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INTERACTION OF CAT GENOMIC DNA WITH MANGANESE(II) p-AMINOBENZOATE COMPLEX

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ABSTRACT

The complex of bis-(p-aminobnzoato)tetraquamanganese(II) was synthesized according to the literature method. The interaction between bis-(p-aminobnzoato)tetraquamanganese(II) and cat genomic DNA was studied by gel electrophoresis, fluorescence spectroscopy, UV-visible and viscosity methods. The results suggest that the complex binds with DNA by intercalating through the ligand. The intrinsic binding constant *Kb* of $[Mn(p\text{-}H_2N\text{-}C_6H_4COO)_2].4H_2O$ (MnPAB) was determined as $2.9 \times 10^5\ M^{-1}$. The binding constant shows that the complex binds to DNA. Also all the other experimental results proved that $[Mn(p\text{-}H_2N\text{-}C_6H_4COO)_2].4H_2O$ could attach to DNA mainly by intercalative binding mode.

Keywords: Manganese(II); DNA binding; Interaction; Gel electrophorsis; *p*-aminobenzoic acid

INTRODUCTION

The interactions of biological metal complexes with DNA can result in advances in pharmacy and recognition of a lot of diseases[1–5]. Many different of methods for DNA-based diagnostics have been studied in the past years[1–7]. The interaction of metal complexes with DNA is an area of intense attentions to both inorganic chemists and biochemists[8]. The plenty of studies came up with that DNA is the major intracellular target of antitumor drugs because of the interaction between small molecules andthese compounds can cause DNA damage in cancer cells, preventing the division of cancer cells and ending in cell death[9-12]. Metal complexes interact with double helix DNA in two ways which are covalent and noncovalent interactions that include three binding modes, such as intercalation, groove binding and external static electronic effects. Among these metal complexes, cisplatin reacts with DNA to cause inhibition of DNA replication and cell death by forming several covalent base modifications, and some other compounds. Most notably, complexes of Pt(II), cisplatin and carboplatin have found their way into the pharmaceutical as potent antitumor drugs[13]. The fact that some naturally occurring antitumor antibiotics require metal cofactors for their biological activity has also stimulated the development of metal complexes, with objective of obtaining new pharmaceutical agents and probes for DNA–protein contacts and DNA structure[14]. Transitional metal compounds and their complexes have attracted wide interest because of their ability to bind with DNA by noncovalent interaction[15]. However, most of such compounds have been employed to coordinate with metal centers such as ruthenium, platinum, rhodium and osmium[16-21]. Little attention paid to the first row transition metal *p*-aminobenzoate complexes and much less attention on the antitumor activity of *p*-aminobenzoate complexes. Among the first row transition metal ions, the manganese ion occupies an important position due to the involvement of this element in various biological systems[22].

In this paper, the $[Mn(p\text{-}H_2N\text{-}C_6H_4COO)_2]\cdot 4H_2O$ (MnPAB) was synthesized according to literature report[23]. The interaction between $[Mn(p\text{-}H_2N\text{-}C_6H_4COO)_2]\cdot 4H_2O$ (Fig. 1) and DNA has studied by gel electrophoresis, fluorescence spectroscopy, UV-visible and viscosity methods.

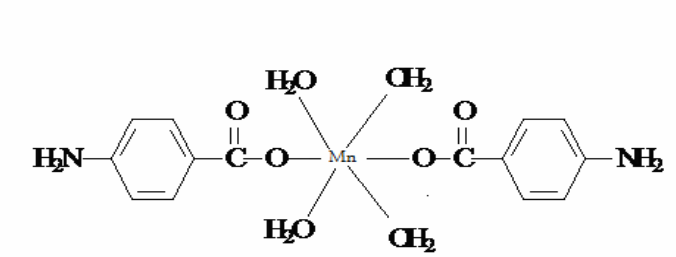

Fig.-1:The Structure of $[Mn(p\text{-}H_2N\text{-}C_6H_4COO)_2]\cdot 4H_2O$ complex.

EXPERIMENTAL

Materials

All reagents and solvents for synthesises were commercially available and used without further purification. Genomic DNA samples were prepared at laboratory of Veterinary school of medicene of Kafkas University in Kars, Turkey. Other chemicals employed were of analytical grade and doubly deionized water were used in all solutions.

Preparation of complex

The compound of bis-(p-aminobnzoato)tetraquamanganese(II) was synthesized according to the literature method[23]. Bis-(p-aminobnzoato)tetraquamanganese(II) was prepared by the reaction of manganese(II) sulfate and sodium p-aminobenzoate in aqueous solution. The mixture was heated to boiling and left in the coldfor 24 h, to give pale-brown crystals.

DNA-binding study

The experiments of the interaction of the complex with genomic DNA were performed in buffer (45 mmol Tris-HCl , 18 mmol EDTA (Ethylenediaminetetraacetic acid) and pH 7.2). All buffer solutions were prepared by using doubled distilled water. Solutions of genomic DNA in the buffer gave UV absorbance around 260, which showed that the DNA was free of protein[24]. The DNA concentration of each nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M cm) at 260 nm[25].

Physical measurements

Viscosity experiments of the complex were conducted on a Ubbelodhe viscometer, sinked in a thermostatic waterbath preserved at 30.0 ^{0}C. Around 300 bp (base pair) genomic DNA samples with average length were prepared by sonication in order to minimize complexities arising from DNA flexibility[26]. Flow time was measured using a digital stopwatch, each sample was measured three times . Data were presented as $(\eta/\eta_0)^{1/3}$versus the concentration of the Mn(II) complex, where η represents the viscosity of DNA in the presence of the complex, η_0 represents the viscosity of DNA alone[27].

Absorption spectra were recorded on a Shimadzu UV–VIS spectrophotometer using cuvettes of 1cm path length. Absorption spectral measurements were performed using DNA stock solutions treated with the Mn(II) complex. For the gel electrophoresis experiments, the cat genomic DNA was interacted with the Mn(II) complex in 45 mM Tris–HCl, 18 mM NaCl buffer, pH 7.2, and the solutions were incubated for 3 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 7.2. The gel was stained with 1 mg/ml ethidium bromide and then photographed under UV light[14]. Bis-(p-aminobnzoato)tetraquamanganese (II) and the buffer solution were added into each of four 10 mL colorimetric tubes, then different amounts of genomic DNA solution were added. The mixture of DNA-metal complex was diluted to the mark and interacted for 20 min at room temperature. The measurements of fluorescence were carried out by using Hitachi Flurospectrophotometer[14].

RESULTS AND DISCUSSION

Fluorescence spectroscopic studies of the interaction between MnPAB and cat genomic DNA was carried out to investigate whether MnPAB could interact with DNA or not. Fig. 2 shows the fluorescence characteristics of the MnPAB interaction. It was observed that MnPAB had the highest emission peak

around 740 nm. The fluorescence intensity of MnPAB was weak in the absence of DNA, but it was gradually increased with increasing concentrations of genomic DNA. It was reported that MnPAB could enter inside of DNA molecule and intercalated into the base pairs of DNA[28]. The hydrophobic circumstance of DNA could facilitate to increase the fluorescence of the $[Mn(p\text{-}H_2N\text{-}C_6H_4COO)_2]\cdot 4H_2O$. Fig. 2 shows that the fluorescence spectra of energy transfer for MnPAB at different concentrations of genomic DNA, and the energy transferring from DNA to MnPAB suggested the intercalative binding mode between MnPAB and DNA[29]. The possible intercalative binding of MnPAB complex with DNA was investigated by the fluorescence spectra of ethidium bromide -DNA system in the presence of MnPAB[30-31]. It was possible that $[Mn(p\text{-}H_2NC_6H_4COO)_2]\cdot 4H_2O$ and ethidium bromide contend each other for the same binding sites of DNA and made weak the fluorescence intensity of ethidium bromide DNA system[30-31].

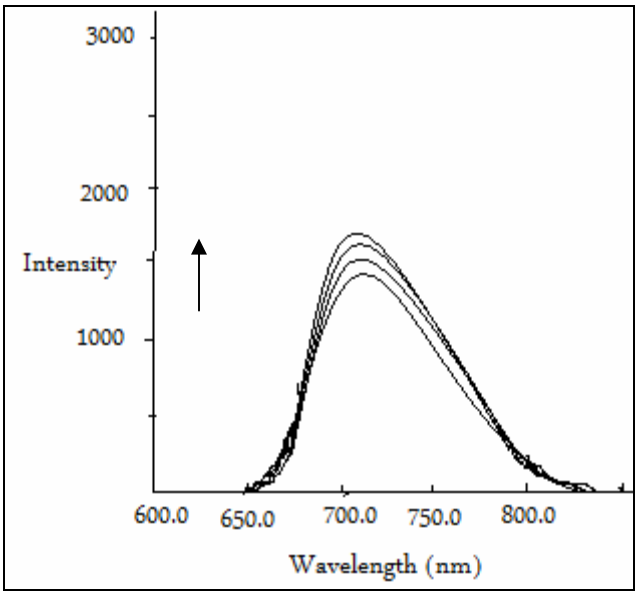

Fig.-2: Fluorescence spectra of $[Mn(p\text{-}H_2NC_6H_4COO)_2]\cdot 4H_2O$ with increasing the concentration of genomic DNA.

MnPAB complex was interacted with cat genomic DNA at different concentrations, and agarose gel electrophoresis of MnPAB-DNA mixture was performed. The gel images of the mixture are shown in Fig. 3. In this work, the effects of increasing concentration of MnPAB, at pH 7.2 on the cat genomic DNA was studied. The changes in intensity, mobility, and other small fragments were examined by agarose gel electrophoresis. The cat genomic DNA originally seemed as a bright band (lane C) at pH 7.2 (Fig. 3). When MnPAB complex was allowed to interact with cat genomic DNA at pH 7.2, it was seen that the DNA band brightness was changed, and no changes occurred at the band of the control DNA (lane C). There were significant increases in intensities of the bands for most of the concentrations of MnPAB. The concentrations of the metal complex were changed from 10, 1 to 0.1 mM. The actual changes in intensity of the bands with the increase in concentration of MnPAB were as follows. First, it was found that (as in the case of unreacted DNA, lane C), the band (lane1 at 10 mM MnPAB concentration was less bright than untreated DNA and the band formed the smear, and the band was almost disappreared. (Fig. 3). Another band (lane 2) at next lower concentration of 1 mM had much more brightness compared with the higher concentration (lane1) and untreated DNA and the band had smear (lane C) (Fig. 3). As for the band (lane 3) at next, the lowest concentration of 0.1 mM had almost the same brightness as compared with lane C, and it had much more brightness compared with lane 1. The electrophoretic mobilities of the bands were very sharply observed to decrease at lane1 as the concentrations of MnPAB were increased from 0.1 to 10 mM (Fig. 3). The change in mobility might be because of a change in conformation of the

DNA. This work makes clear that the complex interacts with the cat genomic DNA, thereby resulting in the formation of stable complex. The binding between DNA and MnPAB is considered to take place mainly through intercalation the participating species.

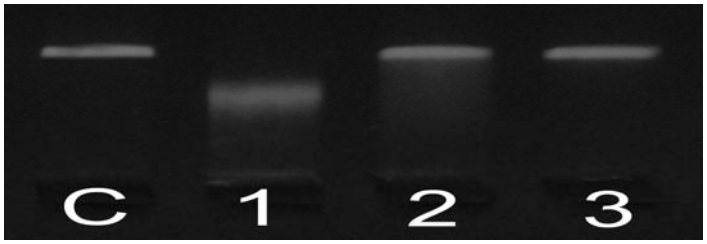

Fig.-3:Agarose gel electrophoresis diagram of cat genomic DNA-$[Mn(p\text{-}H_2NC_6H_4COO)_2]\cdot 4H_2O$. Lane C: untreated cat genomic DNA, Lanes 1–3: DNA + $[Mn(p\text{-}H_2NC_6H_4COO)_2]\cdot 4H_2O$ complex in the concentration of 10,1, 0.1 mM.

Viscosity measurements were conducted to explain the interactions between the complex and DNA. The intercalation model ended in the extending of the DNA helix since the base pairs were separated to compose the binding ligand, directing to an increase in DNA viscosity[32]. In contrast, the intercalation could fold the DNA helix, and decrease its effective length and, its viscosity[33]. The effects of MnPAB on the viscosity of DNA are shown in Fig. 4. The relative viscosity of DNA went up fixed with an increasing amount of compound, which suggests that the complex may bind to DNA through intercalation binding.

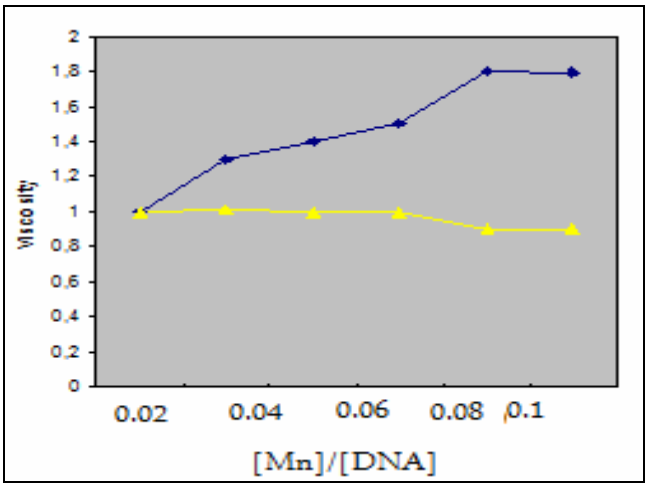

Fig.-4: Effect of increasing the amounts of $[Mn(p\text{-}H_2NC_6H_4COO)_2]\cdot 4H_2O$ (♦) on the relative(▲) viscosity of cat genomic DNA at 30.0 °C.

The hypochromism and a red shift in the absorption spectra show that compounds attach to DNA by intercalative binding mode[37]. The extent of spectral change is related to the strength of binding and the spectra confor intercalators are more perturbed than those for Groove binders[34-35]. The absorption spectra of MnPAB reacted with genomic DNA is shownn in Fig. 5. The absorption spectra of MnPAB **i**n the presence of DNA is at about 250 and 340 nm. This outcome suggests that the complex interacts with DNA and also the result shows that the MnPAB complex binds to DNA by intercalation. In order to compare the binding strength of the complex, the intrinsic binding constant *K*b of MnPAB binding with genomic DNA was determined by spectrophotometric titration, according to the site exclusion equation[36-39]. The intrinsic binding constant *Kb* of MnPAB was determined as 2.9 x 10^5 M^{-1} (Fig. 6). The binding constant shows that the complex binds to DNA.

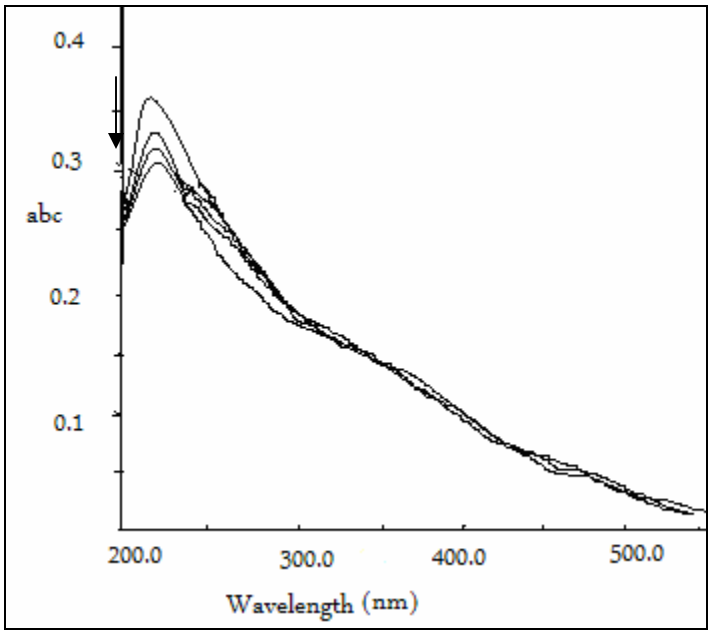

Fig.-5: Electronic spectra of $[Mn(p\text{-}H_2NC_6H_4COO)_2]\cdot 4H_2O$ (10 mM) in the presence of increasing amounts of genomic DNA ([DNA] = 0–25 mM). The arrow indicates the absorbance changes on increasing DNA concentration.

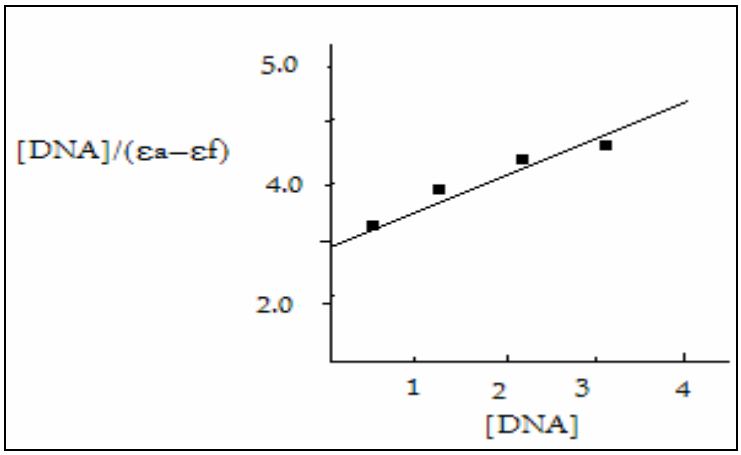

Fig.-6: Plots of [DNA]/(**a**− **f) versus** [**DNA**] for the titration of DNA with the complex

CONCLUSION

The results of gel electrofhoresis, viscosity, absorption spectra, and fluorances methods show that $[Mn(p\text{-}H_2N\text{-}C_6H_4COO)_2]\cdot 4H_2O$ complex binds to DNA by intercalation by the ligand into the basepairs of DNA. The experimental results have prove that $[Mn(p\text{-}H_2N\text{-}C_6H_4COO)_2]\cdot 4H_2O$ could interact with DNA mainly by intercalative binding. This will bring more considerations about the interaction between MnPAB and DNA. It is very useful for studying to design new anti-tumor drugs.

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