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Research Article

Evaluation of DNA Reactivity Properties of Zn(II) Phthalocyanine Complex Carrying Tetrakis(benzhydryloxy) Ligand

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ABSTRACT

Using a variety of techniques, the interaction pathway of zinc(II) phthalocyanine with tetrakis(benzhydryloxy) ligand with DNA was assessed in this article. Electrophoresis, thermal denaturation, viscosity, absorption, and fluorescence spectra were employed to investigate the binding mechanism of the ZnPc complex with DNA. The DNA binding constant (Kb) of the ZnPc compound and the thermal melting profile values of the DNA showed that the binding of the ZnPc compound to CT-DNA is governed by an intercalative interaction mechanism. The Kb was computed to be 1.392 10^6 M^{-1} for the ZnPc compound, which provides very important clues about the binding mechanism. Fluorescence spectroscopy is another important tool for clarifying the ability of ZnPc to interact with DNA. The information obtained from the fluorescence method confirms that ZnPc interacts with DNA by means of intercalative binding. When the findings obtained from electrophoresis experiments were evaluated, the significant decrease in the intensity of CT-DNA bands revealed that ZnPc interacted with DNA through a physical interaction. The data from the viscosity study confirm data previously obtained by other methods. As a result of their findings, they showed that ZnPc interacts with DNA by intercalative binding. All of these informations suggest that ZnP has DNA interaction properties and could be a potential agent that can be used in the treatment of cancer diseases on the basis of its DNA bonding property.

Keywords: Metallic phthalocyanines, DNA binding, Zn(II), thermal melting, intercalative intercation.

1. INTRODUCTION

Phthalocyanines and various derivatives of these compounds are very valuable and useful substances and extensive studies of phthalocyanines have been ongoing for several decades. In this context, phthalocyanine derivatives are considered to enable a wide range of applications in materials science, in particular chemical sensors, liquid crystals, films and sensitizers for the photodynamic treatment of tumors in patients.[1-6] At the same time, they are also distinguished by their superior conductivity properties.[7,8] As time goes on, more of the unexplored functions of these new molecules may come to light. As the properties of such materials emerge, many researchers in the field will turn to the even more fascinating properties of these chemicals.[9] The strong absorbability, emissivity, high photochemical resistivity efficiency in single of oxygen production, modest cytotoxicity property, high flexibility in targeted conformational tuning and specific reagents of phthalocyanines have made them more popular.[10,11]

DNA is a molecule that stores all genetic information and plays a crucial role in anti-turmour drug development by providing cellular activity parameters. Comprehending the performance and the logic behind a drug that binds to DNA may help scientists evaluate level of tumor blocking used in tumor treatment.[12] Phthalocyanine macromolecules, which contain metals in their structure, can react very easily with DNA

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molecules by their layered form. In addition, this type of phthalocyanine compounds can be easily replaced by according to nucleic acid-related target area. For example, the spectral behavior of these chemical compounds can be modulated by preferential incorporation of an atom.[12] Coordination of these compounds with many elements is possible, but it is known that the interactions of phthalocyanine compounds with diamagnetic metals have been widely scrutinized due to their interesting aspects.[13] Extensive publications have demonstrated the importance of these chemical components that have been quaternised with aromatic moieties and have shown possible biological factors.[12]

The primary objective of the study was to interpret the interaction of the previously synthesized compound with DNA. In this research, zinc(II) complex having tetrakis(benzhydryloxy) complex (ZnPc) was prepared from 4-(benzhydryloxy)phthalonitrile.[14] The DNA binding behaviour of ZnPc was investigated using many methods such as spectrophotometric methods, viscosity and gel electrophoresis assays to determine its activity as a potential medicine.

2. EXPERIMENTAL

2.1. Materials

Chemical reagents, other auxiliaries and solvents were obtained from Sigma-Aldrich, a commercial company. The chemicals used were used without any purification. The stock CT-DNA sample used in this study was bought from Sigma-Aldrich company. All the DNA samples used in this study were prepared in a previously prepared buffer containing NaOH with a pH of 7.04 and stored in the refrigerator.

2.2. Methods

Absorption titration and thermal denaturation measurements were conducted using an absorption spectrophotometer manufactured by Cary. A Perkin Elmer emission spectrophotometer was used to measure the fluorescence spectra of the studied ZnPc compound. The viscosity measurements 85ort his study were performed to determine DNA binding mode of the complex utilizing Ostwald model viscometer. Electrophoresis was performed using Scientific Owl electrophoresis apparatus at 25 °C at pH 7.04.

2.2.1. Production of (benzhydryloxy)phthalonitrile Group

The procedure for the preparation of 4-(benzhydryloxy)phthalonitrile ligand was documented in the literature.[14]

2.2.2. Preparation of ZnPc complex

Synthesis and characterization data of the ZnPc complex were previously published in the literature.[14]

3. RESULTS and DISCUSSION

3.1. Synthesis of ZnPc Complex

The structure of the investigated ZnPc compound is shown in Figure 1. ZnPc compound was formed by cyclotetramerisation of 4-(benzhydryloxy)phthalonitrile in N_2 by reflux. The properties of this product were fully characterised using spectroscopic techniques. The results obtained by Agirtas and colleagues proved to be in agreement with the predicted molecular conformation and synthesis and characterisation information for the complex was reported in the literature.[14]

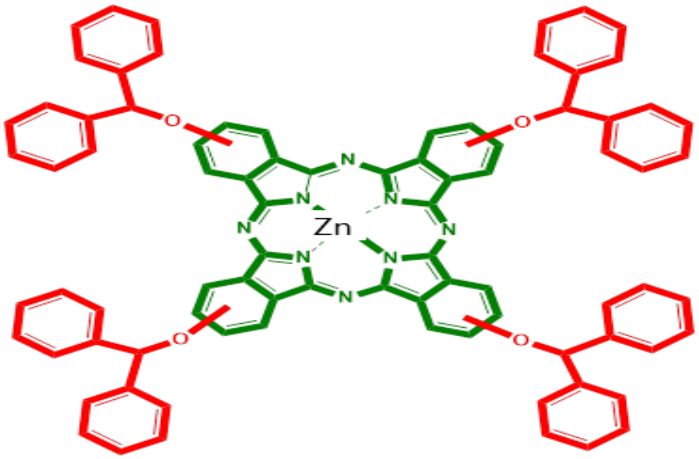

Figure 1. Structural scheme of the ZnPc compound.

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3.2. DNA Interaction Study of ZnPc

CT-DNA sample was solved in buffer and ZnPc was titrated with the DNA by UV/Vis. The absorbance of the DNA sample was checked for protein content and it was observed that the sample did not contain protein.[15] The solution of the substance ZnPc was obtained using DMF solvent and then diluted to the desired amount in a buffer medium at a pH of 7.04. The titrations of the electronic absorption were accomplished by adding incremental amounts of DNA to a specified amount of ZnPc (25 µM). The absorbance spectra after each titration were registered for every application. The binding coefficient (Kb) of this complex was derived by means of the Wolfe-Schimer equation 1.

The absorbance of ZnPc decreased with increasing DNA concentration and saturated above 25 µM DNA concentration. Interaction of ZnPc on DNA resulted in the appearance of two peaks at the wavelengths of 347 nm and 679 nm with a small shift for every peak. The compound showed hypochromism at 347 and 679 nm as shown in Figure 2. The intercalative attachment system is directly related to the term hypochromism, which typically consists of storage interactions between chemical compounds and DNA.[16] It is likely that an intercalative bonding mode with CT-DNA is situated mainly in the planar inner portion of ZnPc with its conjugated ring system. Binding mode with CT-DNA is present in the largely planar central part of ZnPc with its conjugated ring structure. For compound, Kb was found to be 1.392 10^6 M^{-1}. When we compare the absorption values obtained in this study with the values in our previously published studies,[17-19] it is observed that the absorbance values of different phthalocyanine compounds are different. These differences are based on the fact that phthalocyanine compounds contain different substituents and different metal atoms. This makes the compounds different in terms of their properties and structures. Their studied properties are compound-specific.

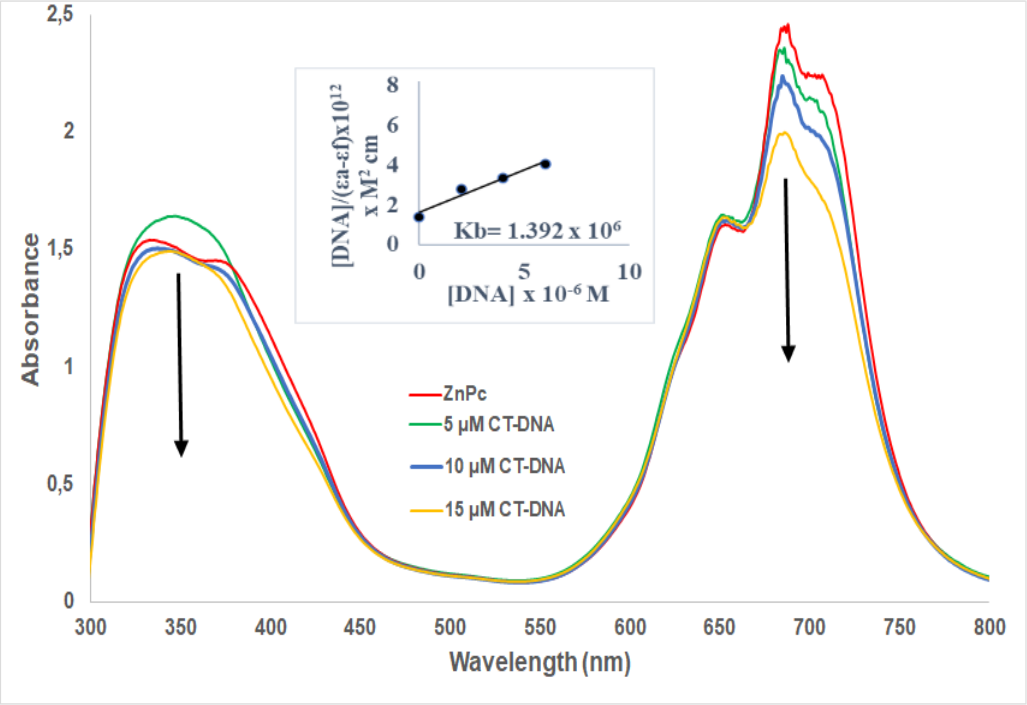

Figure 2. Spectra of the complex of ZnPc (25 µM) at gradually incrementing concentrations of CT-DNA (0-15 µM at pH 7.04). The arrows highlight the decline in absorbance peaks as the amount of DNA is incremented.

3.3. Fluorescence Study of ZnPc Complex

This technique is used to analyse drug-DNA interaction, as emission titration is one of the highly effective and sensitive techniques in research on DNA interaction and offers more information on the mechanism of intercalative binding. The fluorescence titration assay is a technique on the basis of the change in spectrum when a agent binds to DNA. A chemical subtance binds to a biological agent utlizing the intercalative field of DNA. Therefore, emission efficiency may increase significantly when agents bind to the DNA. The binding of material under investigation to intercalativing area on DNA can be monitored by the dropping in the spectrums with elevated concentration of agents.[20] As exhibited in Figure 3, the ZnPc complex without DNA emits a high the spectrum of fluorescence and the

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maximum peak occurred at approximately 508 nm. Just as shown in Figure 3, when the CT-DNA sample is added sequentially on the sample, the emission intensities were found to decrease gradually. These results confirmed that ZnPc attaches to DNA via an intercalating interaction[17-19].

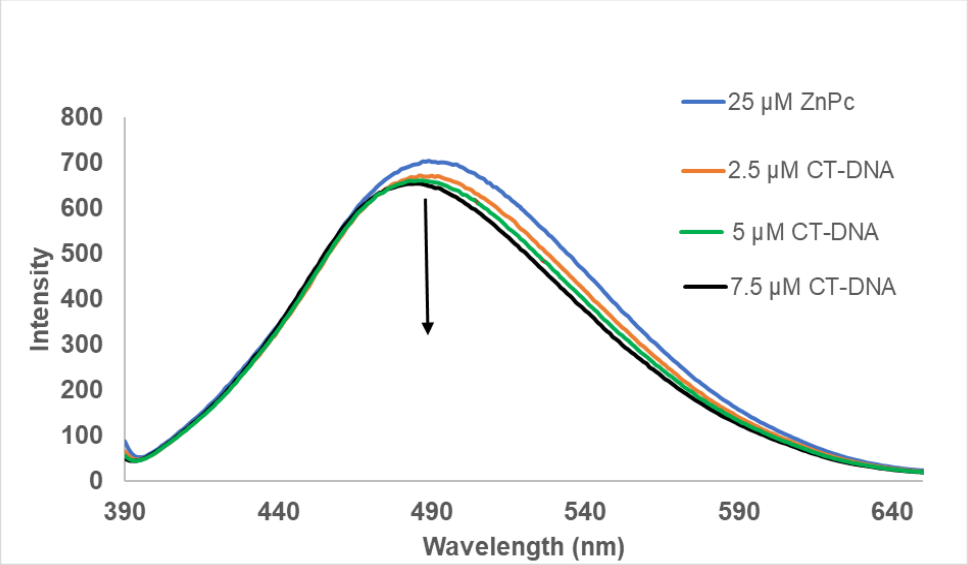

Figure 3. Fluorescent spectra of ZnPc attachment to stranded bases of DNA molecule. The sign of arrow symbolizes the gradual variation in intensity as the amount of DNA rises.

3.4. Viscosity investigation for ZnPc

Since this method is sensitive to the length of DNA, determining the viscosity of DNA can provide basic evidence of DNA binding patterns. In this study, viscosity technique was utilized for elucidation of the interaction mechanism between ZnPc and DNA. This method is also the simplest and most convenient technique for determining DNA binding mode of chemical compounds.[21,22] Fundamentally, when a substance penetrates DNA, DNA elongates as base pairs of DNA move away from each other to hold the attached ligand, this leads to a change in relative viscosity. That is, the compounds interacting by DNA via an intercalation can reduce the effective DNA size by bending the stranded helix and viscosity can decrease correspondingly.[23] However, other situation is that electrostatic and groove type attacment cause very tiny effect upon viscosity of DNA. Figure 4 reveals change in the viscosity if ZnPc is added.

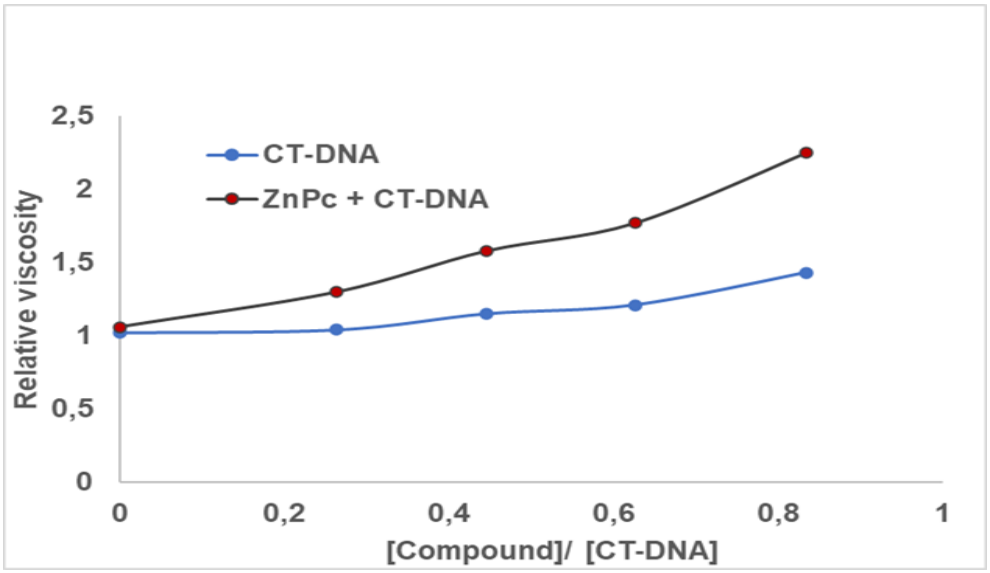

Figure 4. The change caused by the gradually increasing amount of ZnPc upon relative viscosity of DNA is displayed.

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A gradual increase in the viscosity of the solution was observed as the ZnPc concentration was raised. This information proves that the ZnPc compound binds to the DNA via an intercalative echanism[17-19].

3.5. Investigations on Thermal Degradation

The data obtained with this method may give clues as to whether there is an effect on the thermal melting temperature of DNA when it reacts with the ZnPc compound. The melting temperature (T_m) of DNA is often considered if temperature at which almost half of total base pairs are no longer bonding each other. This experimental procedure is a frequently utilized measurement method to observe the binding of chemicals to base pairs. As a general rule, there is an increase in T_m of DNA if subtances bind to DNA via intercalative bonding because of the intercalation of chemicals between DNA bases is involved in the stabilisation of base clusters and consequently in raising the T_m of DNA.

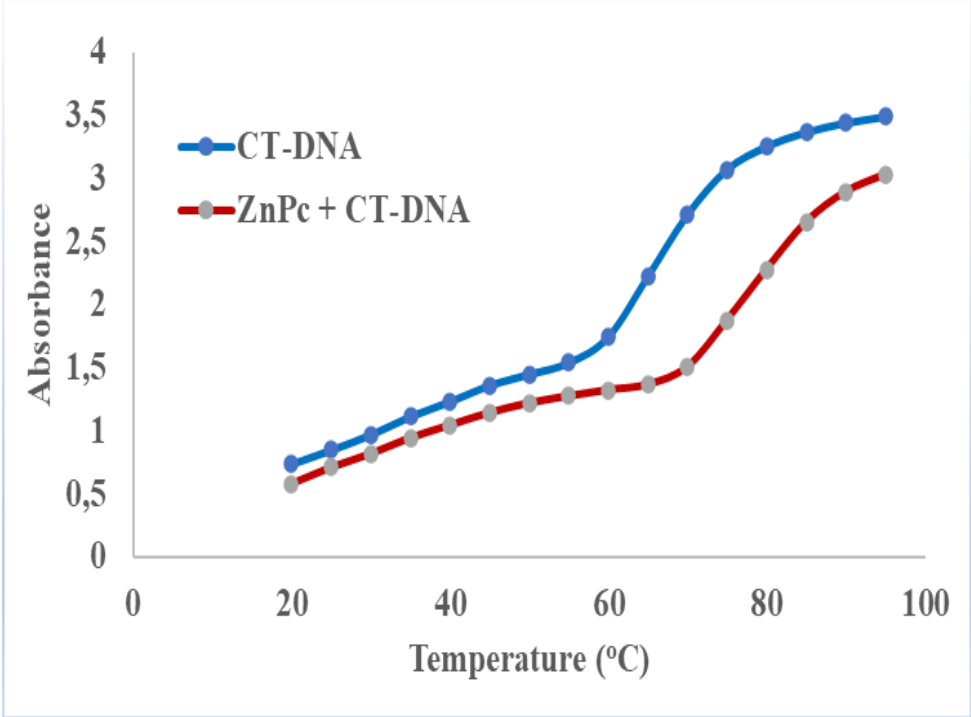

Figure 5. Shift in thermal melting temperature of ZnPc compound upon interaction with CT-DNA.

Melting plots of DNA without and with added ZnPc complex are shown in Figure 5. The thermal melting temperature obtained for DNA before adding ZnPc to the solution medium was 65.3 °C. When ZnPc complex was added to the solution medium in gradual increments, it was found that the thermal melting temperature of DNA increased. After the addition of ZnPc, there was a significant increase in the DNA thermal melting temperature and the Tm value was determined as 74.7 °C[17-19]. The melting temperature of DNA obtained in association with the ZnPc complex was also compared with known intercalators and confirmed that the compound interacts with DNA via intercalative binding.[24-26]

3.6. Gel Electrophoresis Assay

The interaction property of ZnPc compound with DNA was investigated by gel electrophoresis technique. The DNA bands obtained with this technique were visualized with the help of UV light and the bands were photographed. The binding capacity of the compound to DNA was analyzed according to the intensity of the bands. In order to analyze the effect of the ZnPc complex on DNA, the interaction strength was examined by gel electrophoresis method with the addition of the compound in different ratios. In the light of the results obtained in this study, it is clearly seen in Figure 6 that the intensity of the DNA bands detected after the interaction of the ZnPc compound with DNA decreased compared to the free DNA band[17-19].

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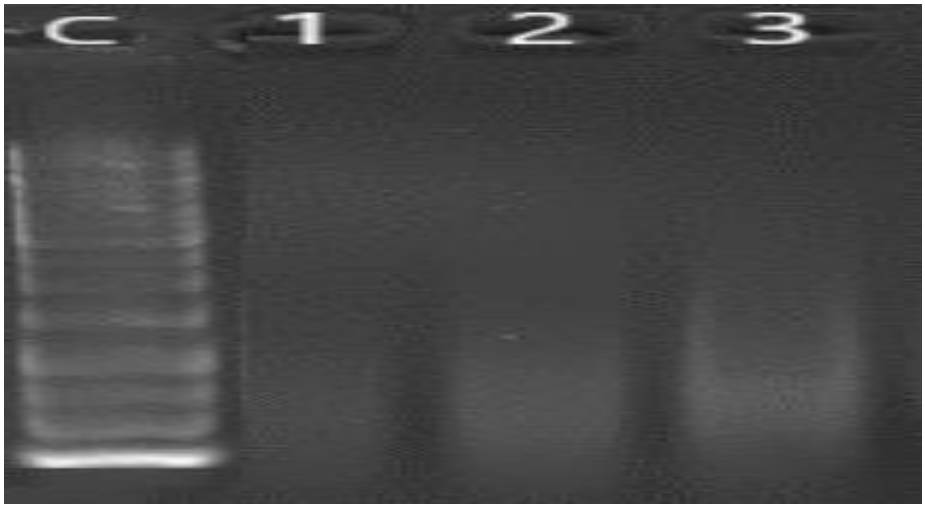

Figure 6. Elucidation of the activity of the interaction between ZnPc and CT-DNA using electrophoresis. Lane C: control CT-DNA. Lanes 1-3: 15 µM CT-DNA + (10, 15, 20 µM) ZnPc.

It was concluded that the decrease in the intensity of DNA bands resulting from the interaction of ZnPc compound with CT-DNA is due to the deformation of the DNA double helix. Previous scientific research report that DNA distortion may be mainly due to backbone fragmentation as a result of targeting of the residues.[27,20] It has been reported that the band intensities shown by EtBr localized in the base pairs in electrophoresis is related to DNA length as well as molecular amount.[28,29] Therefore, the decrease in the band intensities interacting with ZnPc may be due to the interaction of ZnPc with stacked bases within the helix and surface attachment at the active nucleophilic area of DNA.[30-34]

4.CONCLUSION

One of the most prominent studies conducted in the medical field in recent years is the mechanism by which phthalocyanine compounds bind to CT-DNA. In this report, we elucidated the interaction of ZnPc phthalocyanine compound with CT-DNA using different methods. Photophysical techniques, viscosity, thermal denaturation and electrophoresis were used to reveal the interaction of ZnPc with CT-DNA and the information obtained from these methods proved that the ZnPc complex interacts with DNA. The calculated binding constant of ZnPc to CT-DNA was found to be 1.392 10^6 M^{-1} and the data obtained proved that the form of interaction between ZnPc compound and DNA is an intercalative mechanism. The results revealed by the emission titration assay confirmed the binding of the ZnPc complex to DNA. The DNA binding activity of ZnPc complex was also evaluated by thermal melting temperature assay and the obtained results revealed that the compound bonded DNA intercalatively. In addition to above findings, the results obtained from viscosity and gel electrophoresis analyses confirmed that ZnPc reacts with CT-DNA. In conclusion, ZnPc shows strong binding to DNA, suggesting that ZnPc complex may be a potential medical agent.

Conflict of Interest

Authors declare that there is no a conflict of interest with any person.

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