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Studies on Oxidative Cleavage Activity and DNA-Binding of Zinc Complexes with Diaqua*bis*(N,N-diethylenenicotinamide)*bis*(salicylato) and Diaqua*bis*(N,N-diethylnicotinamide)*bis*(4-nitrobenzoato)

A. ARSLANTAS*, H. NECEFOGLU and M. SOZMEN†
Department of Chemistry, Faculty of Arts and Sciences, Kafkas University, 36300 Kars, Turkey
E-mail: arsoz33@gmail.com

The oxidative cleavage and binding of cat DNA with the Zn(II) complexes of diaqua*bis*(N,N-diethylenenicotinamide)*bis*(salicylato) and diaqua*bis*(N,N-diethylnicotinamide)*bis*(4-nitrobenzoato), in an aqueous solutions at pH 7.0, have been investigated as a function of the metal complex-DNA by UV absorption spectrophotometry, viscosity and electrophoresis measurments. In this study, the interactions were compared between DNA and the two zinc(II) complexes. The gel electrophoresis study showed that both of the complexes cleaved the cat DNA under physiological conditions in the presence of H_2O_2. Absorption spectroscopy and viscosity measurement indicate that the complexes bind to DNA by intercalative binding modes. The results confirm for an intercalative interaction of both complex **1** and **2** with DNA, showing complex **1** has more affinity than complex **2**.

Key Words: Zinc(II), DNA, Oxidative cleavage, Electrophoresis, DNA-binding.

INTRODUCTION

The numbers of many small molecules that manage binding and cleaving DNA have attracted great interest over the last decades[1]. Such inexpensive small molecules would potentially be substantial tools in biotechnology, nanotechnology, therapeutic applications and the study of nucleic acid conformations. From this point of view, metal complexes have been found to be espescially useful due to their potential to bind DNA through interactions and cleave the double-helix[2]. The oxidative cleavage of plasmid DNA by different metal complexes has been broadly reported[3]. Many metal complexes draw much attention because of the chemotherapeutic properties[4]. Many researches have shown that bound proteins or synthetic ligands may increase the cleavage effects of the metal ions[5-7]. These types of complexes should be stable and inert in biological environment and water-soluble. Among the metals with biological

†Department of Patology, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey.

properties, great attention has been focussed to zinc complexes and copper complexes for some reasons. First of all, they are the most abundant trace elements present in biological systems and several metalloproteins. In addition, the derivatives of these metals are usually toxic and thus have often been used as biocides[8]. Zinc complexes have been found to interact with DNA *via* various binding modes[9,10]. In particular, zinc(II) complexes in the presence of oxidizing or reducing agents are able to cleave DNA. Studies on oxidative cleavage of DNA by synthetic zinc complexes get much attraction due to its efficacy as agents[10-16].

To further investigate the role of the zinc metal complexes in the interaction with DNA using UV, electrophoresis, viscosity measurements. The Zn(II) complexes of diaqua*bis*(N,N-diethylenenicotinamide)*bis*(salicylato) and diaqua*bis*(N,N-diethylnicotinamide)*bis*(4-nitrobenzoato) studied their interaction with cat DNA in aqueous solution and their oxidative cleavage with cat DNA in the presence of H_2O_2.

EXPERIMENTAL

The materials and solvents were commercially provided and used without purification. Cat DNA was extracted at labrotory of veterinary school of Kafkas university and the DNA was dissolved in distilled water and the concentration of the DNA was determined by absorption spectroscopy using the molar absorption coefficient at 260 nm. The solution of DNA had ultraviolet absorbance at 260 and 280 nm and it showed that the DNA was free of protein[7,16].

Synthesis of $[Zn(C_7H_5O_3)_2(C_{10}H_{14}N_2O)_2(H_2O)_2]$ compound: The Zn(II) complex of diaqua*bis*(N,N-diethylenenicotinamide)*bis*(salicylato) (complex **1**) was synthesized according to the literature reported method[17].

The $[Zn(C_7H_5O_3)_2(C_{10}H_{14}N_2O)_2(H_2O)_2]$ compound (Fig. 1) was prepared by the reaction of 0.01 mol of zinc salicylate and 0.02 mol of diethylnicotinamide in 80 mL water. The mixture was filtered and set aside for crystallization at ambient temperature for several days. The suitable colourless single crystals were obtained[17].

Synthesis of $[Zn(C_7H_5O_3)_2(4\text{-}O_2NC_6H_4COO)_2(OH_2)_2]$ compound: The Zn(II) complex of diaqua*bis*(N,N-diethylnicotinamide)*bis*(4-nitrobenzoato) (complex **2**) was synthesized according to the literature reported method[18].

The $[Zn(C_7H_5O_3)_2(4\text{-}O_2NC_6H_4COO)_2(OH_2)_2]$ compound (Fig. 2) was prepared by the reaction of 4.34 g (0.01 mol) of $[Zn(OH_2)_2(4\text{-}O_2NC_6H_4COO)_2]$ and 2.56 g (0.02 mol) of diethyl-nicotinamide in 100 mL water. The solution was filtered and set aside for crystallization at room temperature for few days. The suitable colourless crystals were produced[18].

Methods: The oxidative cleavage of cat DNA in presence of activating subtance was observed using gel electrophoresis using 10 μM of the DNA in 10 mM *tris*-HCl buffer and 1 mM of complex **1** and complex **2** at pH 7.0 was treated with varying concentration of complex **1** and complex **2**. The mixtures of reaction were incubated at 37 ºC for 3 h. Then, the reaction mixtures were cooled down by adding

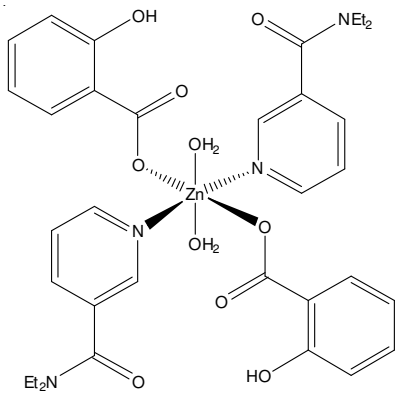

Fig. 1. Structure of the $[Zn(C_7H_5O_3)_2(C_{10}H_{14}N_2O)_2(H_2O)_2]$ compound[17]

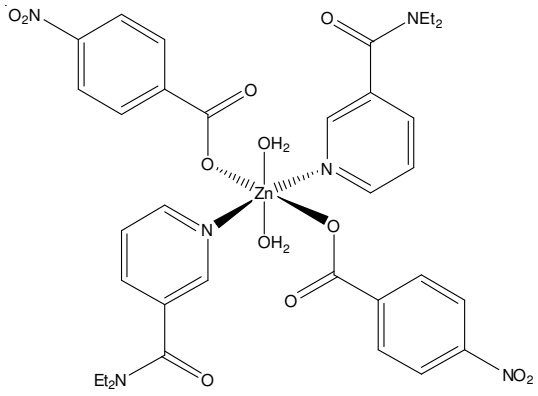

Fig. 2. Structure of the $[Zn(C_7H_5O_3)_2(4\text{-}O_2NC_6H_4COO)_2(OH_2)_2]$ compound[18]

the loading buffer. 15 µL of sample mixtures were loaded on a 2 % agarose gel that contains of ethidium bromide in TBE buffer. The agarose gel was run at 150 V for 3 h and photographed under UV light[7,19].

The binding and cleavage studies of DNA were conducted at room temperature. The concentration of DNA was determined by the absorption coefficient 6600 M^{-1} cm^{-1} at 260 nm[20]. Absorption spectra of complexes **1** and **2** was carried out by taking constant concentrations of the metal complexes with changing concentration of the DNA solutions. The intrinsic binding constants kb were calculated by using succeeding equation, which is [DNA]/ (εa-εf) = [DNA]/ (εb-εf) + 1/Kb (εa- εf)[7,21].

The viscosity experiments were performed on an Ubbelodhe viscometer and plunged into a thermostated water-bath maintained at 30 ± 0.1 ºC. The flow time of viscosity was determined with a digital watch. Each sample was measured four times and an average flow time was computed. Data were indicated as (η/η_0) *versus* binding ratio ([complex **1** and **2**]/[DNA])[22], where η is a viscosity of cat DNA in the presence of the complexes, η_0 is the viscosity of cat DNA alone and t_0 is the flow time. Viscosity values were assessed from the observed[21] flow time of DNA, $\eta = t\text{-}t_0$.

RESULTS AND DISCUSSION

The oxidative cleavage ability of the complex **1** and **2** of zinc(II) was invatigated for cat DNA, which was incubated with each zinc(II) complexes under the same reaction conditions. The cleavage reaction of the DNA strand can be controlled by gel electrophoresis method[7]. When the DNA is exposed to electrophoresis, fast migration will be observed for the Form I of DNA in Figs. 3 and 4. If the cut occurres, the Form I produces a fast moving DNA band than that of Form II[7].

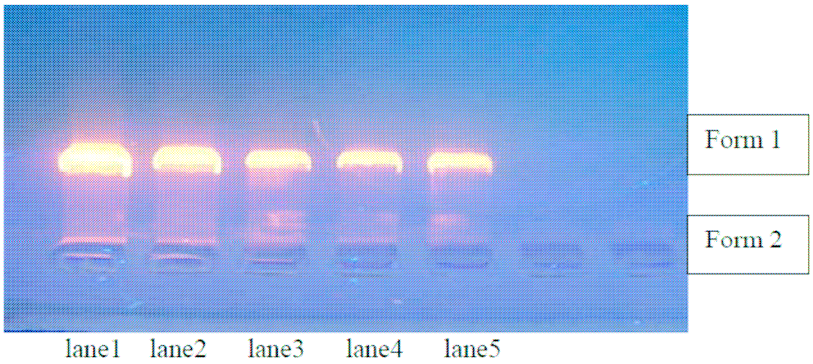

Fig. 3. DNA oxidative cleavage by various concentration of complex **1**. The concentration of H_2O_2 is kept constant at 15 mM and the concentration of the complex is varied. Lane 5 is control DNA in absence of H_2O_2; lane 1, lane 2, lane 3 and lane 4 are DNA + [complex **1**] Each of lanes shows the cleavage experiment in presence of H_2O_2, respectively

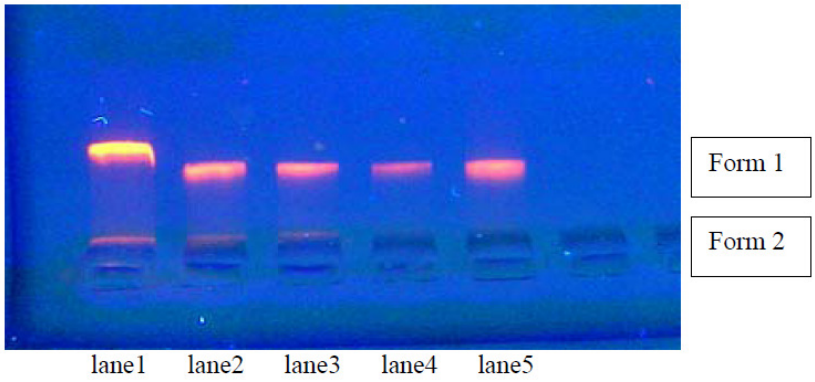

Fig. 4. DNA oxidative cleavage by different concentration of complex **2**. The concentration of H_2O_2 is kept constant at 15 mM and the concentration of the complex is varied. Lane 5 is control DNA in absence of H_2O_2; lane 1, lane 2, lane 3 and lane 4 are DNA + [complex **2**]. Each of lanes shows the cleavage experiment in presence of H_2O_2, respectively

Both zinc complexes are capable of promoting oxidative damage of DNA in presence of H_2O_2 under physiological conditions at pH 7.0 and 37 ° C, shown in Figs. 3 and 4, yet they show different cleavage activities. complex **1** displays the higher DNA cleavage activity than that of the complex **2**. The difference in cleavage behaviour of the complexes **1** and **2** seems to be due to the different ligands and functional groups. The variation in cleavage characteristics of the complex **1** *versus* complex **2** appears to be due to the N,N-diethylenenicotinamide salicylato ligand. From Figs. 3 and 4 it was found that the oxidative cleavage ability of complex **1** was a little higher than that of complex **2**. This difference can also be attributed to the presence of N,N-diethylenenicotinamide salicylato in complex **1**. The control experiments were conducted in the absence of H_2O_2 and in presence of each complex together with cat DNA.

The effect of the concentration of the complexes **1** and **2** and H_2O_2 on DNA oxidative cleavage reaction was studied. In the experiment, the concentration of H_2O_2 was hold at constant value and the concentration of the complexes was changed (Figs. 3 and 4). The results exhibit that the DNA cleavage abilities of the complexes clearly suggest that both complexes are H_2O_2 concentration-dependent.

The interaction of complexes **1** and **2** with DNA was additionally studied by UV-Vis absorption spectra for having evidences about the interaction and binding strength. The electronic absorption spectroscopy is very useful technique which was used for DNA-binding studies[22,23]. The absorption spectra of the complexes **1** and **2** in the absence and presence of cat DNA were performed. The absorption spectra of both complexes in presence of increasing amounts of cat DNA are shown in Figs. 5 and 6. The bands in the UV region were appeared around 373 nm and 315 for complexes **1** and **2**, respectively. In the presence of DNA, the absorption bands of complexes **1** and **2** presented hypochromism and bathochromism[23-26]. The spectroscopic shifting suggest that the complexes have interaction with cat DNA. The intrinsic binding constants of the complex **1** and **2** were 1.78×10^5 M^{-1} and 0.5×10^5 M^{-1}, respectively. These results show that the binding strength of complex **1** is stronger than that of complex **2** complex. The results suggest that there is a correlation between the two zinc complexes with cat DNA. It is presumed that these compounds bind to the DNA through intercalative mode[23-26].

Viscometric technique is an important tool in explaining the mode of interaction of small molecules with DNA. The spectroscopic studies give necessary informations about the DNA binding with small metal complexes, yet they are not enough proofs to support a binding model[27, 28]. The measurements of DNA-complexes viscosities that are susceptible to DNA length, they are supposed the most important tests of

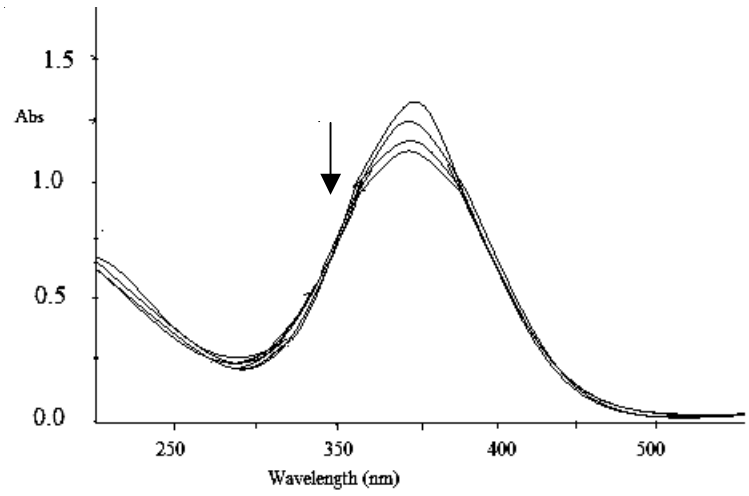

Fig. 5. Absorption spectra of complex **1** in the presence of increasing amounts of cat DNA. Arrow shows the intensities changes upon increasing DNA concentration

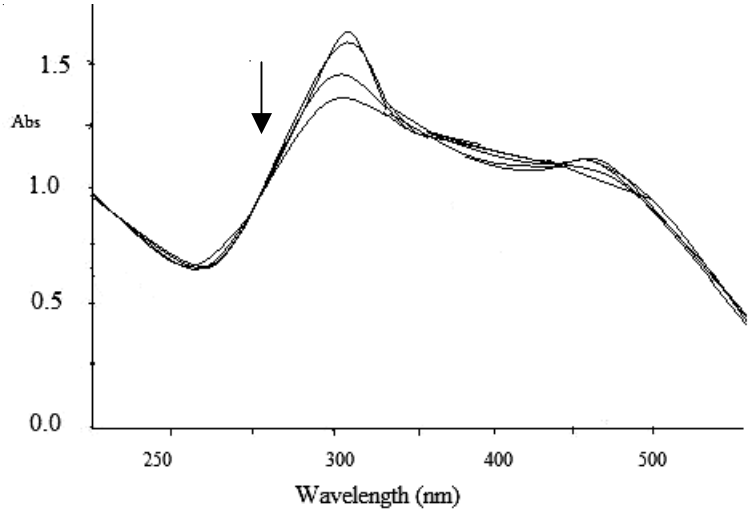

Fig. 6. Absorption spectra complex **2** in the presence of increasing amounts of cat DNA. Arrow shows the intensities changes upon increasing DNA concentration

binding in solution in the absence of structural data[27,28]. Intercalating chemical subtances are anticipated to stretch double helix to accommodate the ligands in between the base leading to an increase in the viscosity of DNA[29]. The values of $(\eta/\eta_0)^{1/3}$ were sketched against [complexes]/[DNA] (Fig. 7). The plot explains positive change in viscosity with increasing concentration of the zinc complexes. The results show that complexes **1** and **2** increase the viscosity of DNA that is consistent with DNA binding suggested above, which is also known to increase DNA viscosity[30]. The viscosity of complex **1** + DNA was found higher than that of complex **2** + DNA.

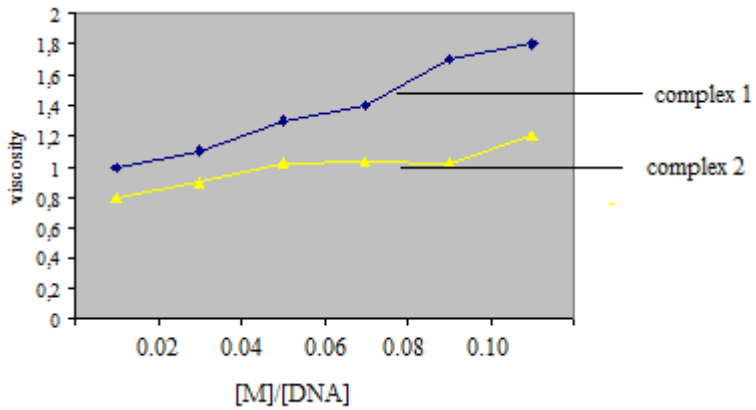

Fig. 7. Effect of increasing amounts of the zinc complexes on the relative viscosity of cat DNA at 30 ºC. Where M reprensents the zinc complexes

Conclusion

Two zinc(II) complexes have been synthesized and their oxidative cleavage and binding of cat DNA abilities were compared. The gel electrophoresis studies indicate that all of these complexes can support the oxidative cleavage of cat DNA at physiological pH in the presence of H_2O_2. The agarose gel electrophoresis show that the zinc(II) complexes bind to DNA by intercalative. The study of these zinc(II) complexes may give important information about the relationship between the ligands of complexes and their DNA reactive activities.

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