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Molecular Spectroscopy Evidence of Berberine Binding to DNA: Comparative Binding and Thermodynamic Profile of Intercalation

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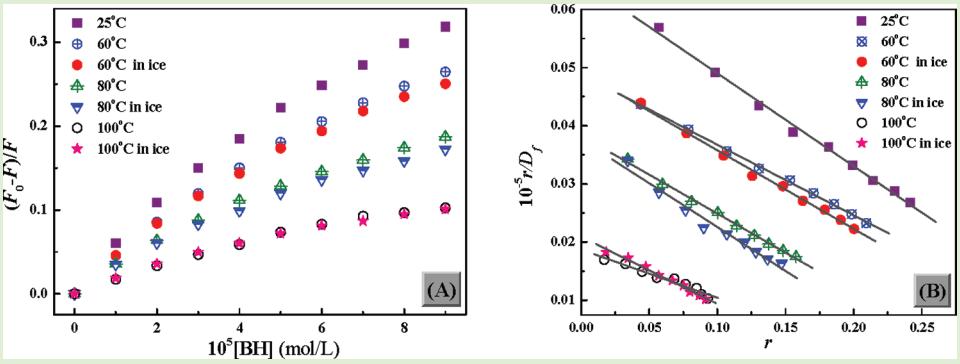
Supporting Information



ABSTRACT: Berberine (BH) is an important traditional medicinal herb endowed with diverse pharmacological and biological activities. In this work, the binding characteristics and molecular mechanism of the interaction between the BH and herring sperm DNA were explored by UV−vis absorbance and fluorescence spectroscopy. In the mechanism discussion, fluorescence quenching, absorption spectra, competition experiment, and iodide quenching experiment studies hinted at an intercalative mode of binding for BH to DNA. Fluorescence studies revealed the binding constant (K) of BH−DNA was ~10^4 L·mol^{-1}. The effects of temperature, chemical denaturants, thermal denaturation, and pH were studied to show the factors of the interaction and provided further support for the intercalative binding mode. The results of thermodynamic parameters ΔG, ΔH, and ΔS at different temperatures indicated that the hydrogen bonds and van der Waals interactions played major roles in the reaction, and the effect of ionic strength indicated that electrostatic attraction between the BH and DNA was also a component of the interaction.

1. INTRODUCTION

Deoxyribonucleic acid (DNA) that contains the genetic instructions for the development and functioning of living organisms is an obvious focus of attraction for many researchers.[1−3] DNA is the target molecule that exists in many drugs, especially antitumor therapies. Consequently, a hot subject of research surrounding DNA has been the recognition and characterization of the interaction of small molecules with DNA as they yield effective information for the development of therapeutic agents in controlling gene expression.[4−6] Therefore, it has formed the nucleus of many faceted research activities for years. The study of the interaction of DNA with small molecules, especially drug molecules, is an important subject in many research fields such as medicinal chemistry, life science, clinical medicine, and so on.[7−9] It is well known that many small molecules can readily interact with DNA double-helix structures through three different modes: ionic, groove, and intercalation binding.[10] However, many actived small molecules can directly interact with DNA, and the factors for these interactions are quite complex, so discussing the reaction modes and kinetic mechanism between the different categories of molecules and DNA is very significant in view of their ability to function as a rational design system for the development of new efficient drugs targeted to DNA, to serve as sensitive molecular reporters for monitoring nucleic acid structure, and so forth.[11−14]

Berberine (Figure 1), an alkaloid isolated from Chinese herbs, is an important traditional medicinal herb. It has been

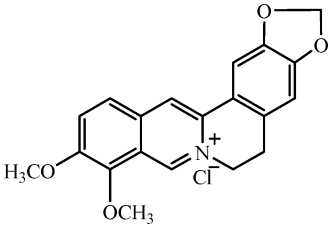


Figure 1. Molecular structure of berberine.

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found to be endowed with diverse pharmacological and biological activities such as analgesic, antibacterial, antitubercular, antitumor activities, and so on.[15−21] Because of these broad uses of berberine mentioned above, more and more researchers have begun to investigate its interaction with biomacromolecules. The interactions between small molecules and DNA studies help us to design new and effective drugs against several diseases. Therefore, the quest for new efficient probes for the fluorometric detection of DNA is on, and there is increasing interest in studying the interactions of drug and dye molecules with the various biological targets. In recent years, many researchers have tried to characterize the binding mechanism of BH to DNA, but it is a pity that the final results showed obvious discrepancy.[22−24] Hence, detailed investigations of the interaction of BH with DNA are of huge significance.

In this Article, the binding mechanisms of berberine to DNA was studied by UV−vis absorbance and fluorescence spectroscopy. High sensitivity of the fluorescence spectroscopy makes fluorescence probes valuable tools in chemistry, materials science, biology, and medicine to look into the properties of environment they are associated with.[25−27] Upon association with biomacromolecules, such as DNA, the emission intensity of the probe increases, and they are useful markers in genomics, as the binding with the host molecule enhances fluorescence emission. In the present study, we demonstrated the intercalative binding mode between BH and DNA; this finding may help to design new efficient probes for the fluorometric detection of DNA, thus replacing many toxic and carcinogenic probes.

2. EXPERIMENTAL SECTION

2.1. Materials. Herring sperm DNA were obtained from Sigma−Aldrich (St. Louis, MO); berberine was obtained from Northeast General Pharmaceutical Factory (Liaoning, China); the buffer Tris and Na_2HPO_4−NaH_2PO_4 (PB) had a purity of no less than 99.5%, and NaCl, HCl, and so on were all of analytical purity; DNA solution was dissolved in buffer solution, and freshly prepared DNA solution was used for the experiments. The purity of DNA was verified by monitoring the ratio of absorbance at 260/280 nm (A260/A280). The concentration of DNA stock solution was determined according to the absorbance at 260 nm by using the extinction coefficients of 6600 $mol^{-1}·cm^{-1}$.[28]

2.2. Methods. The UV−vis spectrum was recorded at room temperature on a U-3010 spectrophotometer (Hitachi, Japan) equipped with 1.0 cm quartz cells. All fluorescence spectra were recorded on a F-4500 spectrofluorimeter (Hitachi, Japan) equipped with 1.0 cm quartz cells and a thermostat bath. The widths of both the excitation slit and the emission slit were set to 10.0 nm. Appropriate blanks corresponding to the buffer were subtracted to correct the fluorescence background.

3. RESULTS AND DISCUSSION

3.1. Absorption Spectra. In general, the hyperchromism and hypochromism were regarded as spectral features for DNA double-helix structural change when DNA reacted with other molecules. The hyperchromism originates from the breakage of the DNA duplex secondary structure; the hypochromism originates from the stabilization of the DNA duplex by either the intercalation binding mode or the electrostatic effect of small molecules.[29] In this work, with a fixed concentration of DNA, UV−vis absorption spectra were recorded with increasing amount of BH. The changes in the UV−vis spectra are shown in Figure 2. It was found that the maximum

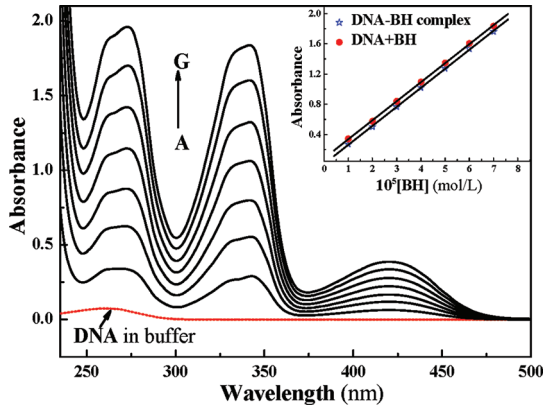

Figure 2. UV absorption spectra of DNA with various concentrations of BH. Inset: Comparison of absorption at 260 nm between the DNA−BH complex and the sum values of circular DNA and BH. c(DNA) = 1.0 × 10^{-5} mol·L^{-1}; c(BH)/(10^{-5} mol·L^{-1}), A−G: from 1.0 to 7.0 at increments of 1.0.

absorption of free DNA was at 260 nm, and the absorption increased after the addition of different concentrations of BH. The inset in Figure 2 shows that the absorption value of simply adding free DNA and free BH was a little greater than the absorption value of DNA−BH complex. This meant that a weak hypochromic effect existed between DNA and BH, and the hypochromism between DNA and different concentrations of BH was 21, 13, 9, 8, 5, 5, and 4%, respectively. For a typical intercalation, the caused red shift was >15 nm, and the hypochromism was >35%.[4] Our results indicated that an intercalation binding actually existed between DNA and BH. This conclusion was further supported from the fluorescence quenching studies, which gave convincing evidence of the intercalation of berberine.

3.2. Fluorescence Characteristics of the BH−DNA System. Because the endogenous fluorescence of DNA is very weak, we used BH as a fluorescence probe to study the interaction between DNA and BH. With a fixed concentration of BH, fluorescence spectra were recorded with increasing amount of DNA (Figure 3). In the absence of DNA, the

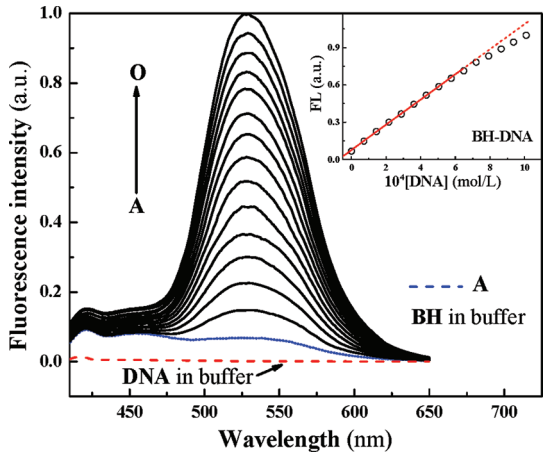

Figure 3. Fluorescence spectrum of BH with various concentrations of DNA. Inset: Relative fluorescence intensity for BH as a function of the DNA for different concentrations. c(BH) = 4.0 × 10^{-5} mol·L^{-1}; c(DNA)/(10^{-4} mol·L^{-1}), A−O: from 0.0 to 10.08 at increments of 0.72.

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original weak spectrum of the BH was characterized by the peak at ~530 nm. With the addition of DNA, the intensity of 530 nm peak fluorescence increased gradually. The experiment results indicated that BH can increase the fluorescence of the system after binding to DNA. The inset in Figure 3 shows that within the investigated concentrations range the fluorescence-increasing sensitivity of BH was proportional to the concentration of DNA.

When we stabilized the concentration of DNA (2.0×10^{-4} mol·L^{-1}) and increased the concentration of BH gradually, we found that the effect of BH on DNA fluorescence intensity (shown in Figure 4) was similar to the result in Figure 3. It was

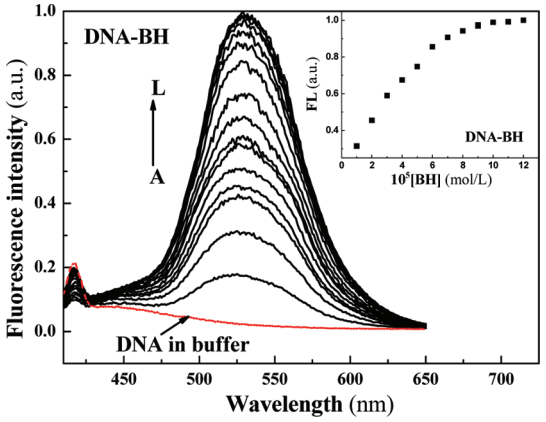

Figure 4. Fluorescence spectrum of DNA with various concentrations of BH. Inset: Relative fluorescence intensity for DNA as a function of the BH for different concentrations. c(DNA) = 2.0×10^{-4} mol·L^{-1}; c(BH)/(10^{-5} mol·L^{-1}), A–L: from 1.0 to 12.0 at increments of 1.0.

observed from Figure 4 that DNA had nearly no fluorescence at ~530 nm, but the fluorescence signal increased by increasing the concentration of BH. The inset in Figure 4 shows that when BH concentration was higher than 8.0×10^{-5} mol·L^{-1}, the change of fluorescence intensity tended to be a constant.

3.3.1. Determination of the Binding Mechanism of BH and DNA. *Iodide Quenching Studies.* To obtain an insight into the mode of binding of the dyes with DNA, the fluorescence quenching in the DNA environment was studied using potassium iodide (KI) as a quencher, and the correlation between the degree of accessibility of each the molecule to the quencher and its steric bulk was examined. A highly negatively charged quencher was expected to be repelled by the negatively charged phosphate backbone of DNA. Accordingly, an intercalative bound small molecules should be protected from being quenched by anionic quencher, whereas the free aqueous complexes and groove binding drugs should be quenched readily by anionic quenchers.[30,31] Therefore, the negatively charged I^- was selected to determine the binding mode of BH to DNA. The fluorescence quenching data were analyzed to obtain the quenching constant by using the well-known Stern–Volmer equation

$$\frac{F_0}{F} = 1 + K_{SV}[Q] \quad (1)$$

where F_0 and F denote the steady-state fluorescence intensities in the absence and in the presence of quencher (BH), respectively, K_{SV} is the Stern–Volmer quenching constant, and $[Q]$ is the concentration of the quencher. Hence, eq 1 was applied to determine K_{SV} by linear regression of a plot of F_0/F against $[Q]$. The values of quenching constants (K_{SV}) of BH by I^- ion in the absence and presence of DNA were calculated to be 26.8 and 18.7 mol·L^{-1}, respectively (Supporting Information, Figure S1). It was apparent that iodide quenching effect was decreased when BH was bound to DNA. These results provided direct evidence that BH molecules have intercalated into the base pairs of DNA.

3.3.2. Competitive Binding between Fluorescent Probe and BH for DNA. Neutral red (NR) is a planar phenazine dye; in recent years, it has been demonstrated that it can bind to DNA by an intercalative mode,[32] and thus it was employed as a spectral probe to investigate the binding mode of BH with DNA in this work. Figure 5 shows the emission spectra of the

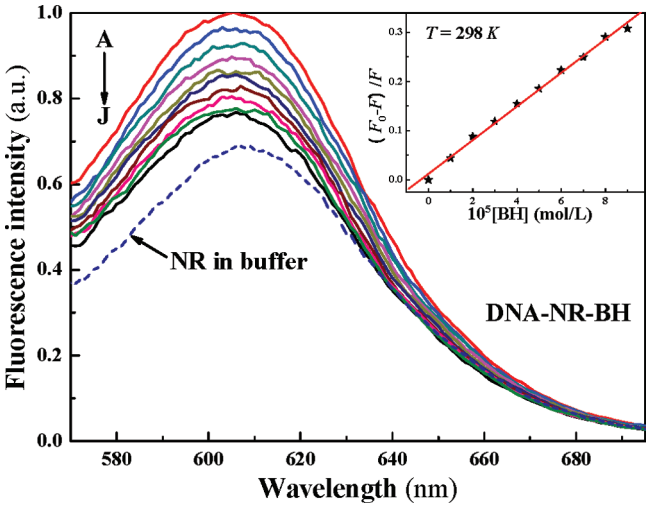

Figure 5. Fluorescence spectrum of DNA–NR with various concentrations of BH. The inset correspond to the Stern–Volmer plots. c(DNA) = 2.0×10^{-4} mol·L^{-1}; c(NR) = 1.0×10^{-5} mol·L^{-1}; c(BH)/(10^{-5} mol·L^{-1}), A–J: from 0.0 to 9.0 at increments of 1.0.

DNA–NR system in the absence and presence of BH. When the concentration of BH was added, a remarkable fluorescence decrease in DNA–NR system was observed. This phenomenon suggested that BH substituted for NR in the DNA–NR and dissociated the NR from the system, which led to a decrease in the emission intensity of the DNA–NR system.

To demonstrate ulteriorly the binding characteristics of DNA and BH, we introduced acridine orange (AO) as the fluorescence probe. AO is a classic intercalating dye,[33] and the structure of the DNA–AO intercalation complex is known from X-ray crystallography.[34,35] The fluorescence of AO increases after binding with DNA due to intercalation, hence, if BH intercalates into the helix of DNA, it would compete with AO for the intercalation sites in DNA and lead to a significant decrease in the fluorescence intensity of the DNA–AO complex. It was observed in Figure 6 that the emission spectra of the DNA–AO system decreased in the presence of BH. This result means that BH can intercalate into the helix of DNA.

3.4. Effect of Temperature. According to the above discussion, we found that the fluorescence intensity of the DNA–AO system decreased regularly with the increase in BH concentration. The different mechanisms of quenching are usually classified as either dynamic quenching or static quenching. In this work, the effect of temperature on DNA–AO–BH fluorescence quenching was studied.

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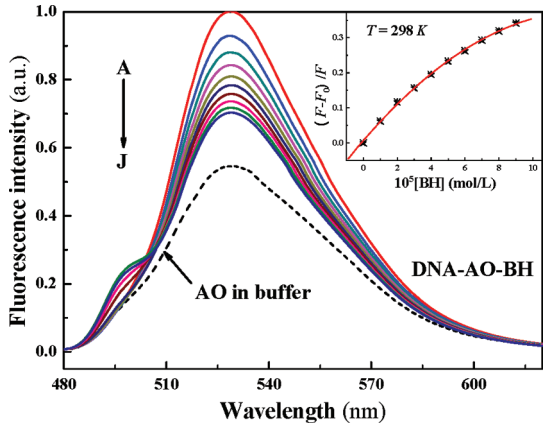

Figure 6. Fluorescence spectrum of DNA–AO with various concentrations of BH. The inset corresponds to the Stern–Volmer plots. c(DNA) = 2.0 × 10^{-4} mol·L^{-1}; c(AO) = 3.0 × 10^{-6} mol·L^{-1}; c(BH)/(10^{-5} mol·L^{-1}), A–J: from 0.0 to 9.0 at increments of 1.0.

3.4.1. Quenching Mechanism. Dynamic and static quenching can be distinguished by their differing dependence on temperature and viscosity.[31] Higher temperatures result in faster diffusion and hence larger amounts of dynamic quenching. Higher temperatures will typically result in the dissociation of weakly bound complexes and, hence, smaller amounts of static quenching. Consequently, we can distinguish the quenching types on the basis of the calculation of K_{SV} received at different temperature.

Table 1 summarizes the calculated K_{SV} from Stern–Volmer plots at each temperature (298, 304, and 310 K) studied; the

Table 1. Stern–Volmer Quenching Constants of BH–DNA (AO) System at Various Temperatures

pH	T (K)	$10^{-3}K_{SV}$/(L·mol^{-1})	R^a	S.D.^{b}
7.2	298	5.641	0.9990	0.0041
	304	4.200	0.9994	0.0025
	310	3.059	0.9998	0.0012

^{a}R is the correlation coefficient. ^{b}S.D. is standard deviation.

results show that the Stern–Volmer quenching constant K_{SV} is inversely correlated with temperature, which indicates that the probable quenching mechanism of the BH–DNA binding reaction is initiated by compound formation rather than by dynamic collision.

3.4.2. Binding Parameters. For a static quenching procedure, the data were analyzed according to the modified Stern–Volmer equation[36]

$$\frac{F_0}{\Delta F} = \frac{1}{f_a K_a}\frac{1}{[Q]} + \frac{1}{f_a} \quad (2)$$

In the present case, ΔF is the difference in fluorescence in the absence and presence of the quencher at concentration $[Q]$, f_a is the fraction of accessible fluorescence, and K_a is the effective quenching constant for the accessible fluorophores, which are analogous to associative binding constants for the quencher–acceptor system.

The dependence of $F_0/\Delta F$ on the reciprocal value of the quencher concentration $[Q]^{-1}$ is linear with the slope equaling to the value of $(f_aK_a)^{-1}$. The value f_a^{-1} is fixed on the ordinate. The constant K_a is a quotient of the ordinate f_a^{-1} and the slope $(f_aK_a)^{-1}$.

When small molecules bind to a set of equivalent sites on a macromolecule, the equilibrium binding constant and the numbers of binding sites can be also analyzed according to the Scatchard equation[37]

$$r/D_f = nK_b - rK_b \quad (3)$$

where r is the moles of ligand bound per mole of protein, D_f is the molar concentration of free ligand, n is binding site multiplicity per class of binding sites, and K_b is the equilibrium binding constant.

Figure 7A,B shows the modified Stern–Volmer plots and Scatchard plots for the BH–DNA (AO) system at different

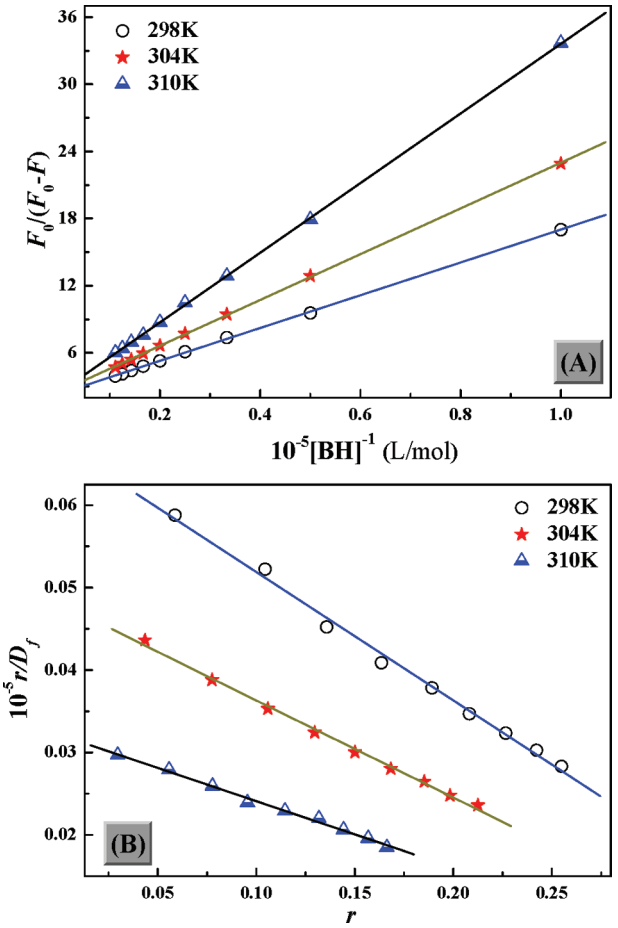

Figure 7. Modified Stern–Volmer plots (A) and Scatchard plots (B) of the BH–DNA (AO) system at different temperatures.

temperatures, respectively. The corresponding results at different temperatures are shown in Table 2. The decreasing trend of K_a, K_b, and n with increasing temperature was in accordance with K_{SV}'s dependence on temperature, as mentioned above, which resulted possibly due to the reduction of the stability of DNA–BH complex.

3.4.3. Thermodynamic Parameters and Binding Modes. The interaction forces between drugs and biomolecules may include electrostatic interactions, multiple hydrogen bonds, van der Waals interactions, hydrophobic and steric contacts within the antibody-binding site, and so on.[38] If the enthalpy change (ΔH) does not vary significantly in the temperature range

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Table 2. Binding Constants and Relative Thermodynamic Parameters of BH−DNA Interaction at pH 7.2

	modified Stern−Volmer method		Scatchard method				
T (K)	$10^{-4}K_a$/(L·mol^{-1})	R^a	$10^{-4}K_b$/(L·mol^{-1})	n	ΔH (kJ·mol^{-1})	ΔG (kJ·mol^{-1})	ΔS (J·mol^{-1}·K^{-1})
298	1.603	0.999 8	1.557	0.503		−23.96	
304	1.237	0.999 8	1.178	0.408	−42.02	−23.59	−60.61
310	0.802	0.999 9	0.807	0.399		−23.23	

[a]R is the correlation coefficient.

studied, both the enthalpy change (ΔH) and entropy change (ΔS) can be evaluated from the van't Hoff equation

$$\ln K_b = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (4)$$

where K_b is analogous to the associative binding constants at the corresponding temperature and R is the gas constant. The enthalpy change (ΔH) is calculated from the slope of the van't Hoff relationship. The free energy change (ΔG) is estimated from the following relationship

$$\Delta G = \Delta H - T\Delta S \quad (5)$$

Table 2 summarizes the values of ΔH and ΔS obtained for the binding site from the slopes and ordinates at the origin of the fitted lines (Supporting Information, Figure S2). The negative sign for free energy (ΔG) means that the binding process is spontaneous. The negative enthalpy (ΔH) and entropy (ΔS) values of the interaction of BH and DNA indicate that the hydrogen bonds and van der Waals interactions played a major role in the reaction.

3.5. Effect of the Ionic Strength. Because the intercalator of BH carries a positive charge and DNA has a negative polyphosphate skeleton, the effect of ionic strength on BH−DNA binding interaction has been studied. In this work, we have employed the strong electrolyte NaCl to decipher the impact of electrostatic interaction between BH and DNA. Some reports showed that very high concentration of NaCl would hinder small molecules from binding with DNA.[39] To facilitate the comparison of the influence of NaCl on the binding of BH to DNA, the quenching constants and binding constants in the presence of electrolyte were analyzed using the Stern−Volmer method and the Scatchard method (Figure 8), respectively. The corresponding results are shown in Table 3. The results show that the binding parameters decreased obviously in the presence of NaCl, These results and plots probability due to the strong electrolyte NaCl screens the electrostatic repulsion between consecutive phosphate groups (negatively charged), prompting the helix to shrink and thereby hinder the intercalator of BH from binding with DNA.

3.6. Effect of Different Degeneration Conditions. Denaturation of the biomacromolecules often results in the release of entrapped probe molecules, leading to modification in the fluorescence behavior of the probe. It has been shown in several studies that the inactivation of many enzymes occurs before protein molecule undergoes significant conformational changes, which can be detected during the process of denaturation by urea, guanidine hydrochloride (GuHCl), and temperature.[40,41] If the binding mode was intercalation, then quenching effect of denatured DNA would be weaker than that of undenatured DNA, for the release of the double strands of DNA. In this study, we discussed the effects of chemical denaturants and thermal denaturation on the binding of BH and DNA, respectively.

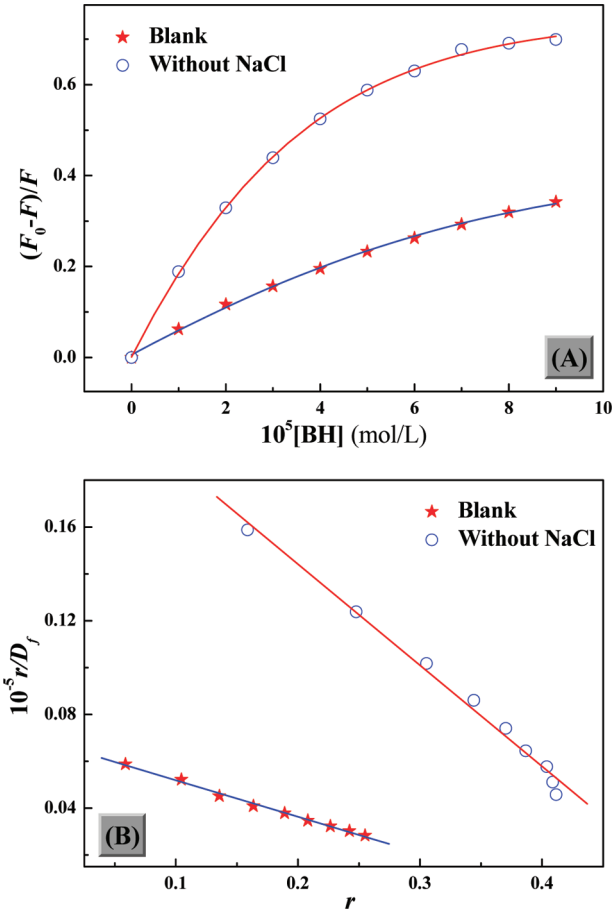

Figure 8. Stern−Volmer plots (A) and Scatchard plots (B) of the BH−DNA (AO) system in the absence and presence of NaCl. c(NaCl) = 0.15 mol·L^{-1}.

Table 3. Binding Constants of BH−DNA Interaction in the Absence and Presence of NaCl

	Stern−Volmer method		Scatchard method	
	$10^{-3}K_{SV}$/(L·mol^{-1})	R^a	$10^{-4}K_b$/(L·mol^{-1})	n
blank	5.641	0.9990	1.557	0.503
without NaCl	16.62	0.9968	4.320	0.620

[a]R is the correlation coefficient.

3.6.1. Effect of Chemical Denaturation. In comparison with either acid or thermal unfolding, chemical agents such as urea and GuHCl are more effective in disturbing the noncovalent interactions. The extent of unfolding is generally greater than that of any other means of denaturation.[41] To investigate their effects on the binding, we introduced urea and GuHCl to the BH−DNA (AO) system. The binding parameters in the presence of denaturants were analyzed using the Stern−Volmer

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method and the Scatchard method (Supporting Information, Figure S3), respectively. The corresponding results are shown in Table 4. The results demonstrated that the binding

Table 4. Binding Constants of BH−DNA Interaction in the Presence of Denaturants

	Stern−Volmer method		Scatchard method	
denaturant	$10^{-3}K_{SV}$/(L·mol^{-1})	R^a	$10^{-4}K_b$/(L·mol^{-1})	n
blank	5.641	0.9990	1.557	0.503
GuHCl	4.845	0.9993	1.033	0.535
urea	4.341	0.9991	0.877	0.554

^{a}R is the correlation coefficient.

parameters decreased in the presence of urea and GuHCl, and this decrease was more intense in the presence of urea. This suggested that both urea and GuHCl were able to liberate the BH from the DNA strands. It is well known that urea and GuHCl are the most frequently used classical denaturants to destabilize the double stranded DNA helix.[42] Hence, we can speculate that these two denaturants make the DNA separate, which weakened the ability of BH intercalating to DNA.

3.6.2. Effect of Thermal Denaturation. Further support for the intercalated binding of BH to DNA is obtained through thermal denaturation experiment because at a certain temperature, local openings of the double helix extend over the full molecule, resulting in a complete separation of the two strands.[43] In our experiment, we heated the native dsDNA solution in water bath for 30 min at each temperature ((60, 80, and 100 °C) and then compared the binding parameters obtained from the two kinds of cooling methods (one is cooling naturally, the other is cooling in an ice−water bath)). Figure 9A,B shows the Stern−Volmer plots and Scatchard plots for the BH−DNA (AO) system at different denatured temperatures, respectively. The corresponding results in Table 5 show that the quenching constant decreased with the increasing temperature, resulting possibly due to the fact that the higher temperature turns more dsDNA to be ssDNA. The release of the double strands of DNA inhibited the small molecule (BH) intercalating between two adjacent base pairs. The quenching constants of the DNA cooling naturally were slight higher than the relevant one cooling in an ice−water bath, maybe because a part of ssDNA turned renaturation under the condition of cooling naturally, but the binding constants of the DNA cooling naturally were slight lower than the relevant one cooling in an ice−water bath.

3.7. Effect of pH. Solution pH plays critically important roles in a variety of chemical and biological processes. As to DNA, the stability of its double-stranded structures is closely related to the environmental pH.[44] In this study, phosphate buffer solution was used to investigate the interaction between BH and DNA. The maximum emission intensity of DNA−BH complex dropped significantly in the pH 12.2 buffer solution (Supporting Information, Figure S4). This suggests that the structure of dsDNA has changed obviously at this value of pH, causing the fluorescence probe (AO) to not intercalate into the DNA easily. Figure 10 presented the effect of pH on the binding of BH and DNA, and the corresponding results are in Table 6. We can find that both of the quenching constants and binding constants are the highest at pH 7.2. In addition, the quenching constants and binding constants in strong acid solution are both much lower than the others, probably due to

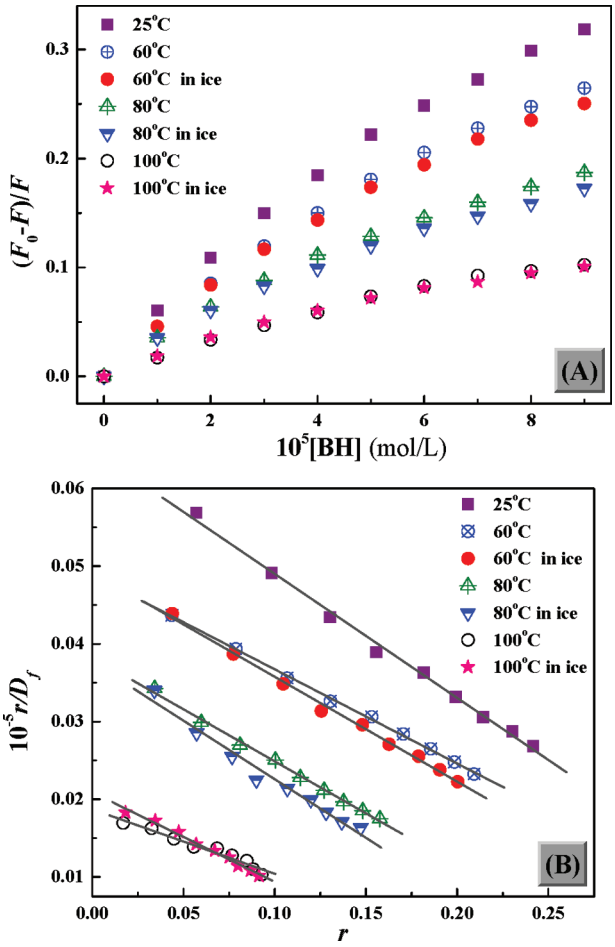

Figure 9. Stern−Volmer plots (A) and Scatchard plots (B) of the BH−DNA (AO) system at different denaturation temperatures.

Table 5. Thermal Denaturation Effects on the Binding of BH−DNA Interaction

	Stern−Volmer method		Scatchard method	
T (°C)	$10^{-3}K_{SV}$/(L·mol^{-1})	R^a	$10^{-4}K_b$/(L·mol^{-1})	n
25	5.641	0.999 0	1.557	0.503
60	4.595	0.999 8	1.182	0.497
60 (cooling in an ice−water bath)	4.419	0.999 4	1.316	0.451
80	3.327	0.998 8	1.307	0.353
80 (cooling in an ice−water bath)	3.046	0.997 7	1.471	0.309
100	1.923	0.999 0	0.810	0.279
100 (cooling in an ice−water bath)	1.902	0.998 9	1.125	0.224

^{a}R is the correlation coefficient.

the acid effects because the strong acid can disturb the noncovalent interactions as mentioned above.

4. CONCLUSIONS

In summary, the present study reports the spectral deciphering of the binding interaction of BH with DNA. From the detailed studies undertaken in the present work, the mode of binding interaction of BH with DNA has been evidenced to be principally intercalative, although the hydrogen bonds and van der Waals interaction between BH and DNA have also been

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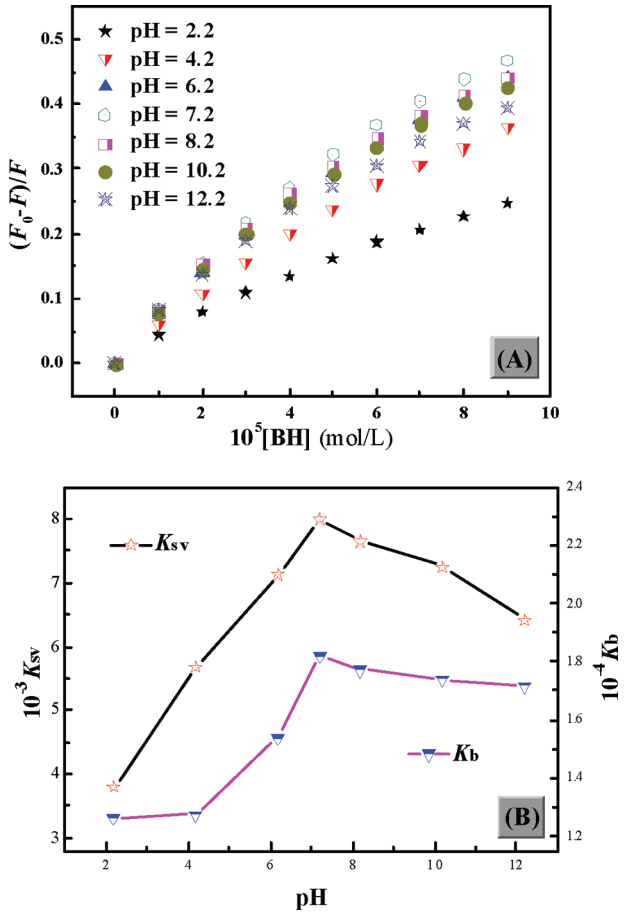

Figure 10. Effect of pH on the binding of BH and DNA. (A) Stern–Volmer plots of the BH-DNA (AO) system at different pH values. (B) Plot of the K_{SV} and K_b of BH–DNA (AO) system versus pH value.

Table 6. Effect of pH on the Binding of BH and DNA

	Stern–Volmer method		Scatchard method	
pH	$10^{-3}K_{SV}/(L\cdot mol^{-1})$	R^a	$10^{-4}K_b/(L\cdot mol^{-1})$	n
2.2	3.795	0.998 7	1.263	0.366
4.2	5.672	0.999 6	1.274	0.496
6.2	7.124	0.999 5	1.538	0.525
7.2	7.982	0.999 6	1.820	0.516
8.2	7.638	0.999 2	1.772	0.499
10.2	7.247	0.999 4	1.737	0.489
12.2	6.416	0.998 6	1.716	0.455

[a] R is the correlation coefficient.

argued. Concurrently, the degeneration conditions study reveals that chemical denaturation and thermal denaturation influence the structure and association stability of DNA. In addition, the effect of temperature, ionic strength, hyperthermia, and pH were also investigated. Our results throw light on the prospective probability of using BH as an efficient DNA probe and might be valuable for clinical research about BH and the theoretical basis for new probe designing.

■ ASSOCIATED CONTENT

Supporting Information

Fluorescence quenching of BH by KI in the absence and presence of DNA; van't Hoff plots of BH–DNA (AO) system; Stern–Volmer plots and Scatchard plots of the BH–DNA (AO) system in the absence and presence of denaturants; and plot of the maximum emission intensity of BH–DNA complex versus pH value. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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