The Study of Interaction Activity of Nickel (II) Phthalocyanine Complex Bearing Tetra Substituted Phenoxy-3-Methoxybenzoic Acid Groups with DNA

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Highlights
• Nickel (II) phthalocyanine compound having phenoxy-3-methoxybenzoic acid.
• Studying of DNA interaction with Ni(II) phthalocyanine.
• Potential medicine of phthalocyanine and binding mode of the compound.

Abstract
Nickel phthalocyanine complex containing 3-methoxybenzoic acid groups was acquired and specified by way of Fourier Transform Infrared, NMR and UV-Visible spectroscopy procedures. Interaction of PcNi with the DNA molecule was examined via electronic absorption spectra, fluorescence spectra, melting point, viscosity, and the electrophoresis technics, respectively. The interaction activity of PcNi against the DNA was examined by way of absorption spectra titrations and the fluorescence spectra, farther by conducting melting point, viscosity procedures in the buffer of a pH 7.02. The obtained outcomes from these methods demonstrated that PcNi indicated substantial binding affinity to the DNA via intercalating by $K_b$ of $1.31 \times 10^6$ m$^{-1}$. Further, the interacting activity of PcNi on the DNA was analyzed by which electrophoresis technique and this procedure indicated that PcNi complex exhibits strong binding affinity on the DNA.

1. INTRODUCTION

Phthalocyanines are one of substantial aromatic compounds and they have interesting biological features such as anticancer, enzyme inhibition, and antimicrobial activities. Also, they have a great potential of applications in the different fields for instance sensors, treatment of cancer disease [1-7]. Phthalocyanine compounds are confirmed to become an accomplished manner of second origin photosensitizer in nowadays because of their marginal toxicity, powerful absorption spectra in the photodynamic therapy and easy chemical alteration [5]. Predominantly researches are centered upon their actions versus cancer and many photosensitizers depending phthalocyanine compounds have been permitted for clinical usage. The one of disadvantages of phthalocyanine compounds in the implementations is the insolubility of these compounds in certain solvents and in the presence of grouping because of the ring interactivity [5].

Recently, investigation for the interaction studies of phthalocyanine metal complexes by DNA molecule have gotten great attention during the recent years to develop new anticancer medicine [8-13]. Many studies on DNA binding of phthalocyanine metal compounds were performed in the reported literature for cancer therapy [14]. Most of the scientific studies concentrated targeting cell cycle and DNA interaction mechanism because the DNA molecule is evaluated the target molecule on medicinal compounds. In the reported studies, the interaction properties of DNA molecule for phthalocyanine metal compounds have
been studied understanding how the tumor hindering activities of the new anticancer medicine are acting [14, 15]. A crucial part of cancer treatment is made up of phthalocyanine compounds that connect to DNA or avoid the DNA resting [16, 17] and therapeutics binding to DNA molecule may modify DNA structure [18]. When drugs connect to DNA, they may generate difference in duplication of DNA molecule and genetic expression [19, 20]. Small compounds are thought to be the main modes of interaction with the DNA molecule, these are intercalative and non-intercalative binding modes [19, 21]. Phthalocyanines may react on DNA by way of intercalative and hydrogen binding mechanisms [22]. In recently, the interaction activities of phthalocyanines on DNA accelerated in the literature due to the hindering of direct or indirect accretion of cancerous tumor [22].

This current study, synthesized Ni(II) phthalocyanine complex 4 (PcNi) having phenoxy-3-methoxybenzoic group was characterized by way of Fourier Transform Infrared, NMR and absorption spectroscopy methods. Interaction properties of the complex by Calf Thymus DNA were searched via absorption, fluorescence, melting point, procedure of electrophoresis and viscosity experiment. The findings from these techniques may pave way for further studies about treatment of cancer.

2. EXPERIMENTAL

2.1. Material and Method

Chemicals such as NiCl₂, K₂CO₃, 4-hydroxy-3-methoxybenzoic acid, methanol, acetonitrile, DMF, DMSO, THF were purchased from Merck company (Darmstadt, Germany). Calf Thymus-DNA, NaCl and Tris-HCl had been commercially obtained from Sigma/Aldrich (Darmstadt, Germany). Supplied chemicals had not been purified before usage. In this study, the NMR experiments had been performed with an Agilent spectrometer (Van YYU, Türkiye). For IR measurements, FT-IR were conducted by Thermo Scientific FT-IR spectrophotometer (Van YYU, Turkey). A Hitachi Spectroscopy (Van YYU, Turkey) and Cary 60 UV/Vis spectroscopy (Karabük Uni, Central Laboratory, Turkey) were used for the UV-Vis absorption titration experiments and for the fluorescence titrations were conducted with an Agilent Technologies Cary spectroscopy. Thermo Scientific Electrophoresis device was utilized for the electrophoresis experiments and Ubelohde viscometer was used to carry out viscosity experiments in a Tris-HCl buffer.

2.2. The Synthesis of Phenoxy-3-Methoxybenzoic Compound (3)

The synthesis and characterization of phenoxy-3-methoxybenzoic compound (3) had been previously reported in the literature [23].

2.3. The Synthesis of the Nickle (II) Phthalocyanine Compound (PcNi)

The nickel (II) compound was synthesized with the reaction of the compound (3) in medium of NiCl₂, which was previously stated in the literature [23]. The yield was 0.017 g (33 %). UV-Vis (THF) λmax (log ε): 672 (5.15) IR spectrum (cm⁻¹):3523, 3066, 2914, 1695, 1598, 1587, 1462, 1408, 1263,1217, 1176, 1116, 1091,1058, 1029, 956, 754, 742. ¹H NMR (400 MHz, DMSO-d₆) δ ppm: 7.11, (Ar-H), 3.82(CH₃), 3.33 (DMSO-d₆) 2.48 (DMSO-d₆), 1.13 (CH₃).
3. RESULTS AND DISCUSSION

Synthesized Nickel (II) phthalocyanine complex was produced with cyclotetramerization of the compound (3) with NiCl$_2$ at fixed temperature and under N$_2$ gas. The synthesis pathway of Ni(II) phthalocyanine compound is as indicated in Figure 1. PcNi was analyzed by spectroscopic procedures like UV-Vis, $^1$H-NMR, IR and the findings had been coherent by anticipated chemical structure. The complex compound of this nickel phthalocyanine dissolves in THF, DMF and DMSO. The FT-IR and $^1$H-NMR spectral data of PcNi compound are given in the Figures 2 and 3, respectively.

![Figure 1. The synthesis pathway of PcNi phthalocyanine complex](image-url)
Figure 2. The FT-IR spectra of PcNi complex

Figure 3. $^1$H NMR spectra of PcNi complex
3.1. DNA Binding Investigations

The binding of PcNi to DNA were examined with absorption titration measurements to determine its binding activities to the DNA [24]. The DNA sample was solved in a Tris-HCl. All the chemical solutions were produced in dimethylformamide (DMF) and then subtilized in the buffer solution at pH 7.02. UV-Vis absorption titrations were conducted in the range of 260–850 nm at 25°C as illustrated in Figure 4. Absorption measurements were implemented by holding amount of PcNi fixed (20 µM) and changeable the amounts of CT-DNA (0 to 3.5 µM) with a rising of amounts of the DNA. UV-Vis absorption spectra were measured following each of addition of the DNA. The PcNi + DNA solution was permitted to incubate for 5 min for each running and changing in absorption spectra were performed at 25 °C. The rises in concentration of CT-DNA, absorbances of the complex slowly declined. Interaction activity of the compound to the DNA were observed via hypochromism of two main absorbance bands, which were located at around 350, 686 nm connected to the red shift as represented in Figure 4. From obtained UV-Vis spectra parameters, the binding constant (Kb) of PcNi by the DNA was specified with Wolfe-Schimer equation [25]. The Kb values for PcNi was determined as $1.31 \times 10^6$ M$^{-1}$, using Wolfe-Schimer equation. In case of mounting the amount of the DNA, the complex PcNi deduced hypochromism by a red shift. As a result, by checking the binding constant value and the tendency of absorption spectra changing by the DNA addition, it can be expected that PcNi interacts by DNA in an intercalative binding.

**Figure 4.** Absorption titrations of PcNi (20 µM) on increasing concentration of DNA (0 to 3.5 µM) in the buffer solution
3.2. The Fluorescence Titration Studies

The fluorescence procedure is also conducted to explain DNA-medicine interaction activities by reason of fluorescence titration is susceptible technique in DNA binding probes. Besides, it can produce additional knowledge for the intercalation [26, 27]. Fluorescence titration was performed by adding CT-DNA for intercalative interaction to additional examine the binding activity of PcNi with DNA. When PcNi was connected to the DNA, the intensity of fluorescence spectrum were dropped slowly. The dropping of intensities of fluorescence spectra proved that PcNi interacts with CT-DNA using hypochromic mechanism. PcNi gives strong fluorescence spectra in the absence of the DNA around 500 nm as illustrated in Figure 5. The powerful fluorescence spectra may be originated from ligands [26,28, 29]. Upon increasing of DNA concentration, intensity of fluorescence spectrum drop for the compound PcNi. The finding demonstrated that PcNi connects to the DNA much probably via an intercalation, which was coincided with UV-Vis absorption titration results.

![Fluorescence titration spectra of nickel (II) phthalocyanine complex (20 µM) in the buffer solution at 25°C in the absence and the presence of CT-DNA. Arrow represents changes in intensity on raising the concentration of CT-DNA](image)

3.3. Viscosity Studies for DNA Binding

In the current study for PcNi complex, viscosity experiments were conducted to explain binding property between PcNi and DNA. An intercalative binding mechanism of binding can yield in the elongation of the double helix of DNA molecule, which can incline rising in viscosity of DNA by the progressive adding of chemical compounds. Nevertheless, non-intercalation mechanism of binding activity can not yield almost rising in viscosity of DNA molecule [28-32]. The obtained results from the viscosity method for PcNi complex were plotted by the [Complex]/[CT-DNA] ratio. As illustrated in Figure 6, the relative viscosity of CT-DNA favorably with an intercalative interaction mode.
Figure 6. The impact of rising amount of \( \text{PcNi} \) complex over the viscosity of DNA (red) in the buffer solution at pH 7.02

3.4. Thermal Denaturation Studies for DNA Binding

The melting temperature studies provide important information with regard to DNA binding activities of a tiny compounds. In this procedure, the solution of a particular amount of Calf Thymus-DNA and the complex was warmed up along 25 °C to 95 °C. The CT-DNA+ \( \text{PcNi} \) had been incubated at particular time each 5 °C and the absorption titration spectra were registered. The absorbance versus temperature chart had been plotted as represented in Figure 7. Thermal melting points of the DNA was found as approximately 70.40 °C as indicated in Figure 7. \( T_m \) values of CT-DNA+complex had been determined as 77.28 °C. Generally, if melting point deference of DNA and DNA+\( \text{PcNi} \) complex is big, interaction is considered to be an intercalative binding mode. If the value is not big, DNA interaction is considered as a non-intercalative [27]. For this study, the value of \( T_m \) for CT-DNA and \( \text{PcNi} \) were observed as 70.40 °C and 77.28 °C, respectively. The findings proved that the \( \text{PcNi} \) connects to DNA using an intercalation.

Figure 7. The \( T_m \) of CT-DNA (blue line) and the \( T_m \) of CT-DNA+\( \text{PcNi} \) (green line)
3.5. The DNA Binding Studies Using Agarose Gel Electrophoresis

As well above procedures, the DNA binding activities of \textbf{PcNi} was analyzed using the gel electrophoresis technique. The migration of CT-DNA+\textbf{PcNi} had been registered after the staining as illustrated in Figure 8. M lane represents DNA ladder. Bands 1, 2 and 3 belong to \textbf{PcNi} with different amount of the DNA. The concentration of CT-DNA enhances from bands 1 to 3 and the amounts of the compound had been kept fixed at 25 µM whereas the concentration of CT-DNA was shifted between the range of 10 to 25 µM. Band intensities of DNA had been registered in the default of \textbf{PcNi} and then intensities of the DNA were studied in the presence of the compounds. As indicated in Figure 8, The band intensities of DNA were reduced and the migration of DNA bands had been slightly smeared due to the neutralization of DNA [30, 33]. These findings verified that \textbf{PcNi} link to DNA molecule.

![Figure 8. Agarose gel electrophoresis studies for \textbf{PcNi} (25 µM) in the buffer upon mounting concentration of the DNA (10-25 µM). Where M refers DNA ladder](image)

4. CONCLUSION

The synthesized tetra substituted the nickel (II) phthalocyanine was characterized with absorption titration, FT-IR and NMR. Interaction of the complex with CT-DNA were analyzed via absorption titrations, fluorescence titrations and viscosity measurement, melting temperature, and gel electrophoresis. The K_b constant was attained using UV-Vis spectroscopy from CT-DNA titrations proposed an intercalation binding mechanism for \textbf{PcNi} complex. The values produced from fluorescence titrations suggested that \textbf{PcNi} binds to DNA via intercalative mode. The result from viscosity method indicated a rising inclination in viscosity of the DNA sample upon adding of \textbf{PcNi}. The result showed that the compound interacts with DNA. All of referred techniques proved that the binding mechanism was an intercalative interaction. DNA interaction activity of \textbf{PcNi} was also investigated via the agarose gel electrophoresis for DNA. The findings showed that \textbf{PcNi} interacts with DNA molecule.

CONFLICTS OF INTEREST

No conflict of interest was declared by the authors

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