p\text{-}H_2NC_6H_4COO)_2(H_2O)_4]_n \cdot 1.5nH_2O$, *bis*-(*p*-aminobenzoato)zinc: $[Zn(p\text{-}H_2NC_6H_4COO)_2(H_2O)_4]_n \cdot 1.5nH_2O$ complex was synthesized according to the reported procedure[27]. The hydrate of ZnPAB (*bis*-(*p*-aminobenzoato)zinc) was obtained by adding a hot dilute aqueous solution of the sodium salt of *p*-aminobenzoate (PABA) to a dilute solution of zinc sulfate. After filteration, the pale-yellow solution was left to stand for several days, after which pale-yellow crystals formed. The crystals were separated and dried in a desiccator over anhydrous $CaCl_2$ at room temperature[27].

DNA samples were dissolved in aqueous solution and all the experiments involving the interaction of compound with goose genomic DNA were conducted in 50 mM *Tris*-HCl, 20 mM NaCl buffer, pH 7.2. Agarose gel (1.5 % w/v) in TBE buffer (45 mM *Tris*, 45 mM boric acid and 1 mM EDTA, pH 7.2). A solution of genomic DNA in this buffer gave, at 265 and 285 nm, ratios of UV absorbance, presenting that the DNA was considerably free of protein[28]. The solution of compound in the presence of DNA were prepared. The changes in absorbance of DNA of an intra ligand (IL) band upon each addition were monitored at the maximum wavelengths 285 and 335 nm for 65, 0.65 and 0.065 mM, respectively. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm[29,30].

RESULTS AND DISCUSSION

The clarification of the interaction between the complex and DNA was studied by viscosity measurements. Optical photophysical studies provide necessary, but not enough, evidences to support a binding model. The measurements of hydrodynamic that are sensitive to length change (such as, viscosity and sedimentation) are considered as the least ambiguous and the most critical tests of binding in solution in the absence of crystallographic structural data[25]. A classical intercalation model ends up in lengthening the DNA helix, as base pairs are splited to accommodate the binding ligand, leading to the increase of DNA viscosity. The effects of $[Zn(p\text{-}H_2NC_6H_4COO)_2(H_2O)_4]_n \cdot 1.5nH_2O$ on the viscosity of DNA is shown in Fig. 1. When increasing the

ratio of Zn(II) complex to DNA, [compound]/[DNA. ZnPAB can increase the viscosity of DNA, which indicates that these compounds bound to DNA by intercalation of the aromatic ring into the base pairs of DNA. This result gives sign of an intercalative binding mode of the complex and also in agreement with the pronounced hypochromism, bathochromism of the complex in the presence of DNA[25].

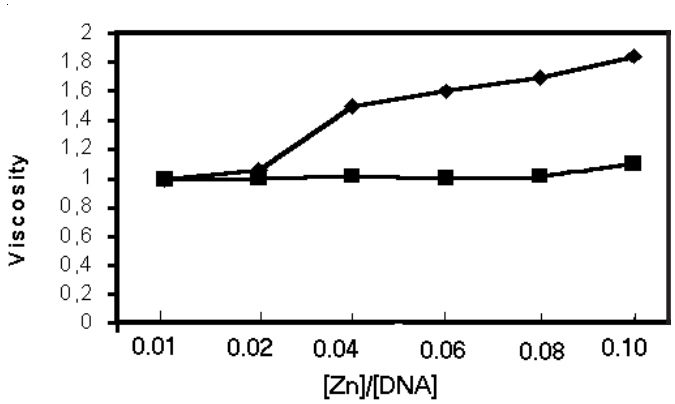

Fig. 1. Effect of increasing concentrations of $[Zn(p\text{-}H_2NC_6H_4COO)_2(H_2O)_4]_n \cdot 1.5nH_2O$ (◆), on the relative viscosities of goose genomic DNA (■), [DNA] = 0.5 mM, at 29.0 (± 0.1) °C

In addition to the above study, the binding of zinc-aminobenzoate with genomic DNA was examined. Absorption titration can monitor the interaction of a metal complex and DNA. In the UV-Visible region, absorption bands appeared from 285 to 335 nm which are believed to charge transfer transitions. In the UV region, the absorption band observed in the Zn(II) complex are attributed to intraligand *p*-*p** transition of the coordinated groups[31]. Electronic absorption spectra are initially used to study the binding of ZnPAB complex with genomic DNA. Addition of increasing concentrations of the complex results in the obvious tendency of hyper-chromism and blue shift of the absorption bands (Fig. 2). In general, complex bound to DNA through intercalation usually results in hypochromism and red shift, due to the strong stacking interaction between aromatic chromophore of the complex and the base pairs of DNA. The absorption spectra of the complex in the absence and presence of DNA are illustrated in Fig. 4. An electrostatic interaction between ZnPAB complex and the DNA can be predicted based on the hyperchromism exhibited and shift in absorbance of the cobalt complex. Because our complex contains the *p*-aminobenzoate, which is expected to be non-planar and possesses a smaller π-system than a classical intercalative interaction is precluded[31]. However, the high hyperchromism effects observed suggest that van der Waals contacts between

the groups of PAB of the complex. The coordination bond of DNA base with zinc can take place through replacement of ligands in the complex[31-33].

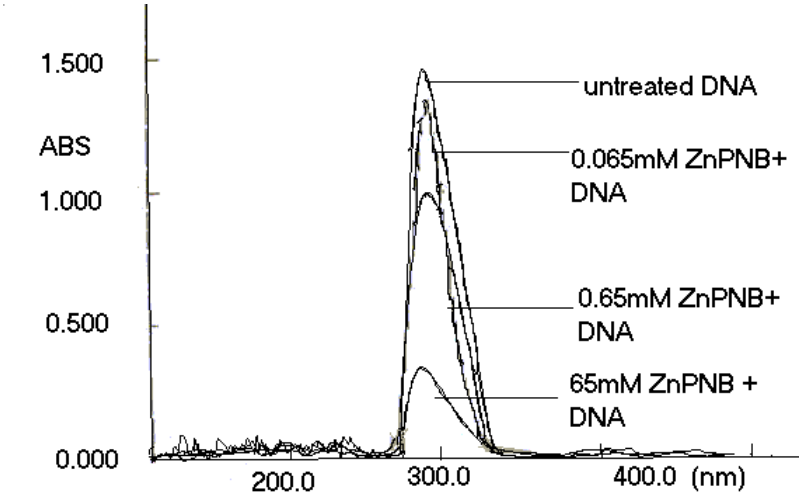

Fig. 2. UV spectra of $[Zn(p\text{-}H_2NC_6H_4COO)_2(H_2O)_4]_n \cdot 1.5nH_2O$ complex in the absence (top) and presence of goose genomic DNA (0.065, 0.65 and 65 mM)

Gel electrophoresis study of $[Zn(p\text{-}H_2NC_6H_4COO)_2(H_2O)_4]_n \cdot 1.5nH_2O$ complex supplies visualization of the interaction of DNA and ZnPAB complex. The complex of genomic DNA with ZnPAB complex was contained at different concentartions and agarose gel electrophoresis was subsequently conducted. Representative gel images are illustrated in Fig. 3. In this study, it was investigated the effect of increasing concentration of ZnPAB, at pH 7.2 on goose genomic DNA. The changes in both intensity, mobility and other small fragments were monitored by agarose gel electrophoresis. Goose genomic DNA originally appeared as bright streaking band at pH 7.2 (Fig. 3), exhibiting that the molecular mass of the DNA covered a wide range of values. When allowed to interact with ZnPAB at pH 7.2, it was found that although the unreacted DNA band was not very bright, there was a pronounced increase in intensity of the band for most of the concentration of ZnPAB. The concentrations of the metal complex were varied from 65, 0.65 and 0.065 mM. The actual changes in intensity of the bands with the increase in concentration of ZnPAB were as follows. First, it was found that (as in the case of unreacted DNA, lane 4), the band (lane1 at 0.065 mM ZnPAB concentration was less brighter than untreated DNA. The other two bands (lane 2 and lane 3) at next higher concentrations 0.65 and 65 mM had much more brightness compared to the lower concentration and

untreated DNA (Fig. 3). The electrophoretic mobility of the band was observed to decrease slightly as the concentrations of ZnPAB were increased from 0.65 to 65 mM (Fig. 3). The change in mobility could be due to a change in conformation of the DNA. While free DNA moves in the electricfield toward the anode, making slow took place at above in the case of ZnPAB complex. This work cleared that the cationic unit of the complex are neutralizing the negative charges of DNA, thereby resulting in the formation of stable complex. The binding between DNA and ZnPAB is considered to take place mainly through electrostatic interactions among the participating species. The ZnPAB-DNA complex is produced spontaneously resulting from the formation of ion pairs between amino groups of *p*-aminobenzoate and the phosphate groups of DNA. In order to prove the binding of the zinc(II) complex to DNA, a gel retardation experiment was conducted on DNA (Fig. 3). The interaction of DNA-ZnPAB, in this work the transportation of the DNA band is made slow as the concentration of zinc(II) complex is increased as well. This clearly proves that the ZnPAB complex neutralizing the negative charges of DNA which could be made easy further because of the increase of *p*-aminobenzoate groups of ZnPAB complex.

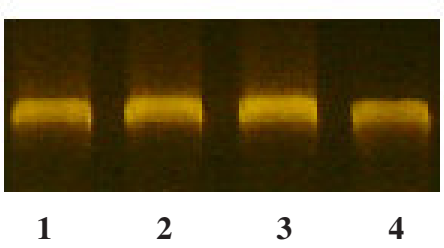

Fig. 3. Interaction between $[Zn(p\text{-}H_2NC_6H_4COO)_2(H_2O)_4]_n \cdot 1.5nH_2O$ and goose genomic DNA in TAE buffer at pH 7.24 in air and incubating for 24 h. Lane 4: untreated goose genomic DNA (100 ng); lanes 1-3: DNA + $[Zn(p\text{-}H_2NC_6H_4COO)_2(H_2O)_4]_n \cdot 1.5nH_2O$ with 0.065, 0.65 and 65 mM, respectively

Conclusion

There is an interaction between DNA and the ZnPAB complex as indicated by the UV spectra.The specrophometric and viscosity studies support an intercalative binding model between the complex and goose genomic DNA. The intercalation generally ends in enforceing of the DNA helix as base pairs are separated to accommodate the binding ligand, then leading to an increase of DNA viscosity. When the concentration of the zinc(II) complex was increased, the relative viscosity of DNA increases with increasing in the concentration of the zinc(II) complex.

REFERENCES

1. V.S. Li, D. Choi, Z. Wang, L.S. Jimenez, M.S. Tang and H. Kohn, *J. Am. Chem. Soc.*, **118**, 2326 (1996).
2. G. Zuber, J.C. Quada Jr. and S.M. Hecht, *J. Am. Chem. Soc.*, **120**, 9368 (1998).
3. S.M. Hecht, *J. Nat. Prod.*, **63**, 158 (2000).
4. K.E. Erkkila, D.T. Odom and J.K. Barton, *Chem. Rev.*, **99**, 2777 (1999).
5. C. Metcalfe and J.A. Thomas, *Chem. Soc. Rev.*, **32**, 215 (2003).
6. I. Haq, P. Lincoln, D. Suh, B. Norden, B.Z. Choedhry and J.B. Chaires, *J. Am. Chem. Soc.*, **117**, 4788 (1995).
7. A. Silvestri, G. Barone, G. Ruise, M.T. Giudice and S. Tumminello, *J. Inorg. Biochem.*, **98**, 589 (2004).
8. M. Navarro, E.J. Cisneros-Fajardo, A. Sierratta, M. Fernandez-Mestre, P. Silva, D. Arrieche and E. Marchan, *J. Biol. Inorg. Chem.*, **8**, 401 (2003).
9. C. Hemmert, M. Pitie, M. Renz, H. Gornitzka, S. Soulet and B. Meunier, *J. Biol. Inorg. Chem.*, **6**, 14 (2001).
10. C.V. Kumar, J.K. Barton and N.J. Turro, *J. Am. Chem. Soc.*, **107**, 5518 (1985).
11. H. Xu, K.C. Zheng, H. Deng, L.J. Lin, Q.L. Zhang and L.N. Ji, *Dalton Trans.*, 2260 (2003).
12. S. Mahadevan and M. Palaniandavar, *Inorg. Chim. Acta*, **254**, 291 (1997).
13. H. Xu, K.C. Zheng, H. Deng, L.J. Lin, Q.L. Zhang and L.N. Ji, *New J. Chem.*, **27**, 1255 (2003).
14. A. Mozaar, S. Elham, R. Bijan and H. Leila, *New J. Chem.*, **28**, 1227 (2004).
15. J.B. Chaires, *Biopolymers*, **44**, 201 (1998).
16. T.M. Bray and W.J. Bettger, *Free Radic. Biol. Med.*, **8**, 281 (1990).
17. S.R. Powell, *J. Nutr.*, **130**, 1447S (2000).
18. I.E. Dreosti, *Mutate. Res.*, **475**, 161 (2001).
19. R.J. Cousins, *Proc. Nutr. Soc.*, **57**, 307 (1998).
20. K.H. Falchuk, *Mol. Cell Biochem.*, **188**, 41 (1998).
21. P. Manikandan, K. Padmakumar, T.K.R.J. Homas, B. Varghese, B. Onodera and P.T. Manoharan, *Inorg. Chem.*, **40**, 6930 (2001).
22. S.A. Willison, H. Jude, R.M. Antonelli, J.M. Rennekamp, N.A. Eckert, J.A. Krause Bauer and W.B. Connick, *Inorg. Chem.*, **43**, 2548 (2004).
23. J.B. Chaires, N. Dattagupta and D.M. Crothers, *Biochemistry*, **21**, 3933 (1982).
24. G. Cohen and H. Eisenberg, *Biopolymers*, **8**, 45 (1969).
25. Q.-L. Zhang, J.-G. Liu, H. Xu, H. Li, J.-Z. Liu, H. Zhou, L.-H. Qu and L.-N. Ji, *Polyhedron*, **20**, 3049 (2001).
26. A. Arslantas, A.K. Devrim, N. Kaya and H. Necefoglu, *Int. J. Mol. Sci.*, **7**, 111 (2006).
27. I.R. Amiraslanov, G.N. Nadzhafov, E.M. Movsumov and Kh. S. Mamedov, *Zhurnal Strukturnoi Khimii*, **21**, 140 (1980).
28. J. Marmur, *J. Mol. Biol.*, **3**, 208 (1961).
29. M.F. Reichmann, S.A. Rice, C.A. Thomas and P. Doty, *J. Am. Chem. Soc.*, **76**, 3047 (1954).
30. R.S. Kumar and S. Arunachalam, *Polyhedron*, **25**, 3113 (2006).
31. M.T. Carter, M. Rodriguez and A.J. Bard, *J. Am. Chem. Soc.*, **111**, 8901 (1989).
32. S.E. Sherman, D. Gibson, A.H.J. Wang and S.J. Lippard, *J. Am. Chem. Soc.*, **110**, 7368 (1988).
33. A.M. Pyle, J.P. Rehmann, R. Meshoyrer, C.V. Kumar, N.J. Turro and J.K. Barton, *J. Am. Chem. Soc.*, **111**, 3051 (1989).

(*Received*: 17 April 2007; *Accepted*: 16 June 2008) AJC-6615