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Microcalorimetric and spectrographic studies on the interaction of DNA with betaxolol

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ABSTRACT

The interaction of calf thymus deoxyribonucleic acid (ct-DNA) with betaxolol (BET) in aqueous buffer solution (pH 7.40) has been investigated using isothermal titration calorimetry (ITC), ultraviolet absorption (UV), fluorescence spectroscopy (FS) and circular dichroism (CD). Thermodynamic parameters, i.e., equilibrium constants, standard changes of enthalpy (ΔH°), Gibbs free energy (ΔG°) and entropy (ΔS°), for the binding process of the drug to the bio-macromolecules have been derived from the calorimetric data. Analysis of the thermodynamic data indicates that there are two classes of binding sites on the DNA molecules being able to coordinate with BET molecules. One class of binding takes place at the sites formed by base pairs, which is synergistically driven by enthalpy and entropy, while the other one takes place on phosphate groups and shown as an entropy driven process. The thermodynamic behavior of the DNA–drug supramolecular system has been discussed in the light of the important weak interactions, hydrophobic force, hydrogen bond and electrostatic force, according to the UV, FS and CD spectra.

1. Introduction

Determination of thermodynamic properties and discovering relationship between the properties and interaction pattern of drugs with deoxyribonucleic acid (DNA) are an important approach of study on biological functions of drugs. To illustrate the influence on the copy and transfer of the nucleic acid induced by the small molecules, or understand the pathogenesis of disease on the gene level, especially found convenient filtrate method of the anticancer drugs in vitro, investigations of the interaction between drugs and DNA always have potential application value (Gorre et al., 2001; Goparajua et al., 2009). Many drugs are known to interact with DNA to exert their biological activities. Generally, drugs influencing DNA can be classified into three categories. Drugs of the first category form covalent linkages with DNA while drugs of the second category form noncovalent complexes with DNA by either intercalation or groove binding. Drugs of the final category cause DNA backbone cleavages (Xia et al., 2007).

Among the several classes of effective cardiovascular medications, β-blockers are widely used for treatment of a variety of cardiovascular disorders such as hypertension, coronary artery disease, heart failure and arrhythmias (Takase et al., 2005). Betaxolol (BET) is an approved clinically used β-blocker (Datta et al., 2006), which produces vasodilation and is effective for the treatment of arterial hypertension, intraocular hypertension (Melena et al., 1999; Canotilho et al., 2006), coronary artery disease (Suzuki et al., 2003) and chronic cardiovascular diseases (Manoury et al., 1987).

Studies on interactions between BET and such bio-macromolecules as proteins and deoxyribonucleic acids are of importance to understand the mechanism of action of the drug, for example, apoptosis of cancer cell induced by the drug. However, reports about the interactions of BET with DNA are sparse. Recently, isothermal titration calorimetry (ITC) has emerged as a powerful, high precision and sensitive technique that can quickly and directly elucidate the complete thermodynamic profiles of small molecules interacting with macromolecules in a single experiment (Saboury et al., 2006; Wolthers et al., 2007; Tellinghuisen, 2007). So calorimetric techniques are far superior compared to several other techniques, it can provide a lot of useful information such as the energetics, binding affinity and so on. In the present work, ITC combining with ultraviolet absorption (UV), fluorescence spectroscopy (FS) and circular dichroism (CD) were used to research the interaction of ct-DNA with BET. Thermodynamic parameters were calculated based on the calorimetric data and were discussed according to the supramolecular structure of the DNA–BET system shown by the spectra.

2. Materials and methods

2.1. Materials

Calf thymus deoxyribonucleic acid (ct-DNA) was purchased from Sigma Company of which the purity was scaled by

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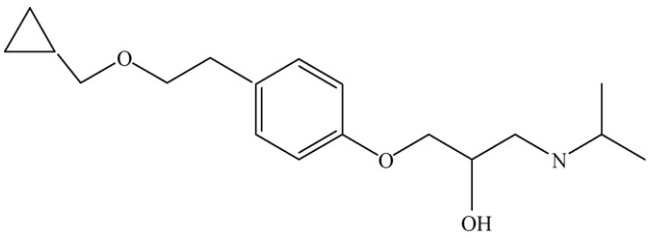

Scheme 1. The molecular structure of betaxolol.

$A_{260}/A_{280} > 1.8$, in aqueous solution (pH 7.40). DNA concentrations were determined by the absorbency of ultraviolet spectrum at 260 nm (Kumar and Asuncion, 1993). Betaxolol hydrochloride (purity >98%) was purchased from J&K Chemical Company and its molecular structure was shown in Scheme 1. Berberine hemisulfate (BR, Alexis), whose purity was 98%, was used as received. Tri-(hydroxymethyl) aminomethane (Tris), hydrochloric acid and sodium chloride were all of analytical grade, and the water used in the experiment was double distilled water, prepared in the presence of basic potassium permanganate. All other reagents were of analytical purity and were prepared with Tris–HCl buffer solution of which the concentration was 0.01 M (pH 7.40).

2.2. Microcalorimetric measurement

Titration calorimetric measurement was completed using a nano-watt scale microcalorimeter supported by TAM 2277 thermal activity monitor (Thermometric, Sweden) and controlled by Digitam 4.1 software. Accuracy of the instrument was ±10 nW in determination of heat-flow power. It had an electrical calibration with a precision better than ±1%, which was regularly verified by measuring the dilution enthalpy of a concentrated sucrose solution (Bai et al., 2002). The 1 mL reaction cell and reference cell were initially loaded with 500 μL ct-DNA solution at the concentration 4.68×10^{-4} M and 750 μL Tris–HCl buffer solution, respectively. BET solutions at the concentration 50 mM was injected into the under stirring reaction cell in 30 portions of 15 μL using a 500 μL Hamilton syringe controlled by a 612 Lund Pump. The interval between two injections was 40 min, which was sufficiently long for the signal to return to the baseline. The system was stirred at 30 rpm with a gold propeller. The heat measurement experiment was startup after the base line became stable so that the heat produced by stirring can be automatically deducted. All experiments were performed at a fixed temperature of (298.15 ± 0.01) K. To deduct the dilution heat of drug and the DNA solutions, titration experiments were also performed for drug solution dropped into Tris–HCl buffer solution and Tris–HCl buffer solution into the DNA solution, respectively. The heat effect caused by DNA dilution was negligible. A representative titration curve was given in Fig. 1.

2.3. Determination of ultraviolet absorption spectra and absorbance at certain wave length

Determination of ultraviolet absorption spectra was carried out using a UV–Vis spectrophotometer (Hp 8453, USA) equipped with a xenon lamp and a 1 cm quartz cuvette of 10 mm light-path. The range of wave length was from 200 to 300 nm and the scan rate at 1200 nm/min. The concentration of the DNA solution in the cuvette was faxed at 30 μM and those of BET solution were ranged between 50 μM (low concentration) and 2.6×10^{-4} M (high concentration).

2.4. Fluorescence spectroscopy

Fluorescence intensity of the DNA–BR–drug aqueous systems was measured using a fluorescence spectrophotometer (LS55, PerkinElmer, USA). The excitation (λ_{ex}) and emission (λ_{em}) wavelengths were 370 and 520 nm, respectively. The corresponding emission spectra were recorded in the range of 450–600 nm. Variously relative amounts of the drug were added into the DNA–BR mixture in which the concentration of DNA and BR were always 70 and 66 μM, respectively.

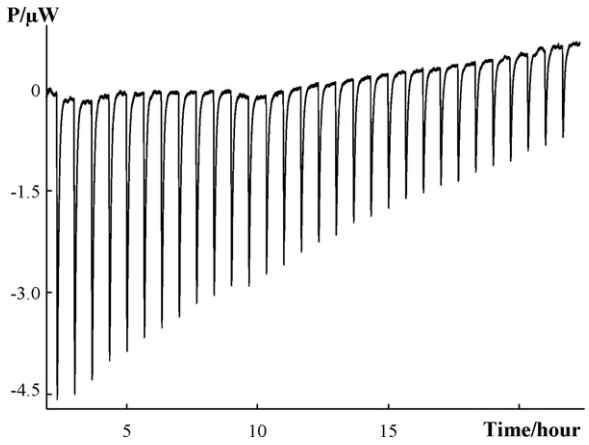

Fig. 1. Variation of heat-flow/electrical power as a function of time, titrant: BET (50 mM); titrand: DNA (4.68×10^{-4} M).

2.5. Circular dichroism

Circular dichroism spectra were recorded on a Jasco J-810 spectropolarimeter (Japan Spectroscopic Ltd., Japan) attached with a Jasco temperature controller and thermal programmer. The spectra were recorded in circular quartz cells of 1 cm path length. The lamp-house system was in protection of nitrogen atmosphere with the flow rate at 5 L/min during the experiment. Spectra were measured as the average of twice scans from 220 to 320 nm at a scan rate of 100 nm/min and the obtained results expressed as millidegrees (mdeg).

3. Results and discussion

3.1. Thermodynamic assumptions of the binding system

The basic assumptions on binding process of DNA with BET which can be regarded as a kind of ligand in the system may be expressed as reported in literature (Aki et al., 1999; Nielsen et al., 2000). (a) Each DNA molecule may have several (i) classes of binding sites, which can bind with the same ligand molecules. (b) All the sites within a certain class are thermodynamically identical. (c) The i classes of binding sites are assumed to be mutually independent, so that the binding ratio on one class of sites does not affect that of another class of sites. (d) Each site can only bind with one ligand molecule. On the basis of the above assumptions and the supposition that the binding of drug to DNA can be regarded as adsorption abiding the Langmuir's model (Wiseman et al., 1989; Lin et al., 1991), the following equations can be obtained.

$$\theta_i = \frac{K_i c_L}{1 + K_i c_L} \tag{1}$$

$$c_{L,0} = c_L + c_{P,0} \sum_{i=1}^{m} N_i \theta_i \tag{2}$$

where $c_{L,0}$ and $c_{P,0}$ are the concentration of BET and DNA, assuming that they do not bind with each other when they are in coexistence in the solution. c_L is the concentration of dissociative drug at the equilibrium state of the binding (or adsorption). θ_i, K_i and N_i are the

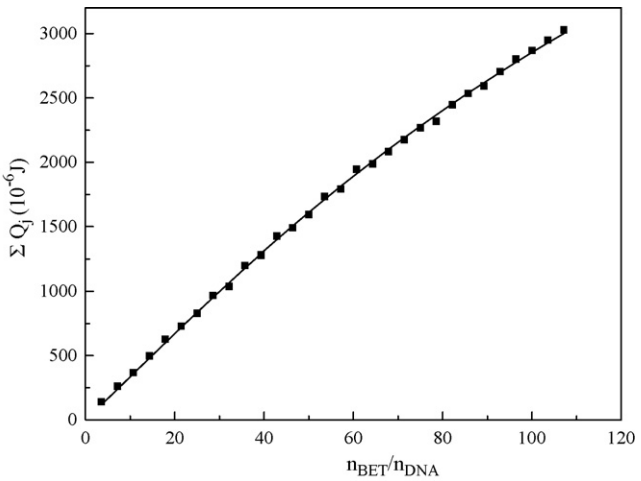

Fig. 2. The non-linear fitting curve of the reaction heat (■) versus the molar ratio of BET to DNA.

binding ratio, equilibrium constant and number of binding sites in class *i*. The following equation can be obtained by combining Eq. (1) with Eq. (2):

$$c_{L,0} = c_L + c_{P,0}\sum_{i=1}^{m}\frac{N_i K_i c_L}{1 + K_i c_L} \tag{3}$$

The heat of the *j*th injection (Q_j) in this experiment may be expressed as

$$Q_j = c_{P,0} V_{\text{cell}} \sum_{i=1}^{m} N_i \Delta\theta_i \Delta H_i^{\circ} \tag{4}$$

where V_{cell} is the volume of the titrand in the reaction cell, $\Delta\theta_i$ is the increment of binding ratio from $(j-1)$th to *j*th injection, and ΔH_i° is the standard binding enthalpy, i.e., change of standard enthalpy of the process that per mole of the drug molecules are bind with the sites. The concentrations $c_{L,0}$ and $c_{P,0}$ are known quantities and Q_j has been obtained from calorimetric experiment. In fact, Q_j is a function of $c_{L,0}$ and $c_{P,0}$ with N_i, K_i and ΔH_i° as undetermined parameters while c_L and ($_i$ as eliminated variables. The fitting curve of accumulated binding heat, ΣQ_j, versus the molar ratio of the drug to the BSA, which is obtained from non-linear least variance fitting principle and by using of MATLAB 7.01 software, is shown in Fig. 2. By analyzing the deviation between the calculated curve and the experimental data points, it can be reasonably determined that there may be two classes of binding sites on the DNA molecule. So the thermodynamic parameters, i.e., ΔH_1°, ΔH_2°, N_1, N_2, K_1° and K_2° can be obtained from Eqs. (1)–(4). The changes of standard Gibbs free energy (ΔG°) and entropy (ΔS°) can be calculated using the following thermodynamic formulas, Eqs. (5) and (6), and the results were gathered in Table 1.

$$\Delta G^{\circ} = -RT\ \ln K^{\circ} \tag{5}$$

$$\Delta G^{\circ} = \Delta H - T\Delta S^{\circ} \tag{6}$$

3.2. Analysis of thermodynamic parameters

From the data in Table 1, it can be seen that K_1° is larger than K_2°, this indicates that the first class of binding is more stable than the second one, while the number of the second class of binding sites (N_2) is evidently larger than that of the first class of ones (N_1). Probably, from the message brought by values of the equilibrium constants and the numbers of the binding sites, a reasonable judgment about the differentiation between the two classes of binding can be made. A drug molecule may (partly) insert itself into the intra-molecular space formed by the double helixes of the DNA molecule, and combine with the base pairs (bps) at a site, which may belong to the first class. However, it may not enter into the hydrophobic internal part of the macromolecule but only interact with the phosphate groups on the DNA surface at a binding site, which may belong to the second class due to the weaker binding and the large number of binding site.

The interaction forces between bio-macromolecules and ligands may comprise hydrophobic, hydrogen bonds and electrostatic interactions (Gharagozlou and Boghaei, 2008). The changes in enthalpy (ΔH_1°) and entropy (ΔS_1°) of the binding at the first class of sites are, respectively, negative and positive, indicating that this binding is an exothermic and entropy increasing process. In order to understand this phenomenon, we firstly consider the direct attraction between the bps and the drug molecules. This kind of interaction should lead to exothermic effect. On the other hand, the drug molecules must release some water molecules from their hydration layers when they are approaching to the DNA molecules, this hydrophobic effect make positive contribution to either enthalpy or entropy. Meanwhile the water molecules originally coordinated to the DNA molecules are extruded by drug molecules in the combining process, and this dehydration process also makes positive contribution to either enthalpy or entropy. The negative enthalpic change indicates that the interaction of the bps with the ligand molecules dominates this class of binding. As the total result, the first class of binding is exothermic and entropy increasing process, i.e., both the entropy effect and heat effect make contribution to the negative change of standard Gibbs free energy (ΔG_1°). In other words, this class of binding is enthalpy–entropy synergically driven process in the viewpoint of thermodynamics, while this class of binding process is mainly driven by entropy because $T\Delta S_1^{\circ} \gg |\Delta H_1^{\circ}|$. Furthermore, owing to $\Delta H_1^{\circ} < 0$ and $\Delta S_1^{\circ} > 0$, the hydrophobic interaction between the macromolecule and the ligand molecule plays a major role in this class of binding process (Ross and Subramanian, 1981).

The positive enthalpy and entropy values indicate that the second binding is an endothermic and entropy increasing process. Because $T\Delta S_2^{\circ} > \Delta H_2^{\circ}$ and the entropy increasing effect result in $\Delta G_2^{\circ} < 0$, the second class of binding is entropy driven process. According to literature (Ross and Subramanian, 1981), the positive enthalpic and entropic changes indicate that hydrogen bonds and electrostatic interaction are the primary acting force of this binding process. The drug molecules loose some water molecules from their hydration shells when they are attracted by electrostatic force and hydrogen bond, approaching to the binding sites. Simultaneously, the hydration layer of DNA molecule at the binding sites is also partly destroyed. The dehydration processes are endothermic and

Table 1
Thermodynamic parameters for the binding of BET to DNA at 298.15 K.

Class of sites	*N*	10^{-3} K°	ΔH° (kJ mol^{-1})	ΔG° (kJ mol^{-1})	ΔS° (J mol^{-1} K^{-1})
First	65.0 ± 0.2	18.29 ± 0.31	−0.97 ± 0.50	−24.33 ± 0.40	78.35 ± 0.50
Second	137.0 ± 0.3	9.81 ± 0.32	1.16 ± 0.03	−22.78 ± 0.04	80.29 ± 0.40

N is the possible largest number of binding sites; K° is equilibrium constant of the binding processes; ΔH°, ΔS° and ΔG° are the standard changes of enthalpy, entropy and Gibbs free energy for per mole BET bound to the DNA.

make a major favorable contribution to the entropy increase, and the positive values of the enthalpic and entropic effects have surpassed the absolute values of those corresponding to the primary acting force.

3.3. Classes of binding sites and UV absorbance at two peaks wavelength

The thermodynamic results show that there were two classes of binding sites on each of the DNA molecules for BET molecule. As further proof for this judgment, the UV absorbance data was determined. According to literature (Moosavi-Movahedi et al., 2004), the plot of $1/\Delta A$ versus 1/[BET] has been drawn (ΔA is the change of observed absorbance of the DNA solution in the presence of different concentrations of BET, see Fig. 3a and b), and then free ligand concentration ($[BET]_{free}$), the number of moles of ligand bound per molar base pair of DNA (ν) and the Scatchard plot (see the inset of Fig. 3c) can be obtained. The binding isotherm curve shown in Fig. 3c is a plot of ν against logarithm of free ligand concentration, which shows the existence of two sets of binding sites. This result is in good accordance with that obtained from the calorimetric data. Since the drug (ligand) is bound to the DNA, cooperativity might be caused in the system.

Cooperativity is an important aspect in DNA–drug complexation as it may influence the biological activity of a drug binding to a continuous array of potential binding sites. The cooperative binding effect has been rationalized as an effect mediated by some conformational changes in the helix structure of DNA. Based on this model, two structurally different conformations may coexist in the DNA molecules (Bhadra et al., 2008; Webb et al., 2001). The inset of Fig. 3c shows the Scatchard plot as a cooperative biphasic changes in the binding process according to literature (Moosavi-Movahedi et al., 2004). The binding of the ligand to form I may lead to a conformational or allosteric change in the DNA structure to form II which exhibits higher binding affinity to the ligand. The form I gets converted to form II when the ligand binds to form I, and at the flex point (which is marked by the arrowhead in the inset of Fig. 3c) of the Scatchard plot all the form I is converted to form II (Bhadra et al., 2008). In form II, the drug can easily bind to DNA.

3.4. Analysis of ultraviolet absorption spectra

The UV spectrometry is one of the most important methods to investigate the interaction between the small molecules and DNA (Chen et al., 2008). The typical absorption peaks of BET and DNA center at 274 and 259 nm, respectively. The mixed solution of BET with DNA presents an absorption peak around 261 nm at low concentrations whereas it shows an absorption peak at about 273 nm at high concentration (see Figs. 4 and 5). As shown in these figures, the absorption peaks of the mixed solutions are not the simple superposition of the characteristic absorption peaks corresponding to the drug and DNA.

Hyperchromism and hypochromism are the proper spectrum property, corresponding to the helix structure and the steric configuration of DNA. Hyperchromism may be attributed to the interactions between small molecules and DNA molecules, and the change of DNA helix structure and hypochromism can be assigned to a relatively strong interaction between the electronic states of the intercalating chromophore and that of the DNA base (Sakurai et al., 2001). According to the Long theory (Long and Barton, 1990), the hyperchromism and the bathochromic effect are the signs of drugs interacting with DNA by the insert function. When the small molecules interact with DNA molecules by the insert function, the π electron clouds of the DNA and drug molecules participate in the interaction, the maximal absorbing peaks shift toward higher wavelengths and the absorbency increased. When the small

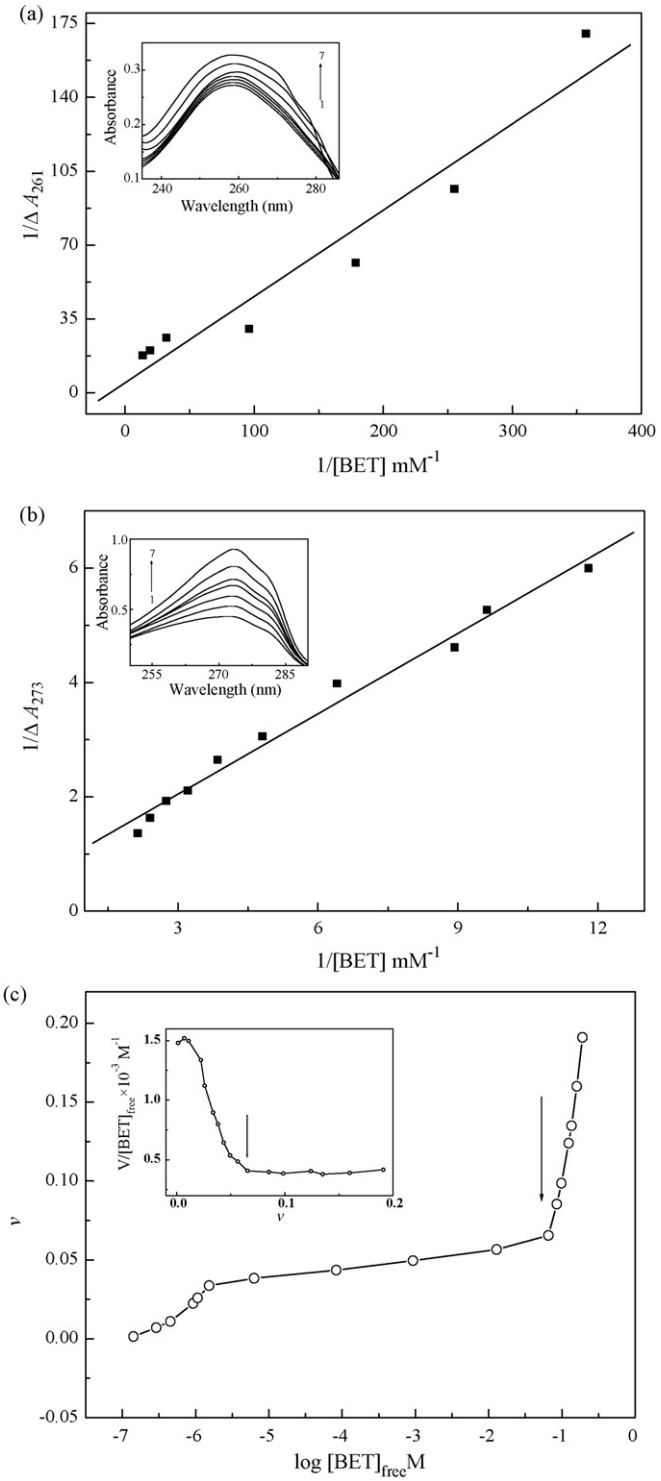

Fig. 3. (a) The plot of $1/\Delta A$ versus 1/[BET]. The inset: UV spectra of the DNA interacting with BET in low concentration range, [BET]/(μM) (1–7): 0, 2.80, 3.92, 5.60, 10.4, 31.2 and 52.0. (b) The plot of $1/\Delta A$ versus 1/[BET]. The inset: UV spectra of the DNA interacting with BET in high concentration range, 10^4 [BET]/(mol/L) (1–7): 1.56, 2.08, 2.60, 3.12, 3.64, 4.16 and 4.68. (c) Binding isotherm (ν vs. $\log[BET]_{free}$) for DNA(BET complexes where $\nu = [BET]_{bound}/[DNA]$ and $[BET]_{bound} = [BET]_{total} - [BET]_{free}$. The inset: Scatchard plot for the binding of BET–DNA complexes. The arrow shows the change in the interval of the curve, under the effect of different kinds of interactions.

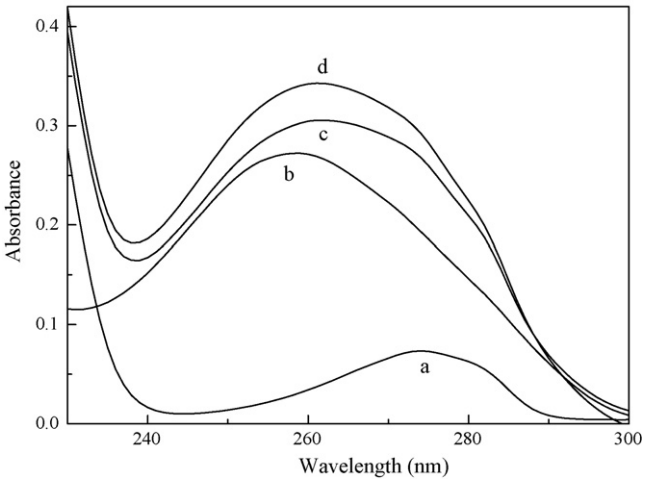

Fig. 4. The UV absorption spectra of the interaction between BET and DNA at the low concentration in the buffer solution (pH 7.40), [DNA] = 70 μM, [BET] = 50 μM. (a) BET; (b) ct-DNA; (c) the simple superposition of (a) and (b); (d) the mixed solution.

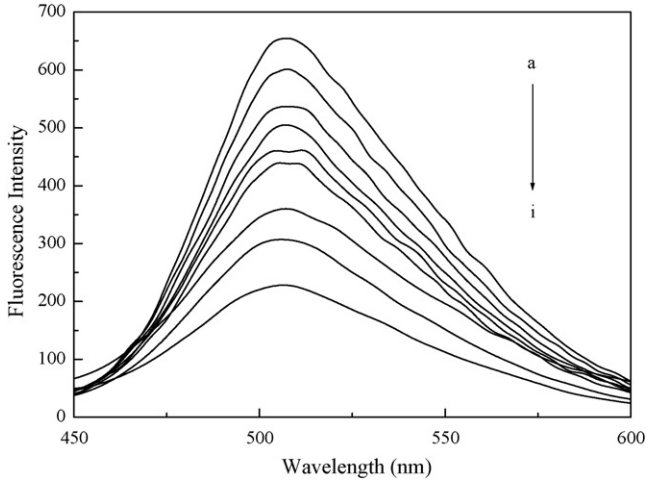

Fig. 6. Emission spectra of DNA-BR solution with increasing concentration of BET ([BR] = 66 μM, [DNA] = 70 μM). The arrow shows the intensity changes upon increasing the concentration of the drug. From (a) to (i) the concentration of BET is 0, 20, 40, 60, 80, 100, 156, 208 and 260 μM.

molecules interact with DNA by the electrostatic function, the peak position is fixed but the intensity of the peak changed.

The discrepancy between the low and high concentration solutions is the different extent of the red shift and the absorbency. The maximal peak of DNA makes red shifts for 2 and 14 nm at low and high concentration solutions, respectively. By comparing the spectrum of DNA solution with that of DNA–drug solution (Figs. 4 and 5), it can be observed that the addition of low concentrations of drug leads to a slightly bathochromic effect and obvious hyperchromicity changes, while that of high concentrations of the same drug results in both quite obvious bathochromic effect and hyperchromicity changes. These results indicate that the interaction of BET–DNA system is mainly the electrostatic function at low concentrations, and that of the system is chiefly the insert function at high concentrations (Long and Barton, 1990).

3.5. The effect of BET on the binding of berberine to DNA

No luminescence is observed for BET in any solvent or even in the presence of DNA. To further understand the interaction of the DNA with BET, berberine (BR) has been applied as probe in our work. BR is a natural plant product obtained from various species of Berberis, belonging to the camptothecin family of drugs. It has been traditionally used to fight a number of infectious organisms, and its sulfate, hydrochloride, and chloride forms are used in pharmaceutical medicine as antibacterial agents (Megyesi and Biczók, 2007; Domadia et al., 2008; Tian et al., 2008). Being different from highly toxic fluorescent probe such as ethidium bromide (Ren and Chaires, 1999; Jadhav and Maiti, 2008), BR can be widely applied as a non-toxic fluorescent probe (Xia et al., 2007). It has an extremely weak intrinsic fluorescence emission spectrum with a peak wave length (λ_{max}) at 550 nm in aqueous solution, but it can emit quite intense fluorescence in the presence of DNA due to its strong intercalation between the bps of double-stranded DNA and it has been demonstrated being a perfect intercalator (Islam and Kumar, 2008).

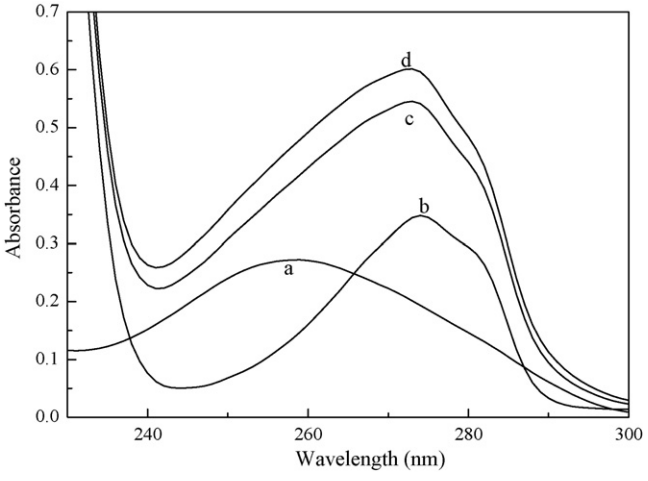

Fig. 5. The UV absorption spectra of the interaction between BET and DNA at the high concentration in the buffer solution (pH 7.40), [DNA] = 70 μM, [BET] = 2.6×10^{-4} M. (a) ct-DNA; (b) BET; (c) the simple superposition of (a) and (b); (d) the mixed solution.

Our experiments show that the fluorescence intensity of the DNA–BR system is weakened by adding BET, and the quenching effect becomes more obvious with increase of BET concentration. It has been reported that the enhanced fluorescence can be quenched, at least partially, by the addition of a second drug to DNA–BR interaction system (Baguley and Bret, 1984). The extent of quenching fluorescence of DNA-bound BR can be used to determine the extent of binding between the new drug molecules and DNA molecules. So steady-state competitive binding experiments using BET as a quencher may provide further information for study on the binding of the drug to DNA. The emission spectra of DNA binding with BR in the absence and the presence of the drug are shown in Fig. 6. From the curves in this figure, a slight decrease of the emission intensity is observed in the range of low concentrations (<156 μM). Nevertheless, the quenching effect becomes quite stronger in the higher concentration (≥156 μM) range of BET.

To better understand the mechanisms of the DNA–BET interactions, the Scatchard plots belonging to the inhibitory effect of BR binding with DNA in the absence and in the presence of BET were obtained according to literature (Moosavi-Movahedi et al., 2004) (see Fig. 7). The emission of BR–DNA complex is quenched by BET and the Scatchard plots show that competitive and noncompetitive behavior coexists for such quenching at both low and high concentrations. This result indicates that the binding sites of drug and BR on DNA molecule are not exactly the same. When drugs interact with DNA they can compete with BR and insert themselves into the double helix made up of bps, as well as bind with the phosphate groups of DNA by the electrostatic interaction and hydrogen bonds.

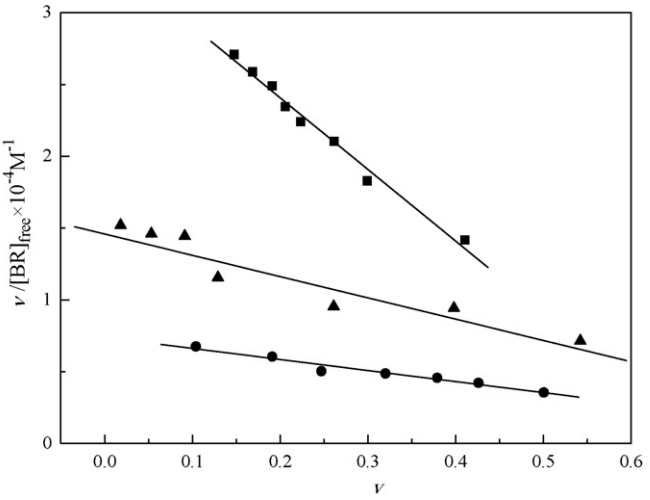

Fig. 7. The Scatchard plots of the binding of BR to DNA in the absence of BET (■), and in the presence of BET: 5.2 μM (▲), 260 μM (●). The DNA concentration was 44.5 μM and BR concentration varied from 20 to 100 μM.

3.6. Circular dichroism study

CD spectroscopy is a useful technique in diagnosing the changes of DNA morphology during drug–DNA interactions as the CD signals are quite sensitive to the mode of DNA interactions with small molecules (Chen et al., 2008). The CD spectrum of DNA is the representative B-conformation when no ligand/drug is in existence, which contains a positive band at 275 nm due to base group-stacking and a negative band at 245 nm due to the right-handed helicity (Sahoo et al., 2008). The changes in CD signals of DNA, observed for the interacting system, may often be assigned to the corresponding changes in DNA structure (Lincoln et al., 1997). Thus electrostatic interaction of small molecules shows less or no perturbation on the base-stacking and helicity bands, while intercalation enhances the intensities of both the bands stabilizing the right-handed B-conformation of ct-DNA (Maheswari and Palaniandavar, 2004).

The CD spectra of the DNA in absence and presence of increasing concentrations of BET are shown in Fig. 8, which can show the influence of the ligand on the conformation of DNA molecules. The BET molecules do not exhibit any CD signal in the region of 220–320 nm,

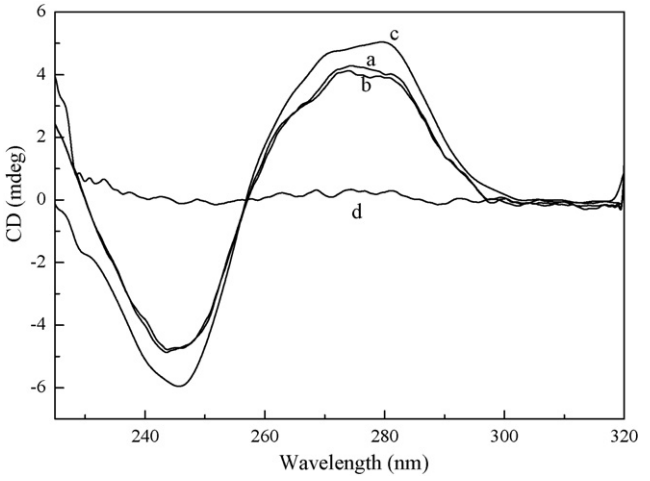

Fig. 8. CD spectra of ct-DNA (44.5 μM) in absence (a) and presence of BET. The concentrations of BET were: (b) 10 μM, (c) 0.45 mM and (d) free BET (0.26 mM) is along the abscissa.

however, they can cause some change in the CD signal of the chiral DNA molecules. In fact, a slight decrease in the positive band at low concentration of BET (10 μM) can be observed with no change in band shape or induction of a new band. No conformational change and/or significant unwinding of DNA base pairs are observed nor is indication of any other form of DNA. These results indicate that the electrostatic interaction played a more important role in this case (Shi et al., 2006). The CD spectrum at higher concentration of BET shows an intensity increase of both positive and negative bands without shift in the peak positions, suggesting a typical strong intercalation involving π-stacking and stabilization of the right-handed B form of ct-DNA (Maheswari and Palaniandavar, 2004). Thus the CD spectral results are consistent with those of ITC, UV and FS experiments, showing that cooperativity is existent in the interaction system.

4. Conclusions

The binding of DNA with BET has been investigated in detail by isothermal titration calorimetry and three kinds of spectroscopy. Analyzing of the thermodynamic data indicates that there are two classes of sites on ct-DNA molecules for binding BET molecules. The first class of binding process is enthalpy–entropy synergically driven and the second one is predominantly entropy driven. All the results of the spectral experiments confirm that there are two different behaviors for binding of BET to DNA. One binding process is the ligand molecules associating with phosphate groups and takes place mainly at low ligand concentrations, while the other one is the drug molecules combining with bps, which mainly occurs at high ligand concentrations. The CD spectra indicate that the conformation of the DNA molecule in solution is slightly changed by the drug molecules.

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