

Study of The Interaction of A Zinc (II) Phthalocyanine Complex with a 2,4-Di-Tert-Butylphenoxy Substitute with Calf Thymus DNA

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Summary: Zinc (II) phthalocyanine complex was previously obtained from the compound of 4-(2,4-di-tert-butylphenoxy)phthalonitrile and characterized by spectroscopic methods like elemental analyses, absorption, NMR and infrared spectra. In this research conducted, the interaction properties of Zn(II) phthalocyanine compound containing 4-(2,4-di-tert-butylphenoxy) substitute by calf thymus DNA (CT-DNA) were investigated by absorption titration, fluorescence, viscosity, gel electrophoresis and melting temperature methods. The K_b value for this complex was found to be $2.58 (\pm 0.32) \times 10^6 \text{ M}^{-1}$. The K_b value supports that the complex binds to base pairs of DNA through intercalative bonding mechanism. The data obtained from fluorescence, viscosity and melting temperature studies were confirmed by the data obtained from absorption titration. In addition to the above methods, a gel electrophoresis study was also performed and this technique confirmed that the complex interacts with DNA. In the light of these data, Pc-Zn compound can be considered as a potential drug after further research on the complex.

Keywords: Zinc complexes, Phthalocyanines, DNA interaction, Absorption titration, Intercalative binding.

Introduction

From past to present, phthalocyanine compounds and different metal complex derivatives have been and continue to be the subject of many scientific investigations due to their interesting properties. The synthesizing a vast diversity of replaced phthalocyanines both metal containing and non-metal-containing phthalocyanine species have been published in the literature [1-3]. Among the main reasons for the increasing interest in the chemistry of phthalocyanines nowadays, these chemical compounds and the vast majority of derivatives of such compounds offer remarkable features for applications in the field of materials science [1, 4]. Phthalocyanine compounds and their diverse derivatives are employed in laser printing [5-9], data storage, liquid crystals, photosensitive agents, sensors [10], electrochromic compounds [11] and photodynamic cancer therapeutics. In a further, due to the capability of specific phthalocyanine derivatives for being able to effectively photo inactivate tumor cells *in vivo* and viruses, which has received significant focus. The generation of these compound derivatives with improved pharmacokinetic and photodynamic characteristics is highly dependent on detailed learning about structure-activity relationships of this type of chemical compound [12].

Cancer, one of the deadliest diseases for humanity in today's modern world, continues to be the most dangerous disease that humanity has not yet found a complete solution to this health problem and creates a major health problem. Although there are drugs and techniques developed from the treatment of certain types of cancer in today's world, the desired point has not yet been reached in cancer treatment. Owing to the fact that the DNA molecule forms the basis of life, we can say that the interaction and reaction of various transition metal complexes like phthalocyanine metal complexes with DNA have become an important field of study. DNA molecules have a negative charge and can react very strongly with positively charged chemical agents. Positively charged porphyrins, phthalocyanines and derivatives of these compounds are of great interest due to the role they play in the human body, their proliferation capacities in various cancer cells, magnetic behavior and their optical characteristics and their interactions with DNA. In this context, phthalocyanine compounds and their transition metal complexes and their reactions with DNA molecules have recently attracted considerable attention [13-21]. Phthalocyanines and metal-containing phthalocyanine complexes and the mechanisms of DNA binding of these chemical agents and the development of new target drugs have become

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a very popular research area and DNA interactions continue to attract interest in the context of medicinal and photodynamic research [22, 23]. Developing effective, environmentally friendly, and easily applicable synthetic methods is of the utmost importance. Furthermore, the need to develop more environmentally friendly synthesis methods for producing both unsubstituted and substituted phthalocyanines typically relies on the use of alternative energy sources such as microwave and UV radiation [24]. Reference values can serve as effective criteria for initially assessing the environmental sustainability of a synthesis based on the waste products produced. This methodology could contribute to the development of more environmentally friendly synthetic methods for the synthesis of phthalocyanine rings [25].

In this research, an investigation was carried out to elucidate the interaction mechanism of Pc-Zn complex with DNA, which was previously synthesized and characterized by our working group. Absorption spectra, fluorescence, melting temperature, viscosity and electrophoresis techniques were used to investigate the DNA bonding properties of this complex. In this current research, all tests were conducted at constant pH and in a Tris-HCl buffer solution containing 25 mM of Tris-HCl + 30 mM NaCl.

Experimental

Materials and instruments

For Calf Thymus DNA, A_{260}/A_{280} ratio was around 1.8 and other consumables utilized in this research were procured from the commercial companies Merck and Sigma-Aldrich. All solvents and reagents used in the experiments were analytical grade and were not subjected to any additional purification process. DMF was used to prepare all solutions used for DNA interaction analyses. The Pc-Zn complex used to determine DNA binding activity was prepared and characterized according to reported procedures by Agirtas [26]. Cary 60 UV/Vis spectrophotometer was utilized for spectra obtained in electronic titration experiment and melting temperature measurements. Viscosity data were determined by Ostwald viscometer and DNA bands were obtained and visualized using the Thermo Scientific Electrophoresis system.

Synthesis

Synthesis of Pc-Zn complex

The compound, 4-(2,4-di-tert-butylphenoxy)phthalonitrile, as well as the zinc(II)

phthalocyanine complex having 2,4-di-tert-butylphenoxy substitute (Pc-Zn) used in DNA interaction were previously synthesized and characterized by Agirtas and reported in the literature [26].

Results and Discussion

Synthesis and characterization

The structural formula of the compound is presented in Fig. 1 [26]. Previously synthesized and characterized Pc-Zn phthalocyanine complex was reported to be soluble in solvents such as chloroform, dichloromethane, THF, toluene, DMF and DMSO [26]. In this research work, the results of the elemental analysis and spectral experiments (IR and UV/Vis) verified that the synthesized compound is in agreement with the prescribed formulation [26]. The IR spectrum data of Pc-Zn compound (KBr) ν_{max} (cm^{-1}) 3071, 2960, 2912, 2862, 1594, 1486, 1313, 1277, 1249, 1088, 840 and UV/Vis (DMF) λ_{max} (log ϵ) (nm) values were determined as 358 (3.68), 612 (3.40), 680 (4.09) [26].

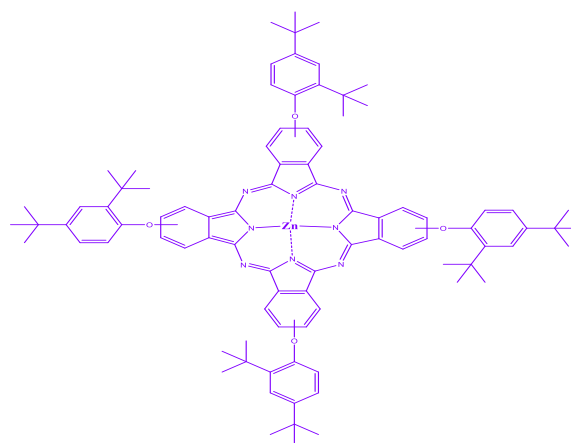


Fig. 1: Basic chemical structure of the previously synthesized Pc-Zn [24].

Binding property of Pc-Zn to DNA

Absorbance analysis for the Pc-Zn complex

To understand DNA interacting activity of Pc-Zn and the nature of interaction, absorption-titration experiments were performed. Comprehending the binding mechanisms of chemical agents to DNA is of great importance. The binding of phthalocyanines to DNA involves several fundamental interaction mechanisms, which can be summarized as follows like intercalation and electrostatic bonding. The intercalating interaction mechanism can be expressed as a type of

binding that takes place as a consequence of the entry of the molecules or derivatives amongs base pairs of DNA. In short, intercalating is an interacting process that occurs in hydrophobic and polar part of DNA linker [27]. As a result, this compound was observed to interact with CT-DNA through intercalation due to hypochromism. The K_b value of the compound was observed to be close to the K_b of ethidium bromide which is a classical intercalator [27]. The mode of binding of the phthalocyanine compound to DNA is the most important influence factor explaining wavelength shift and hypochromic or hyperchromic takes place. The appearance of significant decrease and displace in the absorbance spectra is considered as evidence of intercalating as a consequence of the storage among the bases and the phthalocyanine compound. Data obtained as a result of electronic spectrum measurements provide very important contributions to the elucidation of the structures of compounds such as phthalocyanines. Phthalocyanines have a characteristic electronic spectrum with two dominant absorption bands, one UV with a B band at around 300-400 nm and one visible with a Q band at 600-730 nm. All of the titration measurements performed for the analysis experiments conducted to search the interaction of Pc-Zn complex with DNA were performed at pH 7.09. Concentration of CT-DNA utilized in the absorption titration measurements was set at 20 μM . For this method, based on the wavelength range of 330-730 nm, electronic spectral measurements were performed. In DMF organic solvent, characteristic Q band transition of the Pc-Zn complex was detected at approximately 615-682 nm and the spectrum of Pc-Zn phthalocyanine compound was recorded about at 347 nm for B bands. UV/Vis spectrums of Pc-Zn+DNA are displayed in Fig. 2.

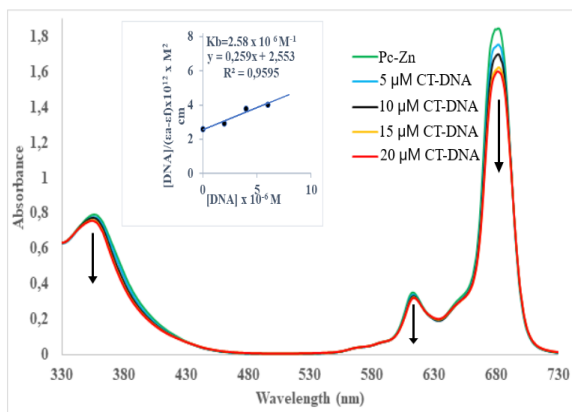


Fig. 2: The absorption spectra of Pc-Zn in the presence and absence of CT-DNA (20 μM) at gradually increasing concentrations.

It was observed that the absorbance spectra decreased when different amounts of CT-DNA were added to the Pc-Zn compound. The decrease in absorbance spectra is an indication that hypochromism occurs. It can be concluded that the Pc-Zn complex binds to DNA by an intercalative interaction mechanism, since the decrease in absorbance intensities at the wavelength of Q and B bands is sufficient as evidence of intercalation. The value of K_b for Pc-Zn was identified using the data obtained from the spectroscopic titration experiment and the Wolfe-Shimer equation (1) [28]. Using equation (1), K_b for Pc-Zn was calculated as $2.58 (\pm 0.32) \times 10^6 \text{ M}^{-1}$.

As a result, the phthalocyanine complex studied in this study is completely different from the complexes studied in the literature, as it is an investigation of the properties related to DNA interaction. This makes the compound different in terms of their properties and structures. The substituted group used in this study is also different from other studies and has a completely different structure. This makes the DNA binding properties of the compound completely different.

Melting temperature measuring for Pc-Zn complex

The basis for the measurement of melting temperature is the value resulting from separation of DNA strands into single strands [29]. The determination of the T_m value is a crucial guiding factor in understanding how the DNA double helix stabilizes under the influence of temperature. Elevations in T_m are highly significant in terms of revealing the strength of the interaction occurring due to binding between DNA and chemicals [30]. The data obtained from the melting stability study are of great importance in terms of revealing whether the DNA molecule follows an intercalative or external binding type mechanism during binding with chemical substances. In general, it has been reported that the traditionally widely used intercalating agents cause a significant increase in the T_m value when they bind to the DNA molecule [31]. In the present study, we first measured T_m of DNA that solution has no Pc-Zn complex and then measured the absorbance at 260 nm after adding the Pc-Zn complex to the DNA solution.

Impact of the Pc-Zn complex over the T_m of DNA is given in Fig. 3, T_m value of pure DNA measured before addition of Pc-Zn complex was found to be approximately 78.6 $^{\circ}\text{C}$, while the T_m value of DNA measured after addition of Pc-Zn to DNA was found to be approximately 86.1 $^{\circ}\text{C}$. The significant melting temperature increase in the experimentally obtained T_m value confirmed that interaction between DNA and Pc-Zn complex is mediated by an intercalative binding mechanism.

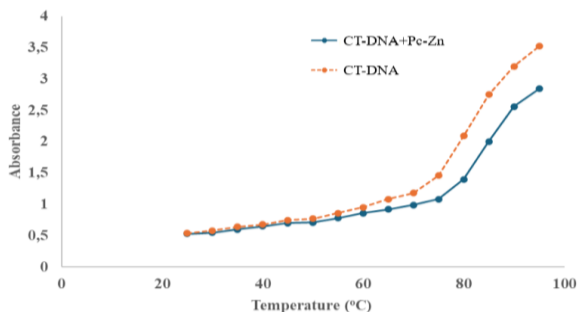


Fig. 3: Impact of the Pc-Zn complex over the T_m of DNA in the buffer medium.

Viscosity assays on interaction of Pc-Zn-DNA

In the case of absence of crystallographic information on the binding of drugs to DNA, the measurement of the movement of liquids is considered to be the most credible and feasible technique for determining the extent and nature of DNA-drug interactions. When a chemical substance interacts intercalatively with DNA, it can lead to a significant increase in the viscosity of the liquid solution of DNA, since the introduction of the intercalator into the system leads to the separation of the base chains and consequently to an elongation of the DNA molecular length. However, non-classical intercalators frequently bend the DNA strand and reduce the viscosity of the DNA [32, 33].

Within the scope of this study, viscosity data were collected at room temperature by means of a viscometer with a certain capacity. The DNA concentration was remained identical and the concentration values of the Pc-Zn complex were enhanced in the range of 0 to 20 μM in various specimens. Viscosity tests were performed three times, sample flow times were recorded and average viscosity values calculated for each trial. The results obtained in the assay are displayed as $[\text{Pc-Zn}]/[\text{DNA}]$ ratio versus relative viscosity. Influences of Pc-Zn complex over viscosity of DNA can be seen in Fig. 4, the viscosity of DNA solution was consistently raised as a result of penetration of Pc-Zn between the DNA base pairs, and this increase in viscosity was interpreted as evidence of elongation in the length of the DNA chain [34]. The observed rises in the relative viscosity of the DNA solution were taken as a clear sign that Pc-Zn attaches to DNA via intercalative mechanism.

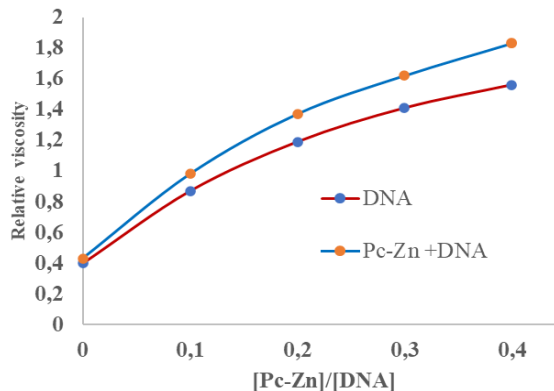


Fig. 4: Influences of Pc-Zn complex over viscosity of DNA in buffer medium.

Gel electrophoresis

The analysis technique of gel electrophoresis is a good method for investigating mechanism of interaction of metal-containing agents with DNA and for detecting the effects of chemical agents on DNA [35]. The binding capacity of the previously synthesized Pc-Zn complex bearing tetrakis[(2,4-di-tert-butylphenoxy) substitute to DNA was probed via gel electrophoresis. The reaction mixture containing CT-DNA (20 μM) and different concentrations of Pc-Zn complex (15, 20 and 25 μM) were prepared in buffer solution was kept to incubate at 37°C for 45 min. At this stage of the study, electrophoresis of the mixture of DNA and complex was carried out at 100 volts using 0.8% agarose gel in buffer solution. The bands were visualized and photographed using UV light.

It was determined that changes took place in intensity of the bands formed as a result of the interaction. Visualisation of DNA electrophoresis bands on an agarose gel is displayed in Fig. 5. The results obtained from this method are shown in Figure 5. It has been discovered that intensity of bands recorded for the complex after binding to DNA decreased compared to the intensity of C band. The reduce in intensity of bands resulting from the binding of Pc-Zn to CT-DNA is considered as evidence that Pc-Zn interacts with DNA. When DNA attached to the Pc-Zn complex, CT-DNA bands were observed to be shortened compared to the control (C) CT-DNA bands [35-37]. These findings clearly verified that Pc-Zn interacts with DNA.

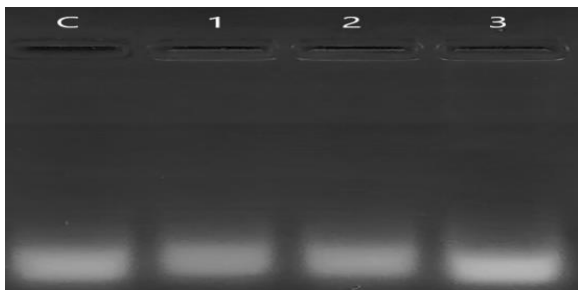


Fig. 5: Visualisation of DNA electrophoresis bands on an agarose gel, control DNA (lane C) and CT-DNA + Pc-Zn complex (lane 1, lane 2 and lane 3), respectively.

Conclusion

In this report, the zinc complex bearing 2,4-di-tert-butylphenoxy substitute was previously prepared from 4-(2,4-di-tert-butylphenoxy)phthalonitrile compound and characterized by spectroscopic methods such as elemental analysis, absorption, NMR and infrared spectra. UV/Vis absorption titration spectra, melting temperature, gel electrophoresis and viscometry were used to analyse the binding activity of this complex to DNA. The results obtained from the techniques utilized reveal that the complex has interaction with CT-DNA and Pz-Zn exhibits a high interaction affinity to DNA. The Kb for the complex was obtained as $2.58 \times 10^6 \text{ M}^{-1}$ via absorption study and increasing in Tm was observed as a result of the interaction of Pc-Zn and DNA. A rising in relative viscosity of DNA was also observed. It is decided that the synthesized Pc-Zn attaches to CT-DNA by an intercalative binding mechanism. In the light of these data, it was reached the conclusion that a drug formulation for cancer disease based on the complex could be effective.

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