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The water soluble ball-type phthalocyanine as new potential anticancer drugs

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ABSTRACT

Compound **1** has been prepared by the reaction of 4-nitrophthalonitrile with triethanolamine. Compound **3** has been synthesized with quaternarization of compound **2** which prepared by cyclotetramerization of compound **1**. The synthesized compounds have been characterized by elemental analysis, ^{1}H NMR, UV–Vis, IR and MALDI-TOF MS spectral data. The interaction of compound **3** with calf thymus DNA was investigated spectrophotometrically. The phthalocyanine-DNA binding mechanism as well as the thermodynamic properties of binding was studied. The thermal denaturation profile, gel electrophoresis studies and the viscosity experiments were also conducted to clarify the mechanism. The replacement of ethidium bromide with phthalocyanine was monitored flourometrically to verify the binding mode. The Stern–Volmer plot has nonlinear characteristic that implies both collisions of molecules and formation of ground state complexes which causes the fluorescence quenching. The experimental results indicate that the synthesized ball type water soluble phthalocyanine binds to calf thymus DNA via mainly intercalation.

1. Introduction

The cancer fighting strategies have been mainly focused on to develop new drugs with better cure and less adverse effects. Photodynamic therapy (PDT) has such a minimal invasive treatment which is recently being used for malignant or benign diseases [1–3]. Development of new photosensitizers is an important task for PDT. There is an increasing interest for development of new phthalocyanines (Pcs) as photosensitizers due to their high efficiency of reactive oxygen species generation upon illumination, high stability, fluorescence, low intrinsic toxicity, high flexibility in structure modification and ease of synthesis [3–5].

In recent years, phthalocyanine (Pc) compounds have been intensively investigated in especially medicine or pharmaceutical industry due to the properties such as antioxidant activity [6–8] and used as photosensitizers in photodynamic cancer therapy [1,5,9]. The special conjugated electronic structure, potential clinical applications in PDT and many useful physicochemical properties of Pcs made scientist synthesize this type of potential drugs as an anticancer chemotherapeutic drug. Pcs generally have large molecular weight and they are hardly soluble in water and organic solvents. The lower solubility of Pcs causes many problems in their application in medicine [10]. It is important to synthesize water soluble Pcs from the point of view of biomedical application [3,10–12]. On the other hand, ball-type Pcs have interesting physical and chemical properties. Synthesis of ball-type Pcs and investigation of their properties such as electrical, electrochemical, gas sensing, non-linear optical and catalytic have been investigated in recent years [13–15].

DNA is a negatively charged huge molecule and it generally tends to bind cationic compounds. In cancer cells, DNA replication rate is much higher than healthy cells. The inhibition of DNA replication in a malign cell is one way of prevention of uncontrolled cell proliferation. High percentages of chemotherapeutic drugs are either directly interact with DNA or inhibit the relaxation of DNA [16]. Recently, the interactions of Pcs with DNA have long been the subject of investigation and development of potential drugs [17]. Two main groups of DNA interactive drugs bind to DNA via intercalation or groove binding mechanism [16].

To the best of our knowledge, this is the first study about the interaction of DNA with a ball type Pc. According to Uslan and Sesalan [18], positively charged Pcs are the most efficient DNA

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binders compared to neutral and negatively charged ones. The high binding tendency of positively charged Pcs made scientist to focus on synthesizing this type of Pcs. In this present work, a new cationic ball type Pc was synthesized. The interaction of this ball type Pc with calf thymus DNA (ct-DNA) was investigated with using UV–Vis spectrophotometer and spectrofluorometer. The experiments were carried out at various temperatures to clarify detailed thermodynamic behavior of Pc itself. The binding mechanism as well as the thermodynamic properties of the binding was investigated. The fluorescence quenching, thermal melting, viscosity and gel electrophoresis experiments were also conducted to elucidate the binding mechanism.

2. Experimental

2.1. Chemistry

Molecular sieves or proper methods were used for solvent driers, and all reactions were achieved under nitrogen atmosphere [19]. 4-nitrophthalonitrile was synthesized according to the literature [20]. All solutions were prepared with using distilled water. All chemicals used are of analytical grade and purchased from Merck and Sigma–Aldrich chemicals and used without further purification. ct-DNA was obtained from Sigma–Aldrich chemicals. To obtain stock ct-DNA solution, 2 mg ct-DNA was dissolved in 1 mL of distilled water and stored overnight in refrigerator. The ct-DNA solutions were used after suitable dilutions. The final concentration of ct-DNA solution was determined spectrophotometrically at 260 nm with using molar absorptivity constant of 13,200 M^{-1} cm^{-1}. IR spectrum was recorded on ATI Unicam-Mattson 1000 spectrophotometer using KBr pellets. Characterizations of compounds were recorded by Shimadzu 1601 UV–Vis spectrometer and DNA binding studies were recorded by Mapada series 6 spectrophotometer. Fluorometric measurements were conducted with Shimadzu RF 5301 fluorescence spectrophotometer. ^{1}H NMR spectrum was obtained by using a Bruker Avence2 400 MHz spectrometer. Elemental analysis was performed on LECO CHNS 932. MALDI-TOF MS analysis was performed with Bruker Daltonics Microflex LT instrument. Melting points are recorded on Electrothermal 9100 digital melting point apparatus. Viscosity measurements were recorded at 24 °C, 20 rpm with a Brookfield CAP 2000 + viscometer.

2.2. Synthesis of compounds

2.2.1. Compound 1

Anhydrous potassium carbonate (0.60 g, 4.32 mmol) was added to the mixture of 4-nitrophthalonitrile (0.50 g, 2.89 mmol) and triethanolamine (0.20 mL, 1.44 mmol) in dry DMF (15 mL) for 2 h with efficient stirring. The reaction mixture was stirred at 50 °C for 5 days. The reaction was monitored by thin layer chromatography (TLC). Then the DMF phase was concentrated in evaporator. The oily product was dissolved in $CHCl_3$ and impurities were filtered off. The filtrate was extracted with water and dried with sodium sulfate and evaporated till dryness. The product was purified by column chromatography with silicagel ($CHCl_3$/methanol (7/3)). The pale yellow solid was soluble in $CHCl_3$, THF, DMF and DMSO. Yield 140.0 mg (24%). Mp 154 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.30 (d, 2H, Ar–H); 8.17 (s, 2H, Ar–H); 7.65 (d, 2H, Ar–H); 4.78 (s, 1H, –OH, disappeared on D_2O addition); 3.84 (s, 2H, HO–CH_2); 3.62 (s, 4H, O–CH_2); 3.31 (m, 6H, N–CH_2). IR (KBr pellet) υ (cm^{-1}) 3420 (–OH); 2969–2835 (Aliphatic–CH); 2229 (–C≡N); 1569 (Ar–C=C); 1236 (Ar–O–C); 744 (Benzene). Anal. Calc. for $C_{22}H_{19}N_5O_3$: C, 65.83; H, 4.77; N, 17.45%, found: C, 65.78; H, 4.72; N, 17.38%.

2.2.2. Compound 2

Compound **1** (100.0 mg, 0.25 mmol) and anhydrous $Zn(CH_3COO)_2$ (12.8 mg, 0.06 mmol) were finely powdered and this solid mixture was heated in a heat and pressure-resistant sealed glass tube at 300 °C. After cooling, the mixture was dissolved in DMF and impurities were filtered off. MeOH was added to the filtrate and was precipitated. The precipitate was filtered, washed with MeOH and acetone and finally dried in vacuum. The dark green solid was soluble in $CHCl_3$, THF, DMF and DMSO. Yield 20.0 mg (18%). Mp: >300 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.49–7.55 (m, 24H, Ar–H); 4.84 (s, 4H, –OH, disappeared on D_2O addition); 3.92 (s, 8H, HO–CH_2); 3.84 (s, 16H, O–CH_2); 3.43 (m, 24H, N–CH_2). UV–Vis ($CHCl_3$) λ_{max}/nm (log ε, dm^3 mol^{-1} cm^{-1}) 682 (5.00), 614 (4.31), 353 (4.98). IR (KBr pellet) υ (cm^{-1}) 3415 (–OH); 2955–2830 (Aliphatic–CH); 1575 (Ar–C=C); 1476 (Ar–C=N); 1264 (Ar–O–C), 745 (Benzene). MALDI-TOF-MS (Dithranol:Formic acid) *m*/*z* Calc.: 1738. Found $[M+HCOOH+H]^+$: 1785.

2.2.3. Compound 3

Methyl iodide (1.0 mL) was added to a solution of compound **2** (100.0 mg, 0.06 mmol) in THF (2 mL) and was stirred at 40 °C for 48 h. After cooling, the organic phase was precipitated with ether and filtered off. Then the solid was washed with ether (2 × 5 mL) and dried in vacuum. The green solid was soluble in water. Yield 80.0 mg (74%). Mp: >300 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.38–7.51 (m, 24H, Ar–H); 4.80 (s, 4H, –OH, disappeared on D_2O addition); 3.88 (s, 8H, HO–CH_2); 3.76 (s, 16H, O–CH_2); 3.40 (m, 24H, N–CH_2); 1.39 (s, 12H, N–CH_3). UV–Vis ($CHCl_3$) λ_{max}/nm (log ε, dm^3 mol^{-1} cm^{-1}) 674 (5.03), 611 (4.67), 338 (5.06). IR (KBr pellet) υ (cm^{-1}) 3416 (–OH); 2957–2834 (Aliphatic–CH); 1574 (Ar–C=C); 1478 (Ar–C=N); 1264 (Ar–O–C), 745 (Benzene).

2.3. DNA binding studies with spectrophotometric titration

All spectrophotometric titrations were conducted at 25 °C. 3 mL 1.2×10^{-4} M Pc and various volumes of DNA solutions were mixed gently and the solution were diluted to a final volume of 4.0 mL with distilled water. A 3.5 mL of prepared solution was pipetted to quartz cell. The spectrophotometric titration data were collected from 300 nm to 750 nm. The thermodynamic characteristics of the system were investigated with the same procedure in temperature range of 298–313 ± 0.2 K.

2.4. Thermal denaturation studies

To differentiate the melting temperature of DNA after binding to Pc, three separate but parallel set of experiment were conducted simultaneously. In the first set, aliquots of solutions containing 3 mL of 1.5×10^{-5} M of Pc, 500 μL of ct-DNA solution (final concentration in 4 mL is 1.96×10^{-5} M) and 500 μL of distilled water was heated from 25 °C to 98 °C gradually. At every 5 °C, the solutions were incubated for 10 min then the UV–Vis spectrums were recorded. In the second set only Pc and in the third, only ct-DNA solution was subjected to the same procedure.

2.5. Viscosity experiments and gel electrophoresis

The viscosity measurements were done at 24 °C, and 20 rpm. The measurements were recorded after addition of DNA aliquots to the Pc solution.

The DNA-Pc solutions were subjected to electrophoresis on 0.8% agarose gel (containing EtBr, 5 mg/mL) prepared in TBE buffer. The samples were loaded on agarose gel with 6 × Loading Dye. 50–1000 bp DNA ladder was used as marker. The electrophoresis