Probing the DNA Interaction Behavior of Tetrakis (benzhydryloxy Phthalocyaninato) Mg (II) Complex

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Summary: In this work, we evaluated the DNA interaction behaviors of previously prepared and characterized peripherally tetra-substituted Mg(II) phthalocyanine containing a tetrakis (benzhydryloxy) group. The 2, 10, 16, 24-tetrakis (benzhydryloxy phthalocyaninato) Mg(II) complex was studied is soluble in organic solvents. During the synthesis and characterization phase, absorption spectroscopy, infrared spectroscopy and ^{1}H NMR were utilized to elucidate the chemical structure of the MgPc complex. For this complex, DNA binding behaviours were investigated with various techniques like electronic absorption spectroscopy, fluorescence emission spectra, electrophoresis and thermal denaturation. To reveal the binding pattern of the MgPc complex to DNA, the binding constant was calculated and found to be 1.73×10^6 M^{-1} for the complex. All the results obtained from the techniques applied showed that MgPc tends to interact with the DNA molecule and confirmed that this interaction occurs intercalatively.

Keywords: DNA interaction, Phthalocyanine, Electronic absorption, Fluorescence spectrocopy, Mg(II) and Thermal denaturation

Introduction

Phthalocyanines are prominent compounds and comprehensive studies on these molecules have been continuing for years. So far, the derivatives of phthalocyanines possess numerous potential implementations in different scientific field, comprising, photosensitizers [1,2], sensors, liquid crystals and films [2-5]. These compounds are also recognized as of their decent conductive features [6-8]. Phthalocyanines have recently been identified as an emerging version of photosensitizers due to their low toxicity, significant phototherapeutic absorption and easy chemical modification [9-13]. Lately, in clinical research, numerious studying were concentrated on the activities of these compounds against cancerious cells [8]. The unknowable complementary features of these complexes may be revealed in the future.

In today's conditions, cancer treatment techniques come into being like chemotherapy and surgery healing [9-13] have been practice at the moment in the field medicine. In the majority of cases, there is a compromise between the surgical procedure and the therapeutic techniques. One of the cancer treatment techniques is photodynamic therapy [13, 14], which relies on photosensitizer and oxygen molecule to cause selective damage to cancer cells [15-17]. In addition, phthalocyanines have the ability to adapt to chemical changes by changing the state of the transition metal and the substituted ligand in order to achieve the desired properties. Phthalocyanine compounds can gain new functionalities because the physical, chemical and optical properties of these compounds can be influenced by the type of metal and the state of the substituted ligands. Due to their physicochemical properties, phthalocyanine complexes have shown great promise in the application of photosensitizers [9, 18-26].

To define the interaction activity of the previously synthesized tetrakis(benzhydryloxyphthalocyaninato) Mg(II) complex (MgPc) [27] with CT-DNA and to elucidate the binding mechanism. The DNA interaction activity of the complex was determined by electrophoresis, absorption spectroscopy, emission spectroscopy and melting temperature experiments.

Experimental

Materials

[Tetrakis-((benzhydryloxy)] substituted Mg (II) phthalocyanine had been synthesized and characterized the previous study [27]. The chemical reagents and calf thymus DNA (CT-DNA) utilized in the present study were procured from Sigma Aldrich.

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All DNA dissolutions used were equipped in the buffer containing NaOH at pH 7.2 and stored in a refrigerator at 4ºC. The stock phthalocyanine complex was prepared and stored at 25 ºC. If needed, all the solutions had been diluted to a desirable volume with tris-HCl buffer. UV/Vis absorption and thermal melting point data had been gathered using Cary absorption spectroscopy and emission titrations were employed by Perkin Elmer emission spectroscopy. Electrophoresis assays had been performed utilized Scientific Owl electrophoresis appliance at pH 7.2 at 25 ºC.

Synthesis 4-(benzhydryloxy) phthalonitrile

The compound of 4-(benzhydryloxy) phthalonitrile was produced properly mechanism informed in published literature [27].

2, 10, 16, 24–tetrakis (benzhydryloxy phthalocyaninato) Mg (II)

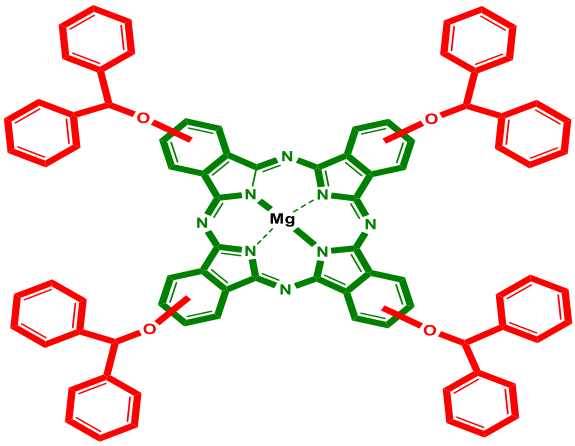
2, 10, 16, 24–tetrakis (benzhydryloxy phthalocyaninato) Mg (II) complex was produced properly mechanism informed in published literature [27].

Results and discussion

The synthesis and characterization of MgPc complex

The chemical structure of Mg (II) phthalocyanine is illustrated in Scheme-1. The synthesis of 4-(benzhydryloxy) phthalonitrile compound was synthesized similarly to previous studied in published literature [27]. In this synthesis, 4-nitrophthalonitrile and diphenyl methanol compounds were put into the solution of K_2CO_3 to produce phthalonitrile compound. The chemical reaction was finished off at 25 ºC in the presence of N_2 gase for a certain period of time. The phthalocyanine complex was obtained with the reaction of 4-(benzhydryloxy) phthalonitrile by $MgCl_2$. The structure of the new produced phthalocyanine complex was enlightened by spectroscopies like UV/Vis, IR, NMR [27].

The IR spectroscopy provides data related the nitrile band that is assumed as a meaningful gap between phthalonitrile compound and phthalocyanine metal complex. The band determines the structural variation. FT-IR^{1}H and NMR data of Mg (II) phthalocyanine complex were previously produced in published paper [27]. The NMR spectrums of MgPc complex indicate small shifts over starting compound. MgPc gives 7.65, 7.31, 7.18, 6.86, 6.62, and 1.35 NMR peaks, respectively [27]. The obtained mass spectrum verified the structure of complex. The 1265.17 value was produced for Mg (II) phthalocyanine is equal to the calculated 1265.17 value [27].



Scheme-1: The chemical structure of MgPc complex.

The interaction of MgPc with DNA

Absorption spectroscopy is one of method to enlighten the mechanism of interacting of molecular compounds with DNA molecule. It is a very beneficial instrumental method which produces knowledge related peak positions in the spectra for the binding of molecular compounds [28-30]. The shifting of bands expresses the power of binding between DNA and phthalocyanine compounds.

The electronic absorption experiments were conducted for MgPc from 250 to 850 nm wavelengths to monitor the absorption spectra at fixed (20 µM) concentration of the complex. The absorbance peaks of the complex indicated the distinctive absorption peaks at around 687 nm and 348 nm in Q and B bands as illustrated in Fig. 1 [31]. When an boosting concentration of the DNA (0-15 µM) was put into 20 µM MgPc, the ultimate electronic spectrum intensities of the complex MgPc were declined and the hypochromism was sighted in the absorption intensity of peak. The observing of hypochromism demonstrates that MgPc bounds to DNA through intercalative mode [32]. The computing the binding constants (K_b) of chemical compounds to DNA can identified. The Kb value was computed for the analyzing the interaction affinity of the MgPc complex by DNA. The Kb of MgPc has been computed to be $1.73 \times 106\ M^{-1}$ [32].

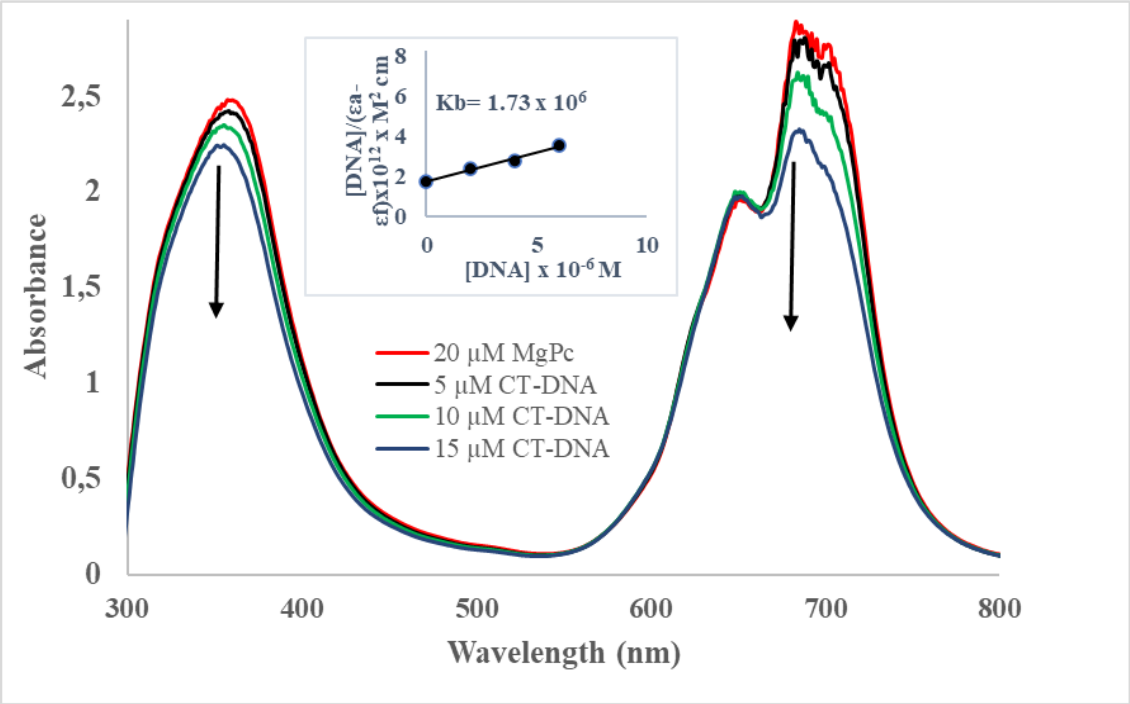


Fig. 1: Electronic spectra of MgPc in the absence and presence of boosting concentrations of CT- DNA.

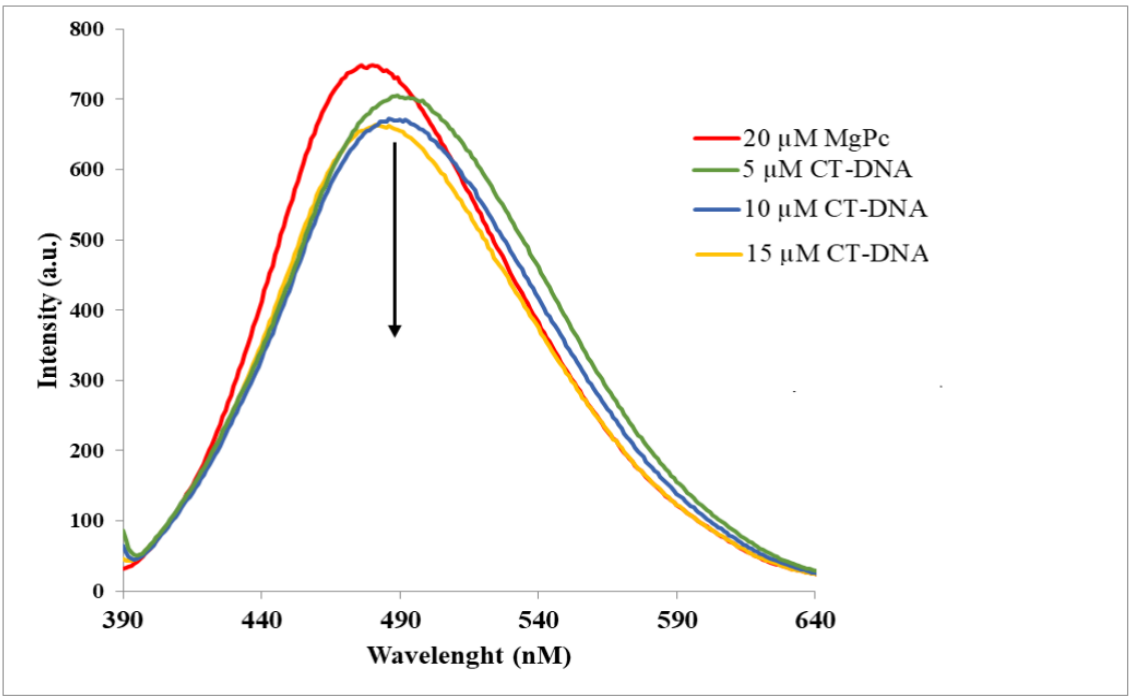


Fig. 2: The changing in emission spectral intensities by adding various amounts of CT-DNA.

Interaction study of MgPc with CT-DNA using Fluorescence spectroscopy

Emission spectroscopy technique can be utilized to examine the attaching activities between metal phthalocyanines. The technique has many advantages over other methods; for example, it is very sensitive and has a wide concentration range [33]. The ways in which therapeutic chemicals interact with DNA can be determined using emission spectroscopy. The technique of emission titration spectra provides more data on drugs and their binding mechanisms to DNA molecule [34, 35]. The binding activity of MgPc to DNA was observed with emission spectrophotometre method in tris HCl buffer at 25 ºC. Fig. 2 displays emission spectra of MgPc in the presence of distinct amounts of CT-DNA. The at constant concentration of MgPc complex (20 µM) was titrated with at distinct amounts of DNA sample (0-15 µM). The emission spectra were observed between around 390 and 600 nm for complex MgPc-DNA sample. The emission intensity of MgPc was observed to be dropped with the adding of DNA, and this finding proposed that MgPc binds to DNA via intercalative binding mechanism.

Thermal denaturation experiment

The melting temperature activity of DNA in the presence of metal complexes may procure understanding of conformational structure differences when temperature increases and provide information respecting the binding activities of chemical compounds to DNA molecule. Studies reported in the literature have revealed that when compounds connect to DNA, the stability of DNA molecule increases. In general, the T_m of DNA increases when metal compounds interact with DNA via an intercalative binding mechanism because the intercalative binding of metal complexes between DNA base pairs leads to the stabilization of the stacking of base pairs of DNA thereby increasing the T_m of DNA. For the most part, electrostatic interaction along the DNA phosphate backbone produces only a small change in melting temperature, whereas an intercalative binding mechanism causes a notable increase in the melting point of DNA due to stabilization of the base-paired duplex of DNA [36, 37].

The T_m curve of DNA in the absence and present of MgPc was as shown in Fig. 3. In the absence of MgPc, the melting temperature experiments were performed for CT-DNA. Melting temperature study of CT-DNA and MgPc showed an acceptable positive change in T_m. It was observed that the T_m for CT-DNA was around 67.7 ºC at pH 7.2. In the presence of MgPc complex, T_m was increased to around 78.7 ºC. The large increment in melting temperature of DNA with MgPc is comparable to that recorded for standard intercalative agents [38-40]. In conclusion, the method data demonstrated that the complex binds to DNA through an intercalative binding mechanism.

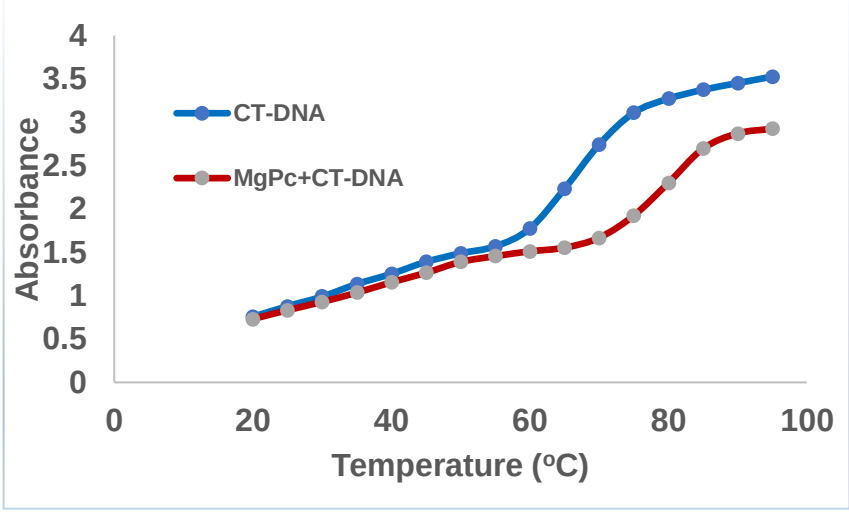


Fig. 3: T_m curves of the DNA in the absence and presence of MgPc, indicating the rising in melting temperature.

The interaction study with gel electrophoresis

In addition to the above studies, in this study, DNA binding activity of MgPc complex was examined utilizing gel electrophoresis technique. Agarose gel electrophoresis is a elementary and very affective technique exercised to separate, define and refine the fragments of molecules such as DNA [41]. Gel electrophoresis study was conducted to find out whether the synthesized complex binds to CT-DNA. Lane C represents control CT-DNA and lanes 1, 2 and 3 consist of MgPc (20 µM)+(CT-DNA (0-15 µM) had been loaded respectively into agarose gel that comprises DNA stain. The samples had been incubated for 30 min. 100 V electric current was implemented the period of time in the experiment. When DNA bands were examined under UV light, MgPc complex was clearly seen to interact with DNA as seen in Fig. 4 [42]. This binding of the complex to the DNA may be due to overlap of the metal complex during binding between bases within the DNA strand or due to surface interaction at the reactive sites of the nucleophile on DNA double helix [43].

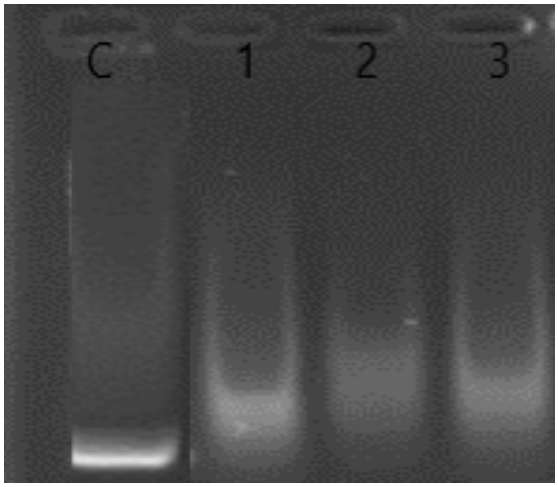


Fig. 4: The interaction study of MgPc with DNA using jel agarose electrophoresis technique. Lane C represents control CT-DNA and lanes 1–3 represent (5, 10 and 15 µM CT-DNA + (20 µM) MgPc, respectively.

Conclusion

In present study, DNA intercation activity of the 2, 10, 16, 24–tetrakis (benzhydryloxy phthalocyaninato) Mg (II) was assayed with various instrumental methods. The DNA interaction investigation of MgPc towards DNA was fulfiled in tris-HCl+NaCl solution of pH 7.2 utilizing absorption titration, emission spectra, thermal melting point and jel eletrophoresis assays. The absorption assay was implemented to find out DNA binding mode of MgPc. The obtained result from assay indicated that MgPc complex interacts with the DNA through an intercalative interaction. Emission spectra assay affirms the result of electronic absorption technique. The Kb binding value from electronic titration was computed as $1.73\ 10^6\ M^{-1}$. Kb value proved that MgPc interacts with DNA through intercalative binding mechanism. Also the binding of MgPc to DNA was examined using thermal melting point and the electrophoresis assays. These both techniques verified that MgPc interacts with DNA. This study of the binding activity of MgPc with DNA aserted that the complex may be evaluate as a potential candidate of a therapeutic agent with further detailed investigation.

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