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Spectroscopic study on the interaction between naphthalimide–polyamine conjugates and DNA

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ABSTRACT

The interaction of naphthalimide–polyamine conjugates with herring sperm DNA was studied by UV/vis absorption and fluorescent spectra under physiological conditions (pH = 7.4). The observed spectral quenching of compounds by DNA and the displacement of EB from DNA-EB complex by compounds indicated that these naphthalimide–polyamine conjugates could intercalate into the DNA base pairs. The UV test also showed that these compounds caused the conformational alteration of DNA. Further caloric fluorescent tests revealed that the quenching mechanism was a static type, which K_{sv} of **1**-DNA, **2**-DNA and **1**-DNA-EB, **2**-DNA-EB **3**-DNA-EB was 1.208×10^4, 7.792×10^3 and 1.712×10^4, 1.287×10^4, 2.874×10^4, respectively, at room temperature. The obtained quenching constant, binding constant and thermodynamic parameters suggested that binding strength was associated with substituted groups on naphthalene backbone, and the type of interaction force included mainly hydrogen bonding and weak van der Waals. The binding process was mainly driven by hydrogen bond and van der Waals. Additionally, the effect of NaCl on compounds-DNA interaction provided further evidence that their interaction modes were dependent on substituted groups.

1. Introduction

The study on the interaction of small molecules with DNA has been the focus of recent research in the scope of life science, chemistry and clinical medicine [1–3]. DNA as carrier of genetic information is a major target for drug interaction because of its abilities to interfere with transcription (gene expression and protein synthesis) and DNA replication, a major step in cell growth and division. A variety of small molecules usually interacts reversibly with DNA in three primary ways, including intercalation of planar or approximately planar aromatic ring systems between base-pairs [4], groove binding in which the small molecules bound on nucleic acids are located in the major or minor groove [4] and binding along the exterior of DNA helix through interactions which are generally nonspecific and are primarily electrostatic [5–8].

The 1, 8-naphthalimide derivatives are the DNA intercalating agents because they consist of a flat, generally p–π deficient aromatic system of which binds to DNA by insertion between the base pairs of the double helix [4]. They exhibit good antitumor activity because their intercalation causes the base pairs to separate vertically, thereby distorting the sugar phosphate backbone and changing the degree of rotation between successive base pairs [9–17]. Polyamines can also bind to DNA by hydrogen bond or electrostatic interactions [18,19]. Naphthalimide–polyamine conjugates have been also proved to exhibit good activity *in vitro* and bind to DNA [20, 21]. However, to date the interactions between different naphthalimide–polyamine conjugates with DNA have been reported rarely. In this work, the interaction between naphthalimide–polyamine conjugates (**1** ~ **3**, Fig. 1) and herring sperm DNA was studied by UV and fluorescence spectroscopy. The binding constants and main sorts of binding force were also investigated.

2. Materials and methods

2.1. Apparatus

UV–vis absorption spectra were measured on a Unicam UV 500 spectrophotometer using a 1.0 cm cell. Fluorescence measurements were performed with a Cary Eclipse spectrofluorimeter.

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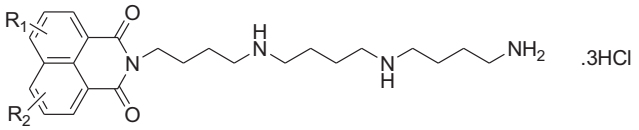

1. R_1=R_2=H
2. R_1=H, R_2=3-NH_2
3. R_1=3-NO_2, R_2=6-NO_2

Fig. 1. Structures of naphthalimide–polyamine conjugates.

2.2. Materials

Naphthalimide–polyamine conjugates **1** ∼ **3** were prepared previously [16,18]. Their solution (2.00×10^{-4} mol L^{-1}) was prepared with the Tris–HCl buffer solution and stored at 4 °C. Herring sperm DNA (Sino-American Biotechnology Company, Beijing, China) was used without further purification, and its stock solution (2.284×10^{-4} mol L^{-1}) was prepared by dissolving an appropriate amount of DNA in doubly Tris–HCl (pH = 7.4) buffer solution and also stored at 4 °C. Ethidium bromide (EB, Sigma Chem. Co., USA) stock solution (1.57×10^{-5} mol L^{-1}) was prepared by dissolving its crystals with the Tris–HCl buffer solution and stored in a cool and dark place.

2.3. Procedures

2.3.1. UV–vis measurement

2 mL solution of compounds **1** ∼ **2** (2.00×10^{-4} mol L^{-1} in Tris–HCl (pH = 7.4) was mixed with 0.0, 0.10, 0.20, 0.30, 0.60, 0.90 1.20, 1.50, 1.80, 2.10, 2.40, 2.70 and 3.0 mL of herring sperm DNA (2.284×10^{-4} mol L^{-1}) respectively. The mixture was diluted to 5 mL with Tris–HCl (pH = 7.4). Thus, two groups of samples were prepared in the concentration of DNA at 0.0, 4.56, 9.13, 13.69, 27.4, 41.08, 54.77, 68.46, 82.15, 95.84, 109.54, 123.23 and 136.92×10^{-6} mol L^{-1}. One contained only compounds **1** ∼ **2** (80×10^{-6} mol L^{-1}) as control, the others contained different concentration of DNA but had the same concentration of compounds **1** ∼ **2**. All the above solution was shaken for 30 min. at room temperature.

2.3.2. Fluorescence measurement

*2.3.2.1. Interaction of compounds **1**∼**2** with DNA.* 2 mL solution of compounds **1** ∼ **2** (2.00×10^{-4} mol L^{-1} in Tris–HCl (pH = 7.4) was mixed with 0.0, 0.10, 0.20, 0.30, 0.60, 0.90 1.20, 1.50, 1.80, 2.10, 2.40, 2.70 and 3.0 mL of herring sperm DNA (2.284×10^{-4} mol L^{-1}) respectively. The mixture was diluted to 5 mL with Tris–HCl (pH = 7.4). Thus, two groups of samples were prepared in the concentration of DNA at 0.0, 4.56, 9.13, 13.69, 27.4, 41.08, 54.77, 68.46, 82.15, 95.84, 109.54, 123.23 and 136.92×10^{-6} mol L^{-1}. One contained only compounds **1** ∼ **2** (80×10^{-6} mol L^{-1}) as control, the others contained different concentration of DNA but had the same concentration of compounds **1** ∼ **2**. All the above solution was shaken for 30 min. at room temperature. Fluorescence wavelengths and intensity areas of samples were measured at following conditions: compound **1**: EX = 345 nm, EM = 355–600 nm; compound **2**: EX = 410 nm, EM = 420–800 nm; temperature: 298, 303 and 310 K.

*2.3.2.2. Interaction of compounds **1** ∼ **3** with DNA-EB complex.* 0.3 mL solution of herring sperm DNA (2.284×10^{-5} mol L^{-1} in Tris–HCl (pH = 7.4) and 0.4 mL EB (1.57×10^{-5} mol L^{-1}) was mixed with 0.0, 0.10, 0.20, 0.30, 0.60, 0.90 1.20, 1.50, 1.80, 2.10, 2.40, 2.70 and 3.00 mL of compounds **1** ∼ **3** (2.0×10^{-4} mol L^{-1}) respectively. The mixture was also diluted to 5 mL with Tris–HCl (pH = 7.4). Thus, three groups of samples were prepared in the concentration of compounds **1** ∼ **3** at 0.0, 4.0, 8.0, 12.0, 24.0, 36.0, 48.0, 60.0, 72.0, 84.0, 96.0 and 108.0 and 120.0×10^{-6} mol L^{-1}. One contained only DNA (13.7×10^{-6} mol L^{-1}) and EB (15.7×10^{-6} mol L^{-1}) as control, the others contained different concentration of compounds **1** ∼ **3** but had the same concentration of DNA and EB. All the above solution was shaken for 30 min. at room temperature. Fluorescence wavelengths and intensity areas of samples were measured at following conditions: EX = 510 nm, EM = 520–800 nm; temperature: 298, 303 and 310 K.

2.3.2.3. Iodide quenching. 0.5 mL solution of compounds **1** ∼ **2** (2.00×10^{-4} mol/L) and 0.5 mL herring sperm DNA (22.84×10^{-4} mol/L) in Tris–HCl (pH = 7.4) were mixed with 0.0, 0.20, 0.40, 0.60, 0.80, 1.00 1.20, 1.40, 1.60, 1.80, and 2.00 mL of KI (2.0×10^{-2} mol L^{-1}) respectively. Meanwhile, 0.5 mL solution of compounds **1** ∼ **2** (2.00×10^{-4} mol/L) was only mixed with 0.0, 0.20, 0.40, 0.60, 0.80, 1.00 1.20, 1.40, 1.60, 1.80, and 2.00 mL of KI (2.0×10^{-2} mol L^{-1}) respectively. The two kinds of mixtures were diluted to 5 mL with Tris–HCl (pH = 7.4) to possess the concentration of KI at 0.0, 400, 800, 1200, 2400, 3600, 4800, 6000, 7200, 8400, 9600, 10,800, 12,000 $\times 10^{-6}$mol L^{-1}. The control groups contained only compounds **1** ∼ **2** (20×10^{-6} mol L^{-1}) and different concentration of KI, the other samples contained different concentration of KI and fixed concentrations of compounds **1** ∼ **2** (20×10^{-6} mol L^{-1}) and DNA (22.82×10^{-6} mol L^{-1}). All the above solution was shaken for 30 min. at room temperature. Fluorescence wavelengths and intensity areas of samples were measured at following conditions: compound **1**: EX = 345 nm, EM = 355–600 nm; compound **2**: EX = 410 nm, EM = 420–800 nm.

*2.3.2.4. Effect of ionic intensity on the interaction between compounds **1** ∼ **2** and DNA.* 1.0 mL solution of compounds **1** ∼ **2** (2.00×10^{-4} mol L^{-1}) and herring sperm DNA 1.0 mL (2.284×10^{-4}mol L^{-1}) in Tris–HCl (pH = 7.4) were mixed with 0.0, 0.10, 0.20, 0.30, 0.60, 0.90 1.20, 1.50, 1.80, 2.10, 2.40, 2.70 and 3.00 mL of NaCl (4.0×10^{-2} mol L^{-1}) respectively. The mixture was diluted to 5 mL with Tris–HCl (pH = 7.4), too. Thus, samples were prepared in the concentration of NaCl at 0.0, 800, 1600, 2400, 4800, 7200, 9600, 12,000, 14,400, 16,800, 19,200, 21,600 and 24,000 $\times 10^{-6}$ mol L^{-1}. One contained only compounds **1** ∼ **2** (40×10^{-6} mol L^{-1}) and DNA (45.68×10^{-6} mol L^{-1}) as control, the others contained different concentration of NaCl but had the same concentration of compounds **1** ∼ **2** and DNA. All the above solution was shaken for 30 min. at room temperature. Fluorescence wavelengths and intensity areas of samples were measured at following conditions: compound **1**: EX = 345 nm, EM = 355–600 nm; compound **2**: EX = 410 nm, EM = 420–800 nm.

3. Results and discussion

3.1. UV spectroscopic characteristics

As shown in Fig. 2, the UV spectrum of naphthalimide–polyamine conjugates (**1** ∼ **2**) in the absence and presence herring sperm DNA was measured by Ultraviolet visible range spectrophotometer except compound **3** which display weak absorption. It was observed that a continuous decrease in the absorbance of compound **1** was followed with the increasing concentration of DNA, implying compound **1** could insert into the base pairs of DNA. Hypochromism and hyperchromism are both spectral feature of DNA concerning changes in its double helix structure. The hypochromic effect of compound **1** is thought result from the interaction between the electronic states of the intercalating chromophore and those of the DNA bases [22]. It is expected that the strength of this electronic interaction would decrease as the cube of the