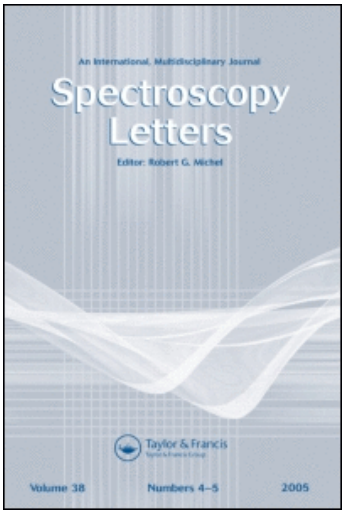

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Spectroscopic and Binding Properties of Berberine to DNA and Its Application to DNA Detection

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SPECTROSCOPIC AND BINDING PROPERTIES OF BERBERINE TO DNA AND ITS APPLICATION TO DNA DETECTION

Key words: Groove binding, Berberine, DNA, Detection

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ABSTRACT

Berberine(BER) binds to the double helical DNA with a high affinity. There is only a much smaller hypochromism and no shifts in the absorption spectra when BER binds to calf thymus DNA(CT DNA). The fluorescence yields increase dramatically

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when BER binds to DNA, with no shifts in the emission maximum. These spectral changes are in contrast to the behavior observed with many fluorescent intercalators. Groove binding rather than intercalation was suggested to be the cause of these spectral changes. Consistent with groove binding, ferrocyanide anion quenching studies showed that the magnitude of K_{sv} of the bound BER was higher than that of the free BER. The addition of salt to the solution releases the DNA-bound drug cation from the groove and causes a decrease in the fluorescence yield. The results of all above studies proved the groove binding of BER to DNA. The large fluorescence enhancements observed when BER binds to DNA and the poor fluorescence yield of BER in the absence of DNA can be used for sensitive detection of DNA. The linear concentration range was 0-20μg/ml. The limit of detection for CT DNA was 12 ng/ml.

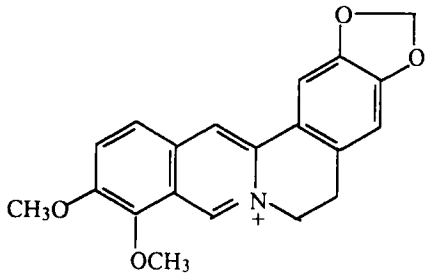
INTRODUCTION

The nature and dynamics of binding small molecules to DNA represents an area of active investigation [1-12]. Studies directed toward the design of site- and conformation-specific reagents provide routes toward rational drug design as well as a means to develop sensitive chemical probes of polymer structure. A systematic investigation of the binding of antibiotics, heterocyclic cations and metal complexes with DNA has revealed several structural and electronic factors that control the DNA binding affinity and sequence specificity of small molecules [7,8]. Binding studies with small molecules are valuable in understanding how proteins recognize and bind to specific DNA sequences. One motivation for these researches has been the use of these small molecules as probes for the detection of DNA in various applications [9].

Small molecules bind to the double helix by two dominant modes, referred to as groove binding and intercalation. Intercalated fluorescent molecules are well protected from the aqueous solvent and water-bound fluorescence quenchers [3,13,14]. In contrast, groove-bound fluorescent molecules may not be protected from water-bound anionic fluorescence quenchers [1]. If K_{sv} (Stern-Volmer quenching constant) of the bound molecule is smaller than that of the free molecule, the interaction between this molecule and DNA should be intercalative binding [3]. In contrast, if K_{sv} of the bound molecule is bigger than that of the free molecule, the interaction between this molecule and DNA should be groove binding [1]. Moreover, groove binding is more sensitive to salt concentration than intercalative binding [1]. Electrostatic hydrogen bonding and hydrophobic interactions contribute to the stability of groove binding [15], whereas intercalative binding of molecules is favored by stacking interactions with the adjacent DNA bases [16].

Berberine(BER) is the active component of Coptis chinensis, one of traditional Chinese medicines. The structure of berberine is given in Scheme 1. Berberine has some pharmaceutical uses, such as in cure of enteritis, dysentery, jaundice, pneumonia, scrofula and metrorrhagia.

The work described here can be divided into two parts. The first part deals with the interaction between BER and DNA using spectroscopic methods. The results of absorption spectra, fluorescence spectra, ferrocyanide quenching studies and salt effect experiment suggested groove binding of BER to DNA. The second part is concerned with a spectrofluorimetric method for the determination of DNA. The linear concentration range was 0-20μg/ml. The limit of detection for CT DNA was 12 ng/ml



Scheme 1 Structure of berberine.

EXPERIMENTAL

Apparatus

All fluorescence measurements were made with a Shimadzu RF-5000 spectrofluorimeter equipped with a 150-W xenon lamp. Absorption spectra were recorded on a Shimadzu UV-2201 ultraviolet-visible spectrophotometer.

Reagents

Commercially prepared CT DNA (Beijing Baitai Biochemical Technology Co., China) was directly dissolved in water at a final concentration of 100 μ g/ml and stored at 4℃. The ratio of absorptions at 260 nm/280 nm is 1.9. A BER solution (2.7×10^{-4} mol/L) was prepared by dissolving 10 mg of berberine chloride(Chinese Assay Institute for Drugs and Biologicals, Beijing, China) into 100 ml of water.

A buffer solution of pH 3.8 was prepared by mixing 50 ml of 0.20 mol/L potassium o-biphthalate and 2.63 ml of 0.20 mol/L HCl and adding water to a final volume of 200 ml.

Procedures

Absorption Spectra. Transfer to a 10-ml standard flask 1.0 ml of buffer solution (pH 3.8) and 1.0 ml of BER solution (2.7×10^{-4} mol/L). Add a known volume of CT DNA standard solution. Dilute to the volume with water and mix. Both the mixed solution and a reagent blank (prepared in a similar manner without BER) are allowed to incubate for 10 min. Record the absorption spectra on a Shimadzu UV-2201 ultraviolet-visible spectrophotometer.

Fluorescence Spectra. Transfer to a 10-ml standard flask 1.0 ml of buffer solution (pH 3.8) and 1.0 ml of BER solution (2.7×10^{-4} mol/L). Add a known volume of CT DNA standard solution. Dilute to the volume with water and mix. Both the mixed solution and the reagent blank (prepared in a similar manner without CT DNA) are allowed to incubate for 10 min. Record the fluorescence spectra of the mixed solution and the reagent blank on a Shimadzu RF-5000 spectrofluorimeter. Measure the fluorescence intensities of the mixed solution (F) and the reagent blank (F_0) with the following settings of the spectrofluorimeter: excitation wavelength (λ_{ex}), 363 nm; excitation slit (EX), 5 nm; emission wavelength (λ_{em}), 530 nm; emission slit (EM), 3 nm. Plot a calibration curve of ΔF ($F-F_0$) vs. the concentration of CT DNA.

Fluorescence Quenching Experiments. The fluorescence quenching experiments with ferrocyanide were performed and the experimental data were plotted according to the Stern-Volmer equation

$$I_0 / I = 1 + K_{SV}[Q]$$

where I_0 and I are the fluorescence intensities in the absence and in the presence of the

quencher (Q). The Stern-Volmer quenching constant K_{SV} was evaluated by linear least-squares analysis of the data in terms of the above equation

RESULTS AND DISCUSSION

Studies of Absorption Spectra

The absorption spectra of BER in the presence of increasing amounts of CT DNA showed that there were only much smaller decreases in the peak intensities with no shifts when BER bound to CT DNA (Fig.1). These results are in contrast to the observed strong hypochromism and red shifts in the absorption spectra when small molecules intercalate into the base stack [2,17]. Therefore, we think that such small changes observed with BER could be due to the groove binding rather than the intercalation of the drug.

Emission Studies

The binding of BER to DNA was further investigated by fluorescence spectroscopy. The addition of CT DNA to a solution of BER intensely increases the fluorescence intensity of the drug (Fig. 2). However, there is no shifts in the emission maximum, even in the presence of an excess of CT DNA. This result is in contrast to the observed large red shifts in the fluorescence maxima when drugs intercalate into the helix [18].

Ferrocyanide Quenching Studies

Since groove binding exposes the bound molecules to the solvent surrounding the helix much more than does the intercalation [19], the ferrocyanide quenching experiment

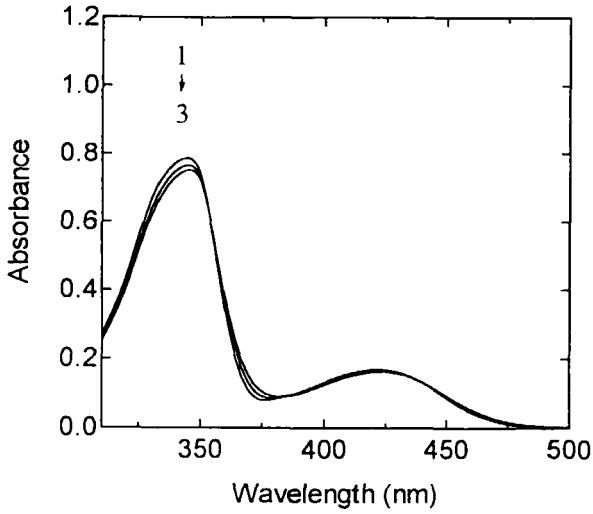


FIG. 1 Absorption spectra of BER. (1) in the absence of CT DNA; (2)-(3) in the presence of CT DNA with the concentrations of 20 and 40 μg/ml, respectively

was chosen to further establish the DNA binding affinity of BER. If BER is intercalated into the helix stack, the magnitude of K_{SV} of the free BER should be higher than that of the bound BER [3]; in contrast, if BER binds to DNA in the groove, the magnitude of K_{SV} of the bound RVN should be higher than that of the free BER [1] In aqueous solutions, ferrocyanide quenched the fluorescence of BER very efficiently. Addition of ferrocyanide to a mixture of BER and CT DNA resulted in increased quenching of the fluorescence intensity (Fig. 3). K_{SV} values for the free BER and the bound BER with CT DNA at 1μg/ml were 147.7 L/mol and 297.1 L/mol, respectively The results showed that the magnitude of K_{SV} of the bound BER was higher than that of the free BER, which suggested the groove binding of BER to DNA [1].

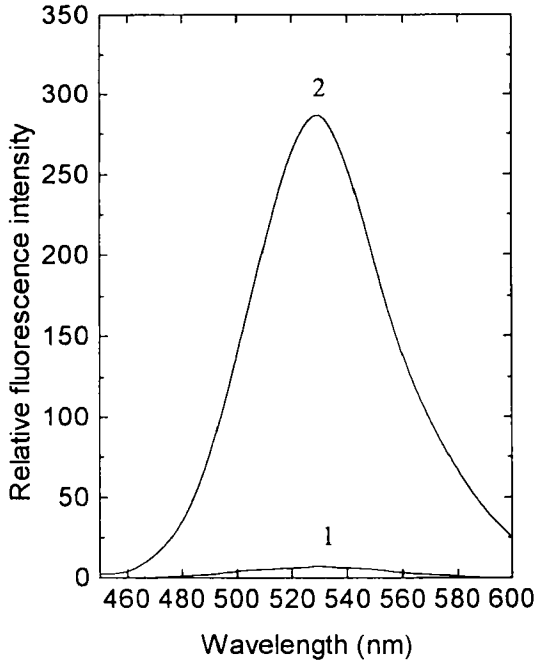


FIG. 2 Fluorescence emission spectra of BER. (1) in the absence of CT DNA; (2) in the presence of CT DNA with the concentration of 40 μg/ml. BER at 2.7×10^{-5} mol/L.

Salt effect

The effect of the ionic strength on the BER fluorescence yield was tested by the addition of NaCl. In the absence of CT DNA, the addition of NaCl to the free BER had minor effect on the fluorescence yield of the drug. However, in the presence of CT DNA, the addition of NaCl to the mixture had major effect on the fluorescence yield of BER (Fig. 4). The quenching constant with NaCl in the absence of and in the presence of CT DNA (1μg/ml) were 7.5 L/mol and 25.7 L/mol, respectively. The strong dependence of the binding on the ionic strength clearly indicates that BER

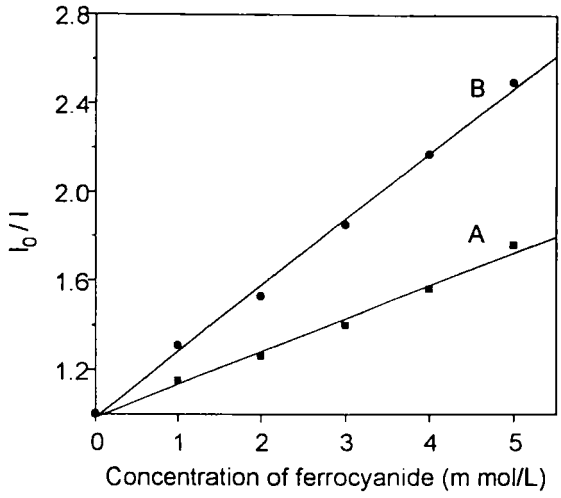


FIG. 3 Quenching of BER fluorescence (1.3×10^{-5} mol/L) by ferrocyanide in the absence of CT DNA (curve A) and in the presence of CT DNA with the concentration of 1.0 μg/ml (curve B).

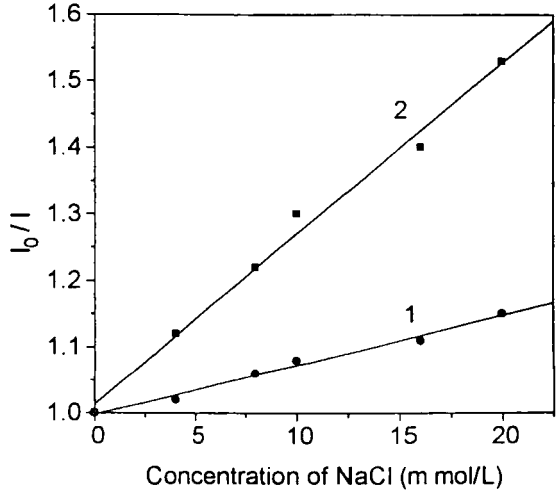


FIG. 4 Quenching of BER fluorescence (1.3×10^{-5} mol/L) by NaCl in the absence of CT DNA (curve 1) and in the presence of CT DNA with the concentration of 1.0 μg/ml (curve 2).

binding to CT DNA is predominantly controlled by electrostatic interactions and this result is consistent with groove binding rather than intercalation into the helix [1].

Application on DNA Detection

First, the effect of pH on ΔF was studied. The concentrations of BER and DNA were maintained at 2.7×10^{-5} mol/L and 1 μg/ml , respectively. The results indicated that the maximum ΔF occurred in the pH range of 3.6 — 3.8 and the ΔF values decreased for other pH values outside this range. In the subsequent studies, a pH of 3.8 was recommended for use. Next, the influence of BER concentration on ΔF was investigated with constant concentration of DNA (1 μ g/ml) at pH 3.8. The results showed that the maximal and constant ΔF was reached when the volume of BER solution(2.7×10^{-4} mol/L) was in the range of 0.9 — 1.2 ml, and the ΔF values decreased outside this range of BER concentration. Thus, 1.0 ml of BER solution (2.7×10^{-4} mol/L) was chosen for use. The experiment results showed that the fluorescence intensities from the mixture of BER and CT DNA were stable for two hours.

The values of ΔF showed a linear relationship with the concentrations of CT DNA. The linear concentration range, limit of detection, correlation coefficient and precision were given in Table 1.

The limit of detection (LOD) was given by the equation, $LOD=KS_0 / S$, where K is a numerical factor chosen according to the confidence level desired, S_0 is the standard deviation of the blank measurements (n=9) and S is the sensitivity of the calibration graph. Here a value of 3 for K was used.

TABLE 1 Analytical Parameters of This Method

	Linear range (μg/ml)	r[a]	LOD[b] (ng/ml)	RSD[c] (n=7)
CT DNA	0—20	0.9995	12	1.0 %

[a] r, correlation coefficient.
[b] LOD, limit of detection.
[c] RSD, relative standard deviation for seven measurements of 1.0 μ g/ml DNA.

CONCLUSIONS

BER binds to the double helical DNA with a high affinity. Upon binding BER to CT DNA, a much smaller hypochromism with no shifts in the absorption spectra of BER and intense fluorescence yield increasing of BER with no shifts in the emission maximum were observed. These spectral changes are in contrast to the behavior observed with many fluorescent intercalators. Groove binding rather than intercalation was suggested to be the cause of these spectral changes. Ferrocyanide anion quenching studies showed that the magnitude of K_{sv} of the bound BER was higher than that of the free BER, which supported the groove binding of BER to DNA. Consistent with groove binding, the addition of NaCl to the solution of BER and CT DNA caused a decrease in the fluorescence intensity of the drug. The large fluorescence enhancements of BER in the presence of DNA and the poor fluorescence yield of BER in the absence of DNA were used for sensitive detection of DNA.

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