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Study on the interaction between the 1,4,5,8-naphthalene diimide–spermine conjugate (NDIS) and DNA using a spectroscopic approach and molecular docking†

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The interaction of herring sperm DNA with the 1,4,5,8-naphthalene diimide–spermine conjugate (NDIS) was studied by UV/vis absorption, fluorescence and CD spectroscopic methods. Compared with the 1,8-naphthalimide–spermidine conjugate (NIS), the values of K_{SV} (quenching constant) and K_b (binding constant) of NDIS were larger, and the hypochromic effect in the UV/vis spectra and the quenching effect in the fluorescence of NDIS were more significant. The interaction mode between NDIS and DNA was mainly groove binding. The fluorescence experiments at varying temperatures showed that the binding process of NDIS and DNA was static, as both hydrogen bonds and hydrophobic forces played a major role in the binding of NDIS and DNA. The CD spectrum indicated that NDIS caused a conformational change, like the B to A-DNA transition, and the tests using KI and NaCl and ^1H NMR spectroscopy indicated that NDIS was not a classical DNA inserter. All the results demonstrated that both the polyamine side chain and the aromatic rings affect the process of NDIS binding to DNA, which is thus obviously different from that of NIS. The conclusion was confirmed by the *in silico* molecular docking experiments.

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1. Introduction

DNA is a major target for drug discovery including antitumor drugs, and it has become more and more important in chemistry, life sciences and medicine.^{1–3} The main reason for this is that drugs can affect the transcription and replication of DNA, and they are expected to further produce more therapeutic effects, be less toxic and have less side effects, especially for cancer treatment. It is universally known that the interacting factors of the direct binding of most small molecules to DNA are very complex. There are four basic binding modes of the interaction of small drug molecules with DNA, which are the following: (1) intercalation, in which the planar or approximately planar aromatic ring systems insert into the base-pairs of DNA;⁴ (2) groove binding, in which small molecules are located in the major or minor groove of DNA⁴ and (3) nonspecific or (4) electrostatic binding, in which small

molecules are located outside of the DNA spiral.^{5–8} The modes and mechanisms of binding of small molecules to DNA have been extensively studied for they usually result in the design and synthesis of new, efficient and DNA-targeting drugs.^{9–13}

It has been already proven that 1,8-naphthalimide and its derivatives can insert into DNA base pairs, because of their own aromatic planar structure, which is the anthraquinone backbone.^{4,14–17} Their intercalation usually causes the base pairs to separate vertically, the sugar phosphate backbone to twist and the rotational degree between successive base pairs to change, thus producing antitumor activity.^{18–26} A polyamine is able to interact with DNA by hydrogen bonding or electrostatic interactions.^{27,28} We also found that naphthalimide–polyamine conjugates displayed good antitumor activities, which are bound to DNA by intercalation and groove binding and further induce the conformational change from the B- to A-DNA type.^{29–32} Besides, there are substituent and side chain effects on the binding process of naphthalimide–polyamine conjugates to DNA.^{31,32} More importantly, a 1,4,5,8-naphthalene diimide–spermine conjugate (NDIS), named 2,7-bis{3-[4-(3-aminopropylamino)butylamino]propyl}benzo[*lmn*]-[3,8]-phenanthroline-1,3,6,8-(2*H*,7*H*)-tetra-one hexahydrochloride, displayed good activities against

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tumor cells and caused cell apoptosis through the mitochondrial pathway mediated by reactive oxygen species (ROS).³³ According to our previous research, the interactive manners between NDIS and DNA may differ markedly from those of NIS because of their different chemical structures (Fig. 1), which has been rarely investigated up to now. Therefore, the interaction between NDIS and DNA was studied by spectroscopy and molecular docking, which was also compared with that of NIS in this paper.³²

2. Materials and methods

2.1. Apparatus

The measurement of the UV-vis absorption spectrum was performed on a Unicam UV 500 spectrophotometer. The fluorescence spectrum was measured using a Cary Eclipse spectrofluorimeter. The measurement of the circular dichroism spectrum was conducted on a Modle 420 SF (USA) automatic recording spectrophotometer. ¹H-NMR spectroscopy was conducted using a Bruker AV-400 model spectrometer (400 MHz).

2.2. Materials

The 1,4,5,8-naphthalene diimide-spermine conjugate (NDIS) was prepared according to ref. 24. Its chemical structure was confirmed by ¹H NMR. Its solution (2.00×10^{-5} mol L⁻¹) was prepared in a Tris-HCl buffer solution (pH = 7.4) for UV and (fluorescence) or a phosphate buffer saline (PBS, pH = 7.4) buffer solution for CD. Herring sperm DNA was bought from the Sino-American Biotechnology Company (Beijing, China), which was directly used without further purification. Its stock solution (2.284×10^{-4} mol L⁻¹ or 11.42×10^{-4} mol L⁻¹) was prepared by dissolving an appropriate amount of DNA in a double Tris-HCl (pH = 7.4) buffer solution (UV and fluorescence) while another stock solution (9.128×10^{-4} mol L⁻¹) was prepared with a PBS (pH = 7.4) buffer solution (CD). An ethidium bromide (EB, Sigma Chem. Co., USA) stock solution (1.57×10^{-5} mol L⁻¹) was prepared by dissolving its crystals in the Tris-HCl buffer solution. The solutions above mentioned were kept at 4 °C.

2.3. Procedures

2.3.1. UV-vis measurement. (1) The tested concentration range of DNA in the mixtures was from 0 to 136.92 μM, and the concentration of NDIS was 80 μM. The experiments were conducted after all the above solutions were shaken for 30 min at room temperature. The measured wavelength range was 200–600 nm on a UV-vis spectrophotometer.

(2) The NDIS–DNA solution was prepared by the following procedures: 0.5 mL solution of NDIS (200 μM) was added to 0.0, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00 and 4.50 mL solution of herring sperm DNA (1142 μM), respectively, and then the mixture was diluted to 5 mL with Tris-HCl (pH = 7.4). Thus, the concentration of DNA was 0.0, 114.2, 228.4, 342.6, 456.8, 571.0, 685.2, 799.4, 913.6 and 1027.8 (μM), respectively, and that of NDIS was 20 μM. The samples were shaken for 30 min at room temperature before measurement.

2.3.2. Fluorescence measurement

2.3.2.1. Interaction of NDIS with DNA. (1) The solutions were prepared as mentioned above in 2.3.1 (1). Fluorescence measurement was at wavelengths of 370–710 nm and the excitation wavelength was 360 nm. The temperatures were 298 K, 303 K and 310 K.

(2) The solutions were prepared as mentioned above in 2.3.1 (2), except the conditions of fluorescence measurement, in which the excitation wavelength and scanning range of emission were the following: EX = 360 nm, EM = 370–710 nm.

2.3.2.2. Interaction of NDIS with the DNA–EB complex. The competitive fluorescence experiment between NDIS and EB with DNA was done under the following conditions: (1) the excitation wavelength is 510 nm. (2) The scanning range of emission is from 520 to 810 nm. (3) The tested temperatures are at 298, 303 and 310 K. Before the fluorescence test was performed, all the above solutions were shaken for 30 min at room temperature. The samples include EB (15.7 μM), DNA (15.7 μM) and NDIS (0–120 μM).

2.3.2.3. Iodide quenching. The iodide quenching test was carried out in the absence or presence of DNA in mixtures of NDIS and KI. Therefore, there are two groups of samples, in which one group has NDIS (20 μM), and KI (0–120 mM) with DNA (22.82 μM) and another group has less DNA than the

Fig. 1 1,4,5,8-Naphthalene diimide-spermine conjugate (NDIS) and NIS. Reprinted from *J. Photochem. Photobiol. B*, 2015, **158**, 1–15.

former. After the above solutions were shaken for 30 min at room temperature, the fluorescence measurement was done under the same conditions as those in 2.3.2.1.

2.3.2.4. Effect of ionic strength on the interaction between NDIS and DNA. The measurement of the ionic strength effect on the binding process of the interaction of NDIS with DNA was conducted with the mixtures containing NDIS (40 μM), NaCl (0–0.024 M) and DNA (45.68 μM). All the above solutions were shaken for at least 30 min at room temperature before the test was performed. EX and EM wavelengths were the same as those in 2.3.2.3.

2.3.3. CD measurement. CD measurement was carried out after NDIS (0–48 μM) was added to DNA (182.56 μM) around 30 minutes and then the test results were reported.

2.3.4. $^1\text{H-NMR}$ spectroscopy. The interaction of NDIS with DNA was determined and the $^1\text{H-NMR}$ spectral data were obtained at 400 MHz using a Bruker Ascend model.

2.3.4.1. Interaction of NDIS with DNA. After DNA (10 mg) and NDIS (3 mg) were placed in the NMR tube and completely dissolved in D_2O , the solution was examined by $^1\text{H-NMR}$ spectroscopy. The chemical shift was described in

parts per million (ppm) and then its $^1\text{H-NMR}$ spectrum was compared with those of NDIS and DNA, respectively.

2.3.4.2. Interaction of NDIS with DNA–EB. NDIS (3 mg), EB (3 mg) and DNA (10 mg) were placed into the NMR tube and then also fully dissolved in D_2O . The $^1\text{H-NMR}$ spectrum was also recorded. The results were obtained and analyzed in line with 2.3.4.2.

2.3.5. Molecular docking. The crystal structure of the herring sperm DNA duplex (5'-GC|GTAC|GC-3') was available according to a reported paper³⁴ (PDB code: 1AL9). With the help of SYBYL 2.0 software (Tripos, Inc., St. Louis, MO 63144), charges of molecules and essential hydrogen atoms of DNA and NDIS were added.^{35,36} *In silico* molecular docking experiments were carried out and the results were obtained using the SYBYL 2.0 platform.

3. Results and discussion

3.1. UV spectroscopic characteristics

The UV spectrum of NDIS interacting with DNA was examined by using an ultraviolet visible range spectrophotometer

Fig. 2 UV absorption spectra of NDIS binding to herring sperm DNA. (A) The DNA concentrations were as follows: 0.0, 4.57, 9.14, 13.70, 27.4, 41.10, 54.80, 68.52, 82.20, 95.93, 109.63, 123.34 and 137.04 μM , respectively. The concentration of the compound NDIS used was 80 μM . The blue line represents herring sperm DNA, in which its concentration was 22.84 μM and had no NDIS. (B) Numbers 1–10 indicated the DNA concentration, which were 0.0, 114.2, 228.4, 342.6, 456.8, 571.0, 685.2, 799.4, 913.6 and 1027.8 μM , respectively. The amount of NDIS used was 20 μM . (C) Plot of $A_0/(A - A_0)$ versus $1/C_{DNA}$ of NDIS interacting with herring sperm DNA from the UV-vis spectra.

(Fig. 2A). As seen from Fig. 2A, there are two maximum absorbance peaks at 362 nm and 383 nm, respectively, while the 1,8-naphthalimide-spermidine conjugate (NIS) only displays one absorbance peak at 344 nm. After increasing concentrations of DNA (0–137.04 μM) were added, the absorption peaks of NDIS continuously decreased and showed a slight red shift. The hypochromism and bathochromic shift are observed from Fig. 2A, which indicated that NDIS could bind to DNA by insertion, and hypochromism and red shift are the features of this binding mode. The empty π^* -orbital of naphthalimide interacted with the π^* orbital of the DNA base pairs, forming a π - π^* conjugate because of their electronic overlap, which resulted in the reduction of the π - π^* transition energy and the π - π^* transition probability. Thus, the red shift and hypochromism occurred.^{37–41} More importantly, the hypochromic rate of NDIS (60.66%) is higher than that of NIS (39.98%), indicating that there was the common effect of polyamines and rings in the process of NDIS binding to DNA compared with that of NIS.

H. A. Tajmir-Riahi *et al.* once reported that polyamines were able to bind to DNA by groove binding.^{42,43} Naphthalimide-polyamine conjugates also have the capability of groove binding to DNA.³² Therefore, in order to study whether NDIS could also bind to DNA through groove binding or not, the molar ratios of DNA to NDIS (c_{DNA}/c_1) were enhanced based on Fig. 2A. The results showed that the phenomena of hypochromism and red shift completely disappeared and the hyperchromic effect arose when the molar ratios of DNA to NDIS were more than 5.5 (Fig. 2B), implying that NDIS could also bind to DNA by groove binding.

These data revealed that NDIS could bind to DNA through the groove and intercalation, and two interactive modes did not coexist, which could be relative to the molar ratios of NDIS to DNA. In a word, the binding mode was mainly intercalation when the concentration of NDIS was far more than that of DNA or it was mainly groove binding when the former was less than the latter.

We also calculated the apparent binding constant (K) of NDIS binding to DNA from the UV spectra using eqn (1), which is as follows:

$$\frac{A_0}{A - A_0} = \frac{\varepsilon_{\text{G}}}{\varepsilon_{\text{H-G}} - \varepsilon_{\text{G}}} + \frac{\varepsilon_{\text{G}}}{\varepsilon_{\text{H-G}} - \varepsilon_{\text{G}}} \frac{1}{K_{[\text{DNA}]}} \quad (1)$$

A and A_0 are the absorption intensity in the presence and absence of DNA, respectively; $\varepsilon_{\text{H-G}}$ and ε_{G} represent the molar absorption coefficient of the NDIS-DNA complex and NDIS, respectively. The values of the apparent binding constant K could be obtained from the intercept and slope by plotting $A_0/(A - A_0)$ against c_{DNA} , (Fig. 2C). The value of K was $1.547 \times 10^3 \text{ L mol}^{-1}$, which is far smaller than that of spermidine and herring sperm DNA ($1.47 \times 10^5 \text{ L mol}^{-1}$). The probable reason is that polyamines could induce aggregation and precipitation of DNA and further weaken its ability to bind to DNA.^{31,42,43}

3.2. Fluorescence spectroscopy

3.2.1. Fluorescence quenching. Similar to UV spectroscopy, the fluorescence spectrum of NDIS binding to DNA was also investigated. As seen in Fig. 3A, NDIS had two peaks, which are 400 and 415 nm, respectively. After different concentrations of DNA (0–137.04 μM) were added, fluorescence quenching quickly occurred, in which the maximum fluorescence quenching rate of NDIS was 91.03%. That of NIS, however, was merely 68.53%.³² This result suggested that DNA could be one target of NDIS and there was a difference between the binding process of NDIS and NIS to DNA because NDIS seems to be a structural dimer of NIS.³²

In order to determine the DNA binding mode of NDIS, ethidium bromide (EB) was used as a fluorescent probe because EB is a known typical DNA inserter and there was a strong peak around 605 nm after EB was bound to DNA (Fig. 4).⁴⁴ Upon addition of NDIS (0–120 μM), the fluorescence quenching happened and its maximum rate was 87.83% (Fig. 4), which was more than that of NIS (77.25%).³² This showed that NDIS could insert into the DNA base pairs because it has the stronger ability to intercalate into DNA than EB. The displacement of EB from EB-DNA resulted in the fluorescence quenching of the DNA-EB complex.^{45,46} This also indicated that the ability of NDIS to bind to DNA was more than that of NIS, and there was a possibility of the formation of the NDIS-DNA complex.^{42,43,47–49} However, NDIS may also bind to DNA by groove binding, which could push EB out from the DNA-EB system.^{50,51} The reason was that the polyamine motif of NDIS could bind to DNA by groove binding and this causes a B- to A-type conformational change.^{42,43}

Based on Fig. 3A, we elevated the molar ratios between DNA and NDIS similar to the UV tests (Fig. 3B). The results showed that the fluorescence quenching vanished and the fluorescence enhancement appeared when their molar ratios were more than 5.5, illustrating that NDIS might bind to DNA by another mode besides intercalation.

3.2.2. Fluorescence quenching mechanism. It is well-known that the mechanisms of fluorescence quenching have two types, namely the dynamic and static quenching processes. The former results from the diffusion effects of the quenching agent and the fluorophore over the lifespan of an excited state, but the latter is the result of ground state complex formation. Therefore, the coefficient of diffusion and quenching constants of bimolecules in dynamic quenching would be larger with increasing temperature. The static one would be less stable and quenching constants would be decreased under the condition of higher temperature because it reduces the generation of the fluorescent complex.⁵² To clarify the interaction mechanism of NDIS binding to DNA or NDIS displacing EB from the EB-DNA complex, the fluorescence quenching experiments were performed at three different temperatures, which are 298, 303 and 310 K, respectively. The type of fluorescence quenching could be analyzed by using the Stern-Volmer equation, which is as follows:^{53–55}

$$F_0/F = 1 + K_{SV}c \quad (2)$$

In eqn (2), F_0 is the maximum fluorescence of the DNA-EB complex or NDIS while F is that of NDIS for DNA-EB or DNA for NDIS, and $[c]$ is the concentration of NDIS or DNA. K_{SV} and K_q represent the quenching constant and the quenching rate constant of biological molecules, respectively, which are used to assess the profile of fluorescence quenching. Finally, τ_0 denotes the average molecular lifespan under the absence of any quencher. It is reported that τ_0 is 10^{-8} s or so.⁵⁶ We had tried to obtain the K_{SV} by plotting F_0/F versus $[c]$ (ESI† Fig. S1 and 2 and Tables S1 and S2), but the linearity is poor. On the basis of the 'sphere of action static quenching model', we analyzed the type of fluorescence quenching by the modified Stern-Volmer equation, which is:

$$[1 - (F/F_0)]/c = (1 - W)c/K_{SV}(F/F_0). \quad (3)$$

In eqn (3), ' W ' is a certain fraction of the excited state while F_0 , F , $[c]$ and K_{SV} have identical meanings to those in eqn (2).^{57,58} The results obtained from the plots of $[1 - (F/F_0)]/c$ versus F/F_0 at three different temperatures are shown in Fig. 5 and 6 and are listed in Tables 1 and 2, respectively. As shown in Tables 1 and 2, the values of K_q were far larger than 2.0×10^{10} , implying the binding process of DNA and NDIS or NDIS and the DNA-EB complex was a static one because 2.0×10^{10} (K_q) is the maximum limitation of dynamic quenching.⁵⁹ However, the values of K_{SV} did not decrease at higher temperature, implying that there is a dynamic quenching process between NDIS and DNA. It is reported that the values of K_{SV} did not necessarily decrease with increasing temperature in the static quenching process.⁶⁰⁻⁶³ So this binding process is static as a whole while it was also accompanied by a dynamic one. In other words, the direct interaction of NDIS with DNA was not a typical static

Fig. 3 Fluorescence spectroscopy of NDIS directly binding to herring sperm DNA. (A) The DNA concentrations were the same as those of Fig. 2A. Scan conditions: EX = 360 nm, EM = 370–710 nm; slits of both EX and EM were 5 nm and 10 nm, respectively. (B) The DNA concentrations were the same as those of Fig. 2B. The scan conditions were the same as those in Fig. 2A.

Fig. 4 Fluorescence spectroscopy of NDIS displacement EB from herring sperm DNA-EB. The concentration of NDIS was 0.0, 4, 8, 12, 24, 36, 48, 60, 72, 84, 96, 108 and 120 μ M, respectively while those of DNA and EB were 13.7 and 15.7 μ M, respectively. Scan conditions: EX = 510 nm, EM = 520–800 nm; slits of both EM and EX were 5 nm.

Fig. 5 The modified Stern-Volmer plot of fluorescence quenching of NDIS directly binding to herring sperm DNA from 298 K to 303 and 310 K.

Fig. 6 Stern-Volmer plot of fluorescence quenching of herring sperm DNA-EB interacting with NDIS from 298 K to 303 and 310 K.

quenching process because there was also a dynamic quenching to some extent in their binding course.

3.2.3. Interaction mode between NDIS and DNA. The process of NDIS binding to DNA or the displacement of EB from the EB-DNA complex was mainly a static one. The binding constant (K_b) could be obtained using eqn (4):^{64,65}

$$\log[1/c] = \log[F/(F_0 - F)] + \log K_b. \quad (4)$$

In eqn (4), F and $[c]$ have identical meanings to those in eqn (2) and K_b has already been described. The values of K_b were obtained from the intercept (K_b) of the $\log [1/c]$ vs. $\log [F/(F_0 - F)]$ plots (Fig. 7 and 8), which are listed in Tables 3 and 4. It was observed that the changed trend of K_b from 298–310 K was not different from that of the K_{SV} , indicating that their binding was average and their complex might exist although this binding process is reversible.⁶⁶ Simultaneously, the values of K_b were also more than those of NIS like the K_{SV} , suggesting that there was the common effect of polyamines and rings in the course of NDIS binding to DNA compared with that of NIS.³² Besides, there was also the severe curvature of $\log [1/c_{DNA}]$ versus $\log [F/(F_0 - F)]$ and $\log [1/c_I]$ versus $\log [F/(F_0 - F)]$ plots of Fig. 7 and 8 (as shown also in Tables 3 and 4), resulting from the interactive mode of NDIS, which was not the same as that of NIS. However, the binding constant (K_b) of DNA-NDIS in Table 3 was less than that of DNA-EB (10^5).^{50,51} This might be the result of NDIS-induced DNA conformational alteration from B to A-type and the NDIS-DNA interaction by groove binding, which weakened the

ability of NDIS to intercalate into DNA.^{42,43} The present results further proved that there was a possibility of NDIS binding to DNA by both intercalation and groove binding.

In the interactions of small organic molecules and biological macromolecules, there are four main types of interactive forces: hydrogen bonding, hydrophobic forces, electrostatic interactions and van der Waals forces, respectively. Usually, entropy change (ΔS°) and enthalpy change (ΔH°) are basically constant if the temperature studied is in the limited range. The thermodynamic parameters of NDIS binding to DNA, therefore, could be calculated according to the van't Hoff equation, which is as follows:

$$\ln(K_2/K_1) = (1/T_1 - 1/T_2)\Delta H^\circ/R \quad (5)$$

$$\Delta G^\circ = -RT \ln K = \Delta H^\circ - T\Delta S^\circ \quad (6)$$

In eqn (5) and (6) above, R and K are the gas constant and binding constant at different temperatures, respectively. Their values, including the entropy change (ΔS°) and enthalpy change (ΔH°), are presented in Tables 3 and 4.

Ross *et al.* gave a description of the positive or negative of the thermodynamic parameter related with different interactive forces during binding of all organic molecules to biological macromolecules, which is as follows:⁶⁷ (1) when the ΔH and ΔS values are bigger than 0, the interaction belongs to a typical hydrophobic interaction; (2) when the ΔH and ΔS values are smaller than 0, the interaction is associated with the van der Waals force and hydrogen bonding; (3) if the ΔS value is bigger than 0 but the ΔH value is smaller than 0, it is characteristic of electrostatic interactions. Interactions with a positive value of ΔS and a negative ΔH value, however, are also regarded as hydrophobic interactions and hydrogen bonding.⁶⁸ From Tables 3 and 4, there are negative ΔH° and positive ΔS° values, implying that the hydrogen bond and hydrophobic forces played an important part in the course of the interaction of DNA with NDIS.⁶⁸

3.2.4. Iodide quenching studies. We could also investigate the binding mode of NDIS and DNA by the iodide quenching effect, because DNA has a negatively charged phosphate backbone, which could repel a highly negatively charged quencher, thus protecting itself from being inserted by the quencher. The groove binding agent, however, could not avoid being quenched.⁶⁹ The values of K_{SV} , including that of NDIS by I^- and the NDIS-DNA complex by I^- , therefore, were obtained from the Stern-Volmer equation, as shown in Fig. 9 and Table 5. From Table 5, NDIS seemed to intercalate into DNA for the quenching effect was weakened after NDIS was bound to DNA. From

Table 1 Quenching constants of NDIS binding to herring sperm DNA

Compound	T (K)	K_{SV} (L mol ⁻¹)	K_q (L mol ⁻¹)	R
NDIS	298	5.316×10^3	5.316×10^{11}	0.9805
	303	5.635×10^3	5.635×10^{11}	0.9792
	310	4.628×10^3	4.628×10^{11}	0.9448

Table 2 Quenching constants of displacement of EB by NDIS from the herring sperm DNA–EB complex

Compound	<i>T</i> (K)	<i>K</i> _{sv} (L mol ^{−1})	<i>K</i> _q (L mol ^{−1})	<i>R</i>
NDIS	298	1.177 × 10 ⁵	1.177 × 10 ¹³	0.9639
	303	1.2813 × 10 ⁵	1.281 × 10 ¹³	0.9694
	310	1.449 × 10 ⁵	1.449 × 10 ¹³	0.9798

Fig. 9, however, the F_0/F in the presence of DNA was more than that in the absence of DNA, suggesting that NDIS was able to bind to DNA through groove binding. Moreover, this interaction was also different from that of NIS, because it was a consequence of the common effect of polyamine chains and rings compared with NIS.³²

3.2.5. Effect of ionic strength on the NDIS and DNA interaction. As mentioned above, DNA is an anionic polyelectrolyte having phosphate groups, so we can analyze the interactive modes of NDIS and DNA through monitoring the spectral change of NDIS. As is known, NaCl is a strong polar substance serving as a controlling agent for the solution ionic strength. In Fig. 10, the fluorescence intensity of small molecules was unchanged after Na⁺ was added. The increasing fluorescence was generally associated with the electrostatic interaction between DNA and small molecules because Na⁺ could compete with the small molecules for the phosphate groups of DNA and further weaken their electrostatic interaction.⁷⁰ The observed effect of NaCl on the interaction of NDIS and DNA indicated that there is no electrostatic interaction between NDIS and DNA.

3.3. Circular dichroism spectroscopy

It is reported that polyamines (putrescine, spermidine, and spermine) have the ability to induce DNA conformational alterations from B-type to A- or Z-type transitions in special sequences, and their analogues also have similar functions, causing DNA to aggregate and precipitate.^{42,43} Circular di-

chroism (CD) is a very important tool to analyze DNA conformational change after it binds to small molecules.^{71–74} So we investigated the binding interaction of DNA and NDIS through CD spectroscopy.

Fig. 11 shows the CD spectrum of NDIS interacting with DNA, in which the free DNA showed the B conformation feature for it has four major peaks of 210 (negative), 221 (positive), 245 (negative), and 280 nm (positive).^{46,75–78} After NDIS is bound to DNA, there was an obvious change of the negative peak about 245 nm resulting from the helical B conformation and an insignificant alteration of the positive peak around 280 nm because of base stacking. This may be due to the presence of two polyamine chains of NDIS in contrast to NIS, and thus the groove binding mode could be predominant.^{32,79} The groove binding will potentially induce circular dichroism and the intercalation mode shows weak induced circular dichroism in the process of NDIS binding to DNA. Besides, NDIS also resulted in an obvious change of two positive peaks, approximately 210 and 220 nm, for there was also base stacking. As shown Fig. 11, there is a decreasing intensity of the negative band of NDIS and a blue shift from 248 to 242 nm after the increasing concentrations of NDIS were added, indicating that the B to A-type conformational alteration of DNA occurred.^{22,46,80,81} In addition, a new peak above 300 nm did not emerge, suggesting that NDIS is a non-classical DNA intercalation agent.^{82,83} These results showed that there was a probability of NDIS binding to DNA

Fig. 7 Plot of $\log [1/c_{\text{DNA}}]$ versus $\log [F/(F_0 - F)]$ of the NDIS binding to herring sperm DNA from 298 K to 303 and 310 K.

Fig. 8 Plot of $\log [1/c_{\text{NDIS}}]$ versus $\log [F/(F_0 - F)]$ of the NDIS replacement EB from herring sperm DNA–EB from 298 K to 303 and 310 K.

Table 3 Binding constants and thermodynamic parameters of NDIS binding to herring sperm DNA

Compound	<i>T</i> (K)	<i>K_b</i> (L mol ⁻¹)	ΔG° (L mol ⁻¹)	ΔH° (kJ mol ⁻¹)	ΔS° (kJ mol ⁻¹)	<i>R</i>
NDIS	298	1.381×10^4	-23.619	12.911	0.1226	0.9126
	303	1.255×10^4	-23.744	12.911	0.1226	0.8982
	310	1.697×10^4	-25.101	12.911	0.1226	0.8872

Table 4 Binding constants and thermodynamic parameters of displacement of EB by NDIS in the herring sperm DNA-EB complex

Compound	<i>T</i> (K)	<i>K_b</i> (L mol ⁻¹)	ΔG° (L mol ⁻¹)	ΔH° (kJ mol ⁻¹)	ΔS° (kJ mol ⁻¹)	<i>R</i>
NDIS	298	1.233×10^5	-29.044	-1.417	0.0927	0.959
	303	1.249×10^5	-29.653	-1.417	0.0927	0.960
	310	1.206×10^5	-30.155	-1.417	0.0927	0.959

Table 5 Dynamic quenching constants of NDIS or the NDIS-DNA system by KI

Compound	<i>K_{sv}</i> in the absence of DNA (L mol ⁻¹)	<i>K_{sv}</i> in the presence of DNA (L mol ⁻¹)
NDIS	27.000	18.100

through the groove and intercalation like NIS.^{82,83} The groove binding to DNA of NDIS seemed to be more obvious than intercalation.

3.4. ¹H-NMR

3.4.1. Interaction of NDIS with DNA. The binding modes of NDIS and DNA are usually determined through the changes in the chemical shifts and line widths in the ¹H NMR spectra.^{44,84} According to the literature: (1) intercalating binding manifests high field shifts of the A-T and the G-C peaks accompanied by some broadening of peaks; (2) groove binding mode has downfield shifts of A-T and the G-C

Fig. 9 Fluorescence quenching plots of NDIS or the NDIS-DNA complex by KI. *c*(NDIS) = 20 μM; *c*(DNA) = 22.84 μM; the KI concentration was 8–120 mM.

Fig. 10 Effects of ionic strength (NaCl) on the fluorescence intensity of the NDIS-DNA complex.

Fig. 11 Circular dichroism spectra of the free DNA (182.56 μM) and its NDIS-DNA system at 16, 32 and 48 μM.

Fig. 12 ^1H -NMR of DNA, NDIS, NDIS-DNA, EB, DNA-EB and the NDIS-DNA-EB complex. A: DNA; B: NDIS; C: NDIS-DNA (3 mg:10 mg); D: EB; E: DNA-EB (10 mg:3 mg); F: NDIS-DNA-EB (3 mg:10 mg:3 mg).

peaks; and (3) electrostatic interactions show that there is no spectral alteration in the down field region of ^1H NMR.⁸⁴

However, three binding modes could be confirmed by ^1H NMR as follows: (1) total line broadening is characteristic of the intercalation; (2) groove binding presents no line broadening and up-field shift in ^1H NMR; and (3) the line broadening and high field shift belonged partially to intercalation into DNA.⁴⁴

The ^1H NMR of NDIS directly interacting with DNA is shown in Fig. 12A–C. From Fig. 12C, the appearance of several new peaks implied the formation of the NDIS–DNA complex. As is shown in Fig. 12A–C, compared with the ^1H NMR spectra of NDIS (Fig. 12B), after DNA (10 mg) were added, the chemical shifts of NDIS (B) were changed from δ 8.61, 4.15, 2.96–3.12, 1.92–2.07 and 1.68–1.70 to δ 8.56, 4.14, 2.98–3.15, 1.92–2.13 and 1.64–1.72, respectively (Fig. 12C), indicating that chemical shifts were up-field. Besides, in contrast to the ^1H NMR spectra of DNA (Fig. 12A), after the addition of NDIS (3 mg), the ^1H NMR signal of A–T and G–C at δ 8.57, 8.29, 8.24, 7.96, 7.36–7.61, 6.12 and 5.50 shifted to δ 8.60, 8.34, 8.28, 7.99, 7.29–7.36, 6.16 and 5.51 (Fig. 12C), respectively, suggesting that they were small down-field shifts. These results illustrated that NDIS could interact with DNA through groove binding.

3.4.2. Interaction of NDIS with DNA–EB. As is known, EB, as a representative of DNA intercalation agents, is used to determine if a small molecule has the ability to insert into DNA. If NDIS could interact with DNA by intercalation and its binding ability was stronger than that of EB, it can replace EB in the EB–DNA complex and, thus, the ^1H NMR signal of EB in the DNA–EB complex has an obvious en-

hancement.^{45,46} Therefore, we studied the ^1H NMR of the herring sperm DNA–EB complex interacting with NDIS.

As is shown in Fig. 12D–F, in comparing the ^1H NMR spectra of DNA–EB (Fig. 12E) with that of the NDIS, DNA and EB mixture (Fig. 12F), after the addition of NDIS (3 mg), the chemical shifts of EB in the EB–DNA complex (Fig. 12E) changed from δ 7.64–7.72, 7.44–7.46, 7.35–7.38, 6.98, and 1.35 (Fig. 12E) to δ 7.63–7.73, 7.44–7.48, 7.23–7.39, 6.91 and 1.37, respectively (Fig. 12F), implying that there is no change in the chemical shifts. However, there is a significant enhancement in the signal of EB in the ^1H -NMR spectra (Fig. 12F) after the addition of NDIS (3 mg). Specifically, in comparing the ^1H NMR spectra of DNA–EB (Fig. 12E) with that of the NDIS, DNA and EB mixture (Fig. 12F), the peak of $-\text{CH}_2$ at 4.57 obviously increased after the addition of 3 mg of NDIS. These results showed that the NDIS could also interact with DNA by insertion.

It is thus speculated that the planar rigid structure of the aromatic rings intercalated into the DNA base pairs and the polyamine chain was bound to the groove at the same time after NDIS was bound to DNA.^{85–87}

3.5. Molecular docking

To further determine the binding mode between NDIS and DNA, we performed molecular docking calculations using SYBYL 2.0 (Fig. 13). The results suggested that there are two binding modes in the process of NDIS interacting with DNA, which were groove and insertion, respectively. Specifically speaking, the planar rigid structure of the aromatic rings interacted with the G–C base pairs of DNA by π – π stacking

Fig. 13 The molecular docking *in silico* of NDIS interacting with DNA. NDIS interacts with DNA by groove binding and insertion. (A) The DNA is expressed by the tube which is colored by the following atom type: (a) C, grey; (b) polar H, blue; (c) Cl, green; (d) N, dark blue; (e) O, red; (f) P, orange. (B) The surface structure of NDIS interacting with DNA.

and thus NDIS intercalated into the DNA base pairs. In two polyamine chains, one polyamine chain was bound to the major groove and the other one interacted with the minor groove through hydrophobic interactions. At the same time, it also bound to the backbone phosphate group of the DNA duplex by hydrogen bonds. The binding energy (ΔG°) calculated from molecular docking was $-13.25 \text{ kJ mol}^{-1}$, which is higher than that of NIS. This was in accordance with K_b of UV-vis absorption and fluorescence experimental results compared with that of NIS. NIS, however, only interacted with DNA by the minor groove and intercalation because it has one polyamine chain.³² Furthermore, the binding energy ΔG° of NDIS was higher than that of NIS.³² At length, the amino groups interacted with the following functional groups at different parts through hydrogen bonds: thymine- O_2 , cytidine- O_2 , N_3 of adenine, and the backbone phosphate group. Besides, there were hydrophobic interactions between DNA and the aminoalkyl chain. Molecular docking supported the experimental results mentioned above.

4. Conclusions

In this paper, we studied the interaction of NDIS and DNA through multiple spectroscopy and molecular docking techniques. We also compared it with that of NIS. On the basis of the observed results, we could reach the following conclusions: (1) there was the common effect of polyamine chains and rings on the interaction between NDIS and DNA. NDIS was very different from NIS in the process of its binding to DNA; (2) the K_{sv} and K_b values of NDIS were higher than those of NIS. The hypochromic effect and fluorescence quenching of NDIS were more obvious than that of NIS; (3) NDIS interacted with DNA by intercalation and groove binding, and the groove binding was the main binding mode; (4) NDIS could cause the B- to A-type conformational alteration but it caused unobvious change of the positive peak around 280 nm compared to that of NIS; (5) the fluorescence quenching in the interaction of NDIS and DNA was a static one; (6) the main interactive forces between NDIS and DNA were hydrophobic forces and H-bonding; and (7) importantly, the molecular docking experiment confirmed that NDIS could interact with DNA through intercalation and groove binding, including the major groove and the minor one, which was different from the binding modes of NIS.

Author contributions

Zhi-Yong Tian, Yu-Xia Wang, Xue-Jun Xu and Chao-Jie Wang designed the research; all authors performed the experimental work; Zhi-Yong Tian, Yu-Xia Wang, Xue-Jun Xu and Chao-Jie wrote the manuscript; all authors discussed, edited and approved the final version.

Conflicts of interest

The authors declare that there is no conflict of interest in this work.

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