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Studies on interaction of an intramolecular charge transfer fluorescence probe: 4′-Dimethylamino-2,5-dihydroxychalcone with DNA

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Abstract—The interaction of a new intramolecular charge transfer probe, namely 4′-dimethylamino-2,5-dihydroxychalcone (DMADHC), with calf thymus DNA has been studied. Compared to the spectral characteristics of the free form in aqueous solution, the fluorescence of DMADHC enhanced dramatically accompanying a blueshift of the emission maxima in the presence of DNA. The absorption and fluorescence spectra, salt concentration effect, KI quenching, fluorescence polarization, and DNA denaturation experiments were given. These results give evidence that the DMADHC molecule is inserted into the base-stacking domain of the DNA double helix. The intrinsic binding constant and the binding site number were estimated. The analytical characteristics were also given.

1. Introduction

In recent years, the application of small molecules for DNA studies has been of great interest of some researchers. The binding of small molecules to DNA has been of great interest due to the importance in understanding the drug–DNA interactions and the consequent design of new efficient drugs targeted to DNA.[1–4] Small molecules interact with DNA primarily through three modes: electrostatic binding, groove binding, and intercalative binding.[5,6] Electrostatic binding is the interaction between cationic species and the negatively charged DNA phosphate backbone that occurs along the external DNA double helix and does not possess selectivity.[5] In groove binding, interactions with the two grooves of the DNA double helix generally involve direct hydrogen bonding or van der Waals interactions with the nucleic acid bases in the deep major groove or the wide shallow minor groove of the DNA helix. Stacking interactions between nucleic acid bases and aromatic ligands are important in defining the intercalative binding, which is defined when a planar, heteroaromatic moiety slides between the DNA base pairs and binds perpendicular to the helix. Therefore, it is apparent that intercalative binding and groove binding are related to the grooves in the DNA double helix but electrostatic binding can take place out of the groove. Understanding the modes of the binding of small molecules to DNA and the factors that can affect the binding is of fundamental importance in understanding DNA binding models in general. Among the three modes, the most effective mode of the drugs targeted to DNA is intercalative binding.[7] Thus, research on the interactive model can provide a start for the design of the structure of new and efficient drug molecules.

Fluorescence probes often have large changes in their spectral parameters such as quantum yield, lifetime, anisotropy, and spectral characteristics after binding to biological macromolecules. Fluorescent probes have been widely used as DNA probes, by which many methods have been developed for the determination of trace amount of DNA. Different probes react with the DNA in different ways. Among these methods, the fluorescence intensity of some probes, such as ethidium bromide,[8] 4′,6-diamidino-2-phenylindole (DAPI),[9] Hoechst 33258,[10] etc., is enhanced by DNA, and the fluorescence of some other probes, such as the photochemical fluorescence probes 9,10-anthraquinone-2-sulfonate[11] and Vitamin K3,[12] etc., is quenched by DNA. Recently, the fluorescence properties of some intramolecular charge

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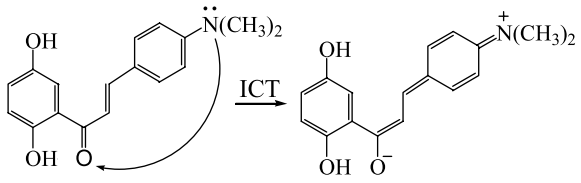

Figure 1. The molecular structure and intramolecular charge transfer process of compound 4′-dimethylamino-2,5-dihydroxychalcone (DMADHC).

transfer compounds involving both electron donor and acceptor moieties have been studied by many researchers.[13–15] The microenvironments of the binding sites of these intramolecular charge transfer probes are expected to be complex in nature and should influence their fluorescence properties. This class of compounds has been extensively used as fluorescence probes for the sensing of metal ions or membranes. A series of substituted flavones, the 4′-(dimethylamino)flavones, show strongly intramolecular charge transfer behaviors and reasonable fluorescence quantum yield.[16,17] Therefore, they have been widely used in revealing the nature of their interaction with different substances and other biological targets (e.g., DNA) at the molecular level. Jin et al. investigated the interaction of an intramolecular charge transfer (ICT) probe, 4′-(dimethylamino)-3-hydroxy-flavone (DMAHF), with calf thymus DNA.[18] This study focused on the mechanism of ICT fluorescence in aqueous solution in the presence of DNA, but the analytical method cannot meet the requirements of determination of the trace amounts DNA because of its low sensitivity (linear range 1.36×10^{-5}–1.28×10^{-3} mol/L, detection limit 2.28×10^{-6} mol/L). Yang et al. showed that 4′-(dimethylamino)-4-amino-chalcone (DMAC) can intercalate into the DNA base pairs and established a new method to determine trace amounts of DNA.[19] However, this method was established at pH 4.29 (not at physiological acidity) because DMAC was supposed to have a protonated form at acidic conditions which interacts with the negatively charged DNA phosphate backbone and leads to an increase in fluorescence intensity. It is obvious that there is still a need for an improved analytical method for DNA determination at physiological acidity conditions.

In this article, we report a detailed study of the interaction of an intramolecular charge transfer probe, namely, 4′-dimethylamino-2,5-dihydroxychalcone (DMADHC), (Fig. 1) with DNA by using steady-state absorption and fluorescence spectrum in aqueous solutions. The essence of the interactive model was discussed. The performance of the analytical system involving the interaction of DMADHC with DNA and the effect of various experimental parameters were investigated.

2. Results and discussion

2.1. Spectral characteristics of DMADHC in various solutions

The normalized fluorescence spectra of DMADHC in solvents of different polarity are shown in Figure 2. As

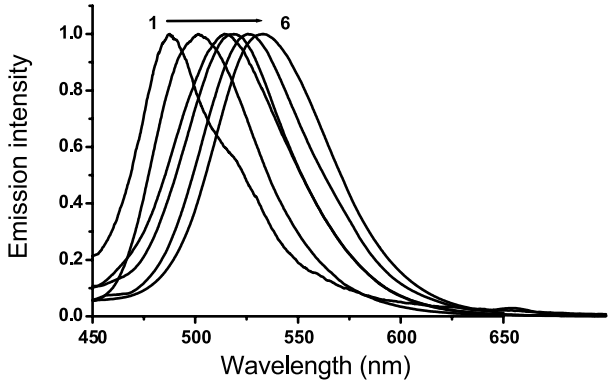

Figure 2. Normalized fluorescence spectra of DMADHC in various solvents: (1) carbon tetrachloride, (2) diethyl ether, (3) tetrahydrofuran, (4) acetone, (5) DMF, and (6) DMSO. The arrow direction indicates increasing solvent polarity.

can be seen, the fluorescence spectrum suffers a strongly bathochromic shift as the solvent polarity is increased. This redshift in the emission maximum, from 487 nm in carbon tetrachloride to 533 nm in dimethyl sulfoxide, observably indicates that intensively photoinduced intramolecular charge transfer (ICT) takes place within the molecule in the singlet excited state. This suggests that the molecule is solvated significantly in the S_1 excited state, resulting in a large difference in dipole moment between the S_1 excited state and the ground state. Analysis of this solvatochromic behavior allows us to estimate the nature of the absorbing and the emitting species. In this study, the widely used Lippert–Mataga equation was employed.[20]

$$\Delta\nu = \frac{2\Delta\mu^2}{4\pi\varepsilon_0 hc\rho^3}\cdot\Delta f(\varepsilon,n) + \text{Constant} \qquad (1)$$

$$\Delta f(\varepsilon,n) = \frac{\varepsilon-1}{2\varepsilon+1} - \frac{n^2-1}{(2n^2+1)} \qquad (2)$$

In this relation, $\Delta\nu$ represents the Stokes shift, $\Delta\mu = (\mu_e - \mu_g)$ the magnitude of the change in the dipole moment from the ground state to the excited state, ε the static dielectric constant, n the refractive index of the medium, h the Plank constant, c the velocity of light in vacuum, ε_0 the permittivity of vacuum, and ρ the Onsager cavity radius, respectively. Figure 3 shows a plot of the Stokes shift versus the orientation polarizability $\Delta f(\varepsilon,n)$. The phenomenon that Stokes shifts increase with solvent polarity reveals an increase in the excited state dipole moment and confirms the ICT character of the excited state, implying potential application of this molecule to probe the local microenvironment.

2.2. Spectral characteristics of DMADHC binding to DNA

The spectral characteristics associated with the binding of DMADHC to DNA are shown in Figures 4 and 5. Binding to DNA causes a progressive shift in absorption spectrum of DMADHC (refer to Fig. 4), and its absorption maximum shifts from 408 to 416 nm. A clear

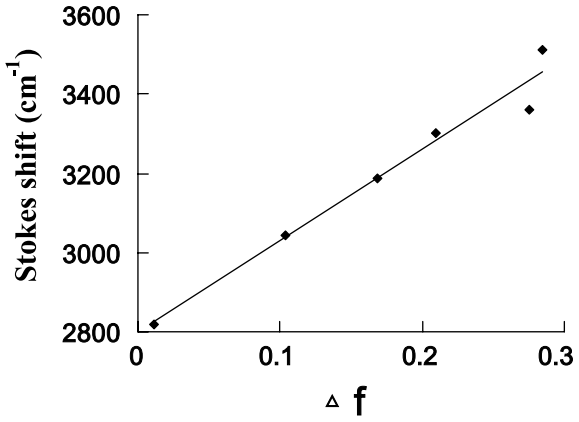

Figure 3. Stokes shifts of DMADHC in various solvents as a function of the solvent orientation polarizability (Δf). (1) Carbon tetrachloride, (2) diethyl ether, (3) tetrahydrofuran, (4) acetone, (5) DMF, and (6) DMSO.

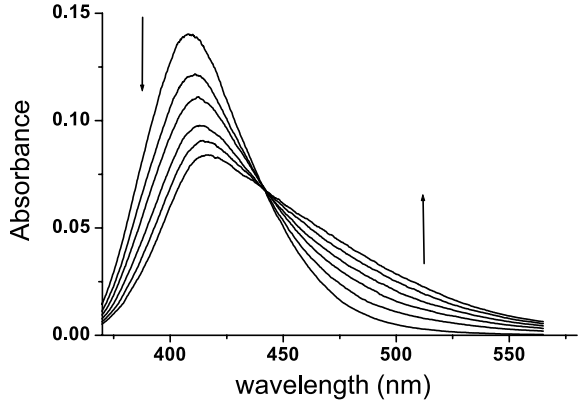

Figure 4. Absorption spectra of 2×10^{-5} mol/L DMADHC with increasing concentrations of DNA at pH 7.4. DNA concentration in base pairs: (mol/L) (1) 0, (2) 8.51×10^{-7}, (3) 2.36×10^{-6}, (4) 4.91×10^{-6}, (5) 8.03×10^{-6}, and (6) 1.20×10^{-5}. The arrow direction indicates increasing concentrations of DNA.

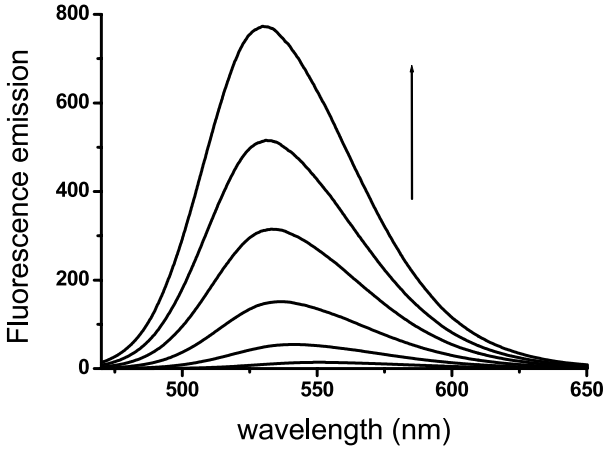

Figure 5. Fluorescence spectra of 1×10^{-5} mol/L DMADHC in different concentrations of DNA at pH 7.4. DNA concentration in base pairs: (mol/L) (1) 0, (2) 8.51×10^{-7}, (3) 2.36×10^{-6}, (4) 4.91×10^{-6}, (5) 8.03×10^{-6}, and (6) 1.20×10^{-5}. The arrow direction indicates increasing concentrations of DNA.

isosbestic point was located at about 440 nm, indicating the existence of two forms of DMADHC, that is, free and bound ones. Each form of DMADHC has a unique absorption. The pronounced hypochromism indicates a strong intercalation of the DMADHC into DNA base pairs.[19]

Figure 5 shows the ICT fluorescence emission of DMADHC in the presence of different amounts of DNA. Addition of low DNA concentration caused noticeable increase of DMADHC fluorescent intensity. With increasing amounts of DNA, the maximum emission wavelength shifts from 552 to 541, 536, 533, 530 or 528 nm, respectively. From the enhancement effect, it can be concluded that the DMADHC molecules have been included into the stacking region of the host DNA where little rotation of the probe occurs during the excited state. The blueshift of the emission maximum can be rationalized of DMADHC binding to a less polar site in DNA. It may also provide information about the size and the flexibility of the binding region of DNA. The fluorescence intensity ratio (F/F_0) for DNA presence and absence systems is in proportion to the concentration of DNA and according to this a new method for determination of DNA has also been established.

2.3. Effect of pH

Figure 6 shows the variation of the fluorescence intensity ratio (F/F_0) with pH of solution. Surprisingly, the fluorescence intensity ratio of DMADHC solution changes only slightly when pH value varies from 6.0 to 9.0, but decreases out of this range. It can be inferred that the pH of solution has a scarce effect on the binding of DMADHC molecule with DNA, except for strong acidic and alkaline environments in which DNA molecules may have been destroyed partly. So pH 7.4 was chosen for the subsequent experiments because it is the physiological acidity.

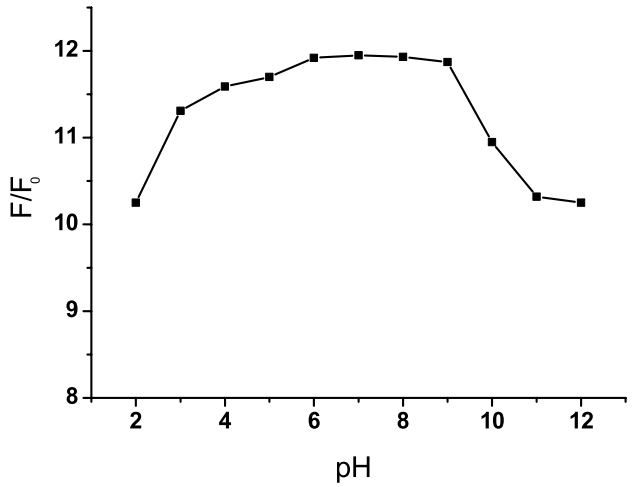

Figure 6. The effect of pH on the fluorescence intensity ratio (F/F_0) of DMADHC binding to DNA. [DNA] = 2.36×10^{-6} mol/L.

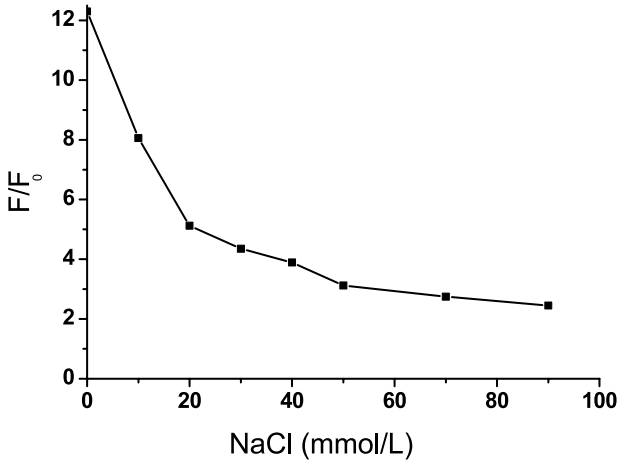

Figure 7. The effect of NaCl on the fluorescence intensity ratio (F/F_0) of DMADHC binding to DNA. [DNA] = 2.36×10^{-6} mol/L.

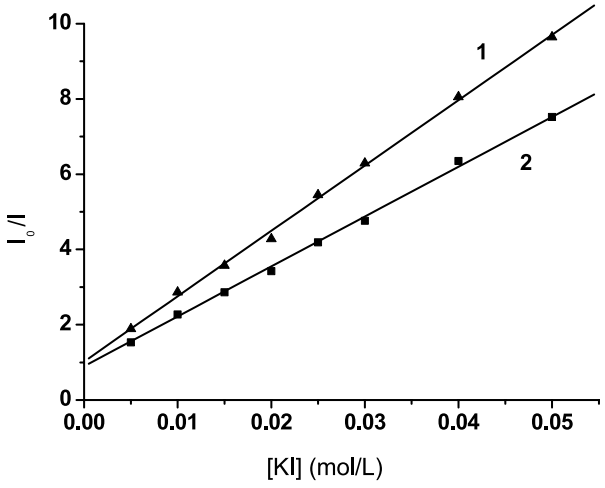

Figure 8. Fluorescence quenching of DMADHC in the absence (line 1) and presence of DNA (line 2). [DNA] = 2.36×10^{-6} mol/L.

2.4. Effect of NaCl concentration

The interaction of DMADHC with DNA is sensitive to the concentration of NaCl. The effect of NaCl on the fluorescence intensity ratio (F/F_0) is shown in Figure 7. With increasing concentration of NaCl, the fluorescence intensity decreases distinctly. From this phenomenon it may be interpreted that the electrostatic repelling interaction among the negative charges of the DNA phosphate backbone decreases when NaCl exist in the system. Therefore, the DNA double helix would hold the small molecules more tightly so that they do not easily intercalate to the binding sites in base pairs of DNA.[21]

2.5. KI quenching experiments

To deduce the interaction pattern of DMADHC with DNA, fluorescence quenching experiments were performed. Stern–Volmer K_{SV} was used to evaluate the fluorescence quenching efficiency. The fluorescence quenching experiment was performed with KI and the experimental data were plotted according to the Stern–Volmer equation

$$F_0/F = 1 + K_{SV}[Q], \tag{3}$$

where F_0 and F are the fluorescence intensities in the absence and presence of potassium iodide, $[Q]$ is the concentration of KI, K_{SV} is the Stern–Volmer quenching constant. The results are shown in Figure 8.

The interaction pattern of the fluorescence probe with DNA can be deduced from the variation of K_{SV}. When small molecules intercalate into DNA base pairs, the double helix of DNA would protect the bound molecules from the anionic quencher, leading to a decrease in quenching. The magnitude of K_{SV} of the small molecules that are bound should therefore be lower than that of the small molecules that are free.[22] In contrast, if the small molecule binds to DNA in the groove, the magnitude of K_{SV} of the bound small molecule should be higher than that of the free small molecule.[23] As shown in Figure 8, binding to DNA resulted in decreased quenching of the fluorescence intensity of DMADHC. K_{SV} values for the free DMADHC and the bound DMADHC with DNA were K_{SV} = 173.7 L/mol and K_{SV} = 132.5 L/mol, respectively. These results indicated that the quenching of DMADHC fluorescence by KI was decreased in the presence of DNA. Therefore, the interaction pattern of DMADHC molecule with DNA is intercalation mode.

2.6. Fluorescence polarization studies

Fluorescence polarization experiments provide an effective parameter for investigating dynamic characteristics of DMADHC in different microenvironments. Small molecules weakly polarize due to the rapid tumbling motion in aqueous media. However, when intercalating to the helix of DNA, its rotational motion should be restricted and therefore the fluorescence polarization should be increased. On the contrary, binding to the phosphate backbone or to the DNA grooves does not result in enhanced fluorescence polarization.[24] Fluorescence polarization can be obtained from the equation below,

$$P = (I_{VV} - GI_{VH})/(I_{VV} + GI_{VH}) \tag{4}$$

Where P is the fluorescence polarization, I_{VV} and I_{VH} are the fluorescence intensities measured through vertically and horizontally oriented polarizers in the excitation beam and emission beam, respectively. G represents the corrected instrumental factor and is equal to I_{HV}/I_{HH}. The results are shown in Figure 9. The addition of DNA leads to an increase in the fluorescence polarization of DMADHC. It illustrates that DMADHC molecule can intercalate into the DNA base pairs, being another proof of the binding mode between DMADHC and DNA.

2.7. Influence of denaturation of DNA

Denatured DNA was produced by heating a native DNA solution in a water bath at 100 °C for 10 min and cooling in ice-water bath immediately. Double

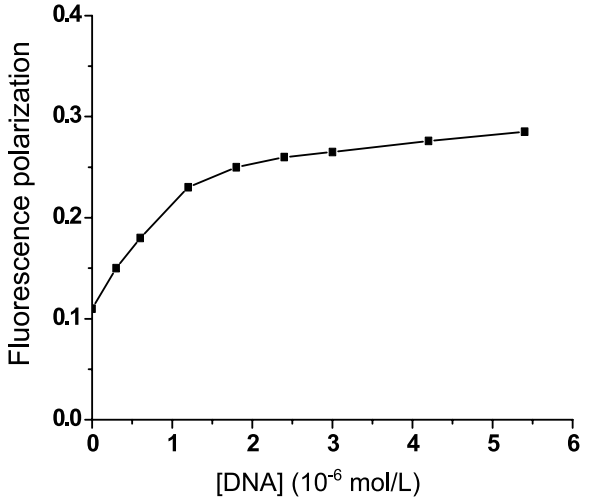

Figure 9. Influence of DNA concentrations on fluorescence polarization.

Table 1. The relative fluorescence intensity of DMADHC in different amounts of native and denatured DNA

Concentration of DNA (mol/L)	Relative fluorescence intensity	
	Native DNA	Denatured DNA
8.51×10^{-7}	54.9	29.6
2.36×10^{-6}	151.1	91.7
8.03×10^{-6}	515.4	346.2

stranded DNA split into two single stranded DNA with the opening of its double helix. The characteristic of DMADHC binding to different amounts of native and denatured DNA are given in Table 1. As can be seen from the table, the enhancement of the fluorescence of DMADHC by denatured DNA is smaller than that of the native DNA, which also supports the intercalation of DMADHC into the helix of DNA.

2.8. Binding constant and binding site number

For the interaction of small molecules with macromolecules, the Scatchard plot is commonly used to characterize the binding properties in terms of measuring the binding site number and binding constant. The data for Scatchard analysis are based on the measurements of absorbance or fluorescence of interacting system,[25]

$$r/c = K(n - r), \tag{5}$$

where r is the molar ratio of bound DMADHC to DNA per base pair, n the binding site number per base pair, K the intrinsic binding constant, and c the concentration of free DMADHC. From the recorded fluorescence titration data, the binding constant and binding site number of DMADHC with DNA can be calculated. The binding constant was 4.6×10^6 L/mol in base pairs and the binding site number was 0.17. This large binding constant indicated that the DMADHC has a strong affinity for DNA base pairs. The binding site number implies only a binding site per six base pairs, giving evidence for an intercalation of DMADHC into DNA according to the neighbor-exclusion model, since groove binding and electrostatic binding usually results in significantly higher binding site number.

2.9. The analytical characteristics

Preliminary studies show that the system has promising analytical prospects in determining trace DNA. The linear calibration graph for determination of DNA was constructed from results obtained under the optimal condition. The fluorescence system reported here was used to determine DNA ranging from 6.98×10^{-7} to 2.41×10^{-5} mol/L with a detection limit (3σ) of 1.80×10^{-7} mol/L. The correlation coefficient is 0.994. This method exhibits good reproducibility, with a relative standard deviation of 2.07% obtained from eight separate determinations for 4.9×10^{-6} mol/L DNA.

3. Conclusions

The fluorescence probe of DMADHC, with intramolecular charge transfer characteristics, is sensitive to the microenvironment. It is found that the new ICT-based fluorescent probe DMADHC could intercalate into the DNA base pairs, inducing a significant enhancement of DMADHC fluorescent intensity. Based on this, a means of sensitive determination of trace DNA is established. On the other hand, because the intercalative model is one of the most important interactive models of drugs and acceptors, DMADHC might be potentially useful in pharmaceutical industries, and DNA damage intercalated by DMADHC could have applications in technical and therapeutic fields. Furthermore, if damage to DNA can be precisely controlled, to the extent that cleavage of the backbone can be limited to a single site, then this opens the door for design of sequence-specific DNA (artificial restriction nucleases).

4. Experimental

4.1. Reagents

The investigated probe DMADHC was synthesized and purified as described in the literature.[26] Purity was conformed by ^{1}HNMR and IR spectroscopy. Calf thymus DNA (ctDNA, Beijing, Beitai Biochemical Co., Chinese Academy of Science, Beijing, China) was commercially purchased and used without further purification. The stock solutions were prepared by dissolving the solid DNA in 10 mmol/L NaCl solution and stored at 4 °C. The concentration was determined according to the absorbance at 260 nm after establishing that the absorbance ratio A_{260}/A_{280} was in the range 1.80–1.90, and the molarities of double stranded DNA solution were calculated based on $\varepsilon_{DNA} = 1.31 \times 10^4$ L mol^{-1} cm^{-1} in base pairs.[27] All other reagents were of analytical-reagent grade and were used without further purification. Double distilled water was used throughout the experiment. All experiments were carried out at pH 7.4 ± 0.1 in a Britton–Robinson buffer containing NaCl (10 mmol/L) and 1×10^{-5} mol/L of DMADHC except

where specifically indicated. All test solutions were incubated at 25 °C for 10 min.

4.2. Apparatus

Steady-state absorption and fluorescence spectra were recorded on TU-1901 UV–vis spectrophotometer (PGENERAL) and F-4500 spectrofluorometer (HITACHI). A 150-W xenon lamp was used as an excitation light source and the excitation wavelength of 403 nm was used. Excitation and emission bandwidths were both set at 5 nm. All pH values were measured with a pHs-2 acidometer (The Second Instrument Factory of Shanghai, China). All experiments were carried out at 20 ± 1°C.

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