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Interaction study between double-stranded DNA and berberine using capillary zone electrophoresis

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Abstract

Two non-self-complementary 17-mer double-stranded DNA (dsDNA) with four different central base pairs were designed to systematically investigate the binding affinity and sequence specificity of berberine with dsDNA by capillary zone electrophoresis (CZE). The data analysis with the Kenndler model proved only low affinity between dsDNA and berberine and suggested some weak binding preference of berberine for AATT-containing to GGCC-containing dsDNA. The binding constant, K_a, between berberine and $dsDNA_{AB}$ was about $(1.0 \pm 0.7) \times 10^3\ M^{-1}$. In addition, the separation of single-stranded DNA (ssDNA) from dsDNA under simple electrophoretic conditions enabled CZE to be a potentially alternative tool to check the extent of DNA annealing, which is usually done by the time-consuming and labor-intensive slab electrophoresis.

Keywords: Berberine; DNA; Capillary zone electrophoresis; Interaction; DNA annealing

1. Introduction

Nowadays, it has been proved that some special base sequences in DNA could be used as potential target sites for anticancer drugs, which might lead to the cleavage or morphological changes of the helix duplexes and other biological processes [1]. Berberine is an active constituent of some Chinese herbal medicines such as *Rhizoma coptidis* ('Huang-Lian') or *Cortex phellodendri* ('Huang-Bo') and belongs to the camptothecin (CPT) family of drugs [2], which are characterized by their ability to induce DNA topoisomerase poisoning and hence the apoptosis of cells. Although the cytotoxicity of berberine is several orders of magnitude lower than that of CPT [3], it retains the anti-cancer activities [3–7], the inhibitory effect on the oxidative damage [8,9], the anti-microbial [10,11] and anti-inflammatory activities [12] without the unwanted side effects of CPT [3], all of which has promoted the research into the interaction between berberine and macrobiomolecules. Most of these studies related to the biological activities and anti-cancer mechanisms of berberine focus on the interaction between the alkaloid and DNA.

To the best of our knowledge, there is still no comprehensive understanding concerning the binding affinity and sequence selectivity of the interaction between berberine and DNA. Li et al. have suggested a high affinity and groove binding characteristic between berberine and double helical DNA by spectral methods in their work [13]. Similarly, the research of He et al. has shown that berberine hydrochloride interacted with calf thymus-DNA in the mode of strong intercalation by UV–Vis spectrum, fluorescent spectrum, fluorescent polorization, etc. [14]. Wang and Yu have also presented that grooving binding was very likely to be the dominant mode in the strong interaction of berberine with DNA by using Fourier transform-surface enhancement raman scattering spectroscopy [15]. In contrast, competition dialysis has shown that berberine preferred to bind poly(dA)-[poly(dT)]$_2$ triplex DNA, while as far as other structural forms of DNA, such as Z DNA, poly A: poly U, poly A: poly (dT) and poly(dGdC), etc. were concerned, berberine exhibited a greatly reduced or no binding ability [16]. Similarly, Das et al. have suggested that spectroscopic and thermodynamic

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studies show berberine could bind and stabilize the DNA and RNA triplexes more strongly than it did to their respective parent duplexes [17]. Recently, other studies described berberine being strongly bound to single-stranded poly(rA) but not double stranded poly(rA) by a mechanism of partial intercalation [18]. This finding is consistent with the suggestion that berberine has only weak affinity with the duplex DNA [19]. As far as the binding specificity is concerned, there is still some discrepancy between the results of different experiments. UV and NMR spectroscopy studies have indicated that non-specific interaction was predominant between berberine and double helix oligodeoxynucleotides and the binding was preferential to AT-rich sequences [20]. Our previous studies using electrospray ionization mass spectrometry and UV/fluorescence titration have suggested no significant or remarkable AT- or GC-rich DNA binding preference of berberine [21]. In addition, the recent studies of the interaction between berberine and nucleotides by affinity capillary electrophoresis have suggested that the interaction with compounds derived from purine was always stronger than those derived from pyrimidine [22].

Compared with other analytical techniques, CE has exhibited its great power in the field of interaction studies, such as systems of protein and ligands or of DNA and small molecules, owing to advantages like short analysis time, low sample size requirement, relatively high separation efficiency and flexible modes of operation for different applications [23–27]. Among all the modes of CE, which are suitable for interaction studies, capillary zone electrophoresis (CZE) requires much less sample and simpler operational conditions than others do and has already been used in different interaction systems [28–31]. These advantages meet the requirements for the study of biomolecular interactions [32]. The studies of the interaction between DNA and drug molecules have been reported before, but most investigations are restricted to model drugs with definite mechanisms of action [28,29].

In this paper, CZE was applied to study the binding performance of berberine with two specially designed 17-mer dsDNA. The electrophoretic performance of the Hoechst 33258 and dsDNA with specific base tracts was also studied as a positive control of DNA binding. Hoechst 33258, which owns a crescent shaped structure, has long been recognized as a model DNA minor groove binder and is widely used as a generalized DNA stain and an active antihelminthic drug as well [33,34]. Numerous experimental results of X-ray crystallography and NMR have shown its site specificity of a 4–6 base pair long AT-tract oligonucleotide when it binds to DNA [35–38]. The interaction between berberine and the dsDNA were quantitatively characterized through changes of the peak parameters on the electropherogram using the Kenndler model [31], which was applicable in the interaction study of low affinity systems compared with the Scatchard model generally used for moderate or high affinity systems [29,30,39–42]. Adsorption of the analytes onto the inner wall may be the cause of poor repeatability of the CE technique. However, most of the published papers have focused on the adsorption of protein. The work described in this paper systematically examines the mobility of DNA in linear polyacrylamide coated and uncoated capillaries and demonstrates the advantages of using a coated capillary in the interaction study with greatly improved repeatability and sensitivity of the electrophoretic performances. In addition, the migrating difference of the 17-mer dsDNA and ssDNA in the polyacrylamide-coated capillary is further studied and could be used as a potentially alternative method to check the annealing extent of the DNA.

2. Experimental

2.1. Apparatus

All the experiments were carried out in the P/ACE MDQ capillary electrophoresis system (Beckman, Coulter, Fullerton, CA, USA) equipped with a photodiode array detector. The uncoated fused-silica capillary tubing, 50 μm i.d. × 31.2 cm, was purchased from the Yongnian Optical Fiber Corp. (Hebei, China), and capillary length to detector of 21 cm. Coating of the capillary with linear polyacrylamide was achieved using described procedures [43].

2.2. Materials and reagents

The non-self-complementary oligodeoxynucleotides:

Strand A: 5′-CTGAGACTAATTGACTG-3′, $M_r = 5209.4$, $\xi = 166{,}200$ L/(mol cm).
Strand B: 3′-GACTCTGATTAACTGAC-5′, $M_r = 5169.4$, $\xi = 165{,}300$ L/(mol cm).
Strand C: 5′-CTGAGACTGGCCGACTG-3′, $M_r = 5211.4$, $\xi = 158{,}000$ L/(mol cm).
Strand D: 3′-GACTCTGACCGGCTGAC-5′, $M_r = 5171.4$, $\xi = 155{,}500$ L/(mol cm).

were purchased as single strand from Sunbiotech Co. Ltd. (Beijing, China). Berberine hydrochloride (see Fig. 1a) was obtained from National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China. Ammonium peroxodisulfate and Tris base were from Amersco Inc. (Solon, OH, USA), while EDTA disodium salt, acrylamide and Hoechst 33258 (see Fig. 1b) were from Sigma (St. Louis, MO, USA). 3-(Trimethoysilyl) propyl ethacrylate (γ-MAPS) and mesityl oxide (MO) (used as a neutral marker in CE) were from Fluka Chemie AG (Switzerland). TEMED was purchased from Promega (Madison, WI, USA). All the samples and buffer solutions were prepared with a Milli-Q water purification system (18.2 MΩ cm, 25 °C) (Millipore, Bedford, OH, USA) and filtered through 0.22 μm pore size filters. The buffer solutions were degassed by sonication before use. All other chemicals were analytical grade unless otherwise indicated.

2.3. Sample preparation

The oligodeoxynucleotides were dissolved in the buffer solution (10 mM Tris, 1 mM EDTA, 50 mM NaCl, adjusted to pH 8.0 with HCl) according to the proportion of 1 OD_{260} per 50 μL buffer solution. Concentrations of the oligodeoxynucleotide

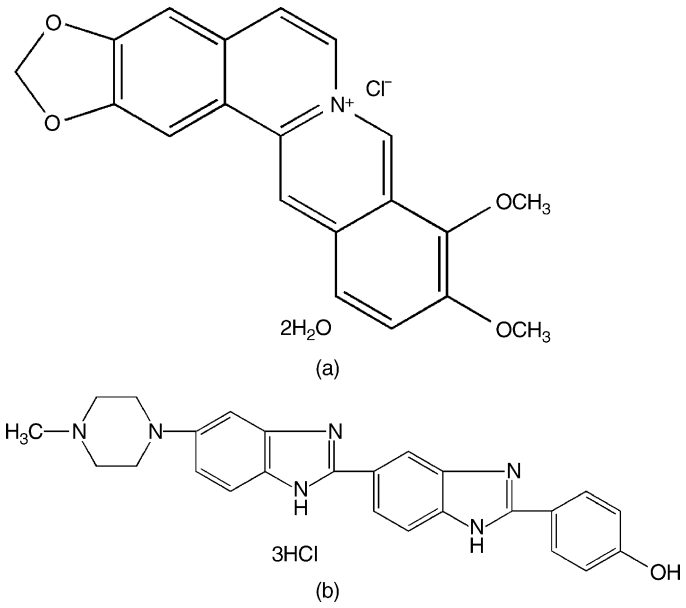

Fig. 1. (a) The chemical structure of berberine and (b) the chemical structure of Hoechst 33258.

solutions were measured by UV biophotometer (Eppendorf AG, Germany) at 260 nm using the molar absorptivities provided by Eurogentec [44]. The annealing of the complementary ssDNA into dsDNA was carried out according to the following steps. Two complementary strands (strands A and B, strands C and D) were mixed together carefully in equal molar amounts with the surface of the solution covered with a layer of mineral oil. These solutions were heated at 94 °C in water bath for 3–4 min and then the machine was unplugged allowing the solutions to cool down naturally to the room temperature. The $dsDNA_{AB}$ (formed by strands A and B) and $dsDNA_{CD}$ (formed by strands C and D) solutions were stored at −20 °C.

The stock solution of berberine hydrochloride (6.12 mg/100 mL) and Hoechst 33258 (2.35 mg/10 mL) were prepared with the buffer solution (10 mM Tris, 1 mM EDTA, adjusted to pH 8.0 with HCl) and stored at 4 °C. A series of sample solutions with constant concentration of dsDNA and increased concentrations of berberine hydrochloride were incubated at 8 °C overnight. While for Hoechst 33258, all the samples were prepared just before use and kept from light as Hoechst 33258 is light sensitive.

2.4. Capillary electrophoresis

The electrophoretic conditions for CZE in a coated capillary were as follows: the running buffer consisted of 20 mM Tris, 1 mM EDTA and was adjusted to pH 7.4 with acetic acid. The temperatures of the cartridge and sample room were both 25 °C. Samples were injected into the capillary using the pressure injection mode at 0.5 psi for 3 s and detected at 260 nm. The separation was carried out under a reverse voltage of 10 kV. Between successive runs, the capillary was rinsed with deionized water and running buffer for 2 min each.

The electrophoretic conditions for CZE in an uncoated capillary were as follows: separations were carried out under a forward voltage of 15 kV. At the interval of successive runs, the capillary was rinsed with 0.1 M NaOH, deionized water and running buffer for 5 min each. Other conditions were the same as those described above.

3. Results and discussion

3.1. Difference between uncoated capillary and linear polyacrylamide coated capillary

Different experimental conditions were explored to improve the repeatability and increase the detection sensitivity. Generally, molecules with $M_r > 100{,}000$ or $pI > 8.4$, tend to be adsorbed onto the inner capillary wall, which is detrimental to the quantification and should be avoided [27]. Although, the molecular weights of dsDNA used in this work were all smaller than 11,000 and they were negatively charged because of phosphate groups, adsorptions of dsDNA samples were still observed in an uncoated capillary, which reflected in the tailing peak and poor repeatability. Fig. 2 shows three successive runs of the same sample containing 10 μM $dsDNA_{AB}$ mixed with 2 μM berberine in the uncoated capillary. The peak #1 was of $dsDNA_{AB}$, the peak #2 was of MO indicating that the EOF and the peak of berberine was too weak to be detected. The migration time of dsDNA in the third run was 30.1% longer than that of the first run. The peak height and the theoretical plate number in the third run were 23.0 and 28.6%, respectively, which were lower than those in the first run. Such a poor repeatability led to the difficulty of both qualification and quantification. However, in the experiments carried out in the coated capillary, the fluctuation in the migration time of every two successive runs of the same sample was well controlled under 0.10%. The signal to noise ratio was ∼1.8 times larger in the coated capillary than that in the uncoated capillary. Compared with the similar phenomenon appearing in analysis of samples containing protein,

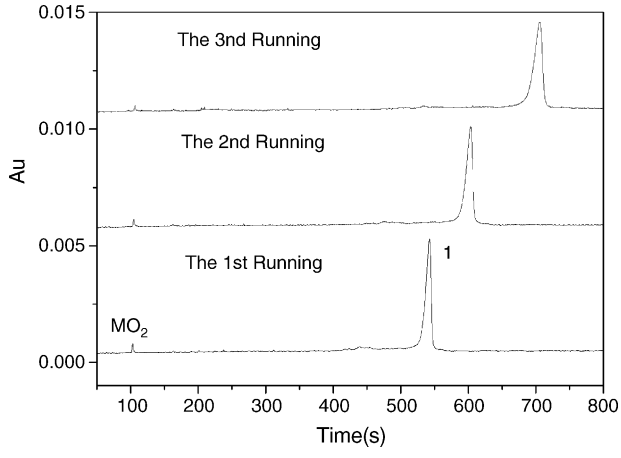

Fig. 2. Electrophoretic performance for three successive runs of the same sample containing 10 μM $dsDNA_{AB}$ mixed with 2 μM berberine in an uncoated capillary. The peak #1 was of $dsDNA_{AB}$, the peak #2 was of MO indicating the EOF. The traces belonged to the 1st run, the 2nd run and the 3rd run from bottom to top. The electrophoretic conditions were described in Section 2.4.

we deduce that the dsDNA is also easily adsorbed on the inner wall of the uncoated infused silica capillary. During each run, a few molecules of dsDNA may be dynamically adsorbed onto the inner wall of the capillary, although the flushing of the capillary with the buffer solutions may alleviate the adsorption status of the inner wall, the number of the molecule adsorbed on the wall seems to be aggrandized gradually with the increase of the running times. The adsorbed molecule layers formed by the former runs may lead to the augment of the distribution ratio for dsDNA in the phase of adsorbed layer and buffer solution, which will in turn result in higher adsorption. However, when the inner wall of the capillary is coated with linear polyacrylamide, the adsorption of dsDNA is largely reduced, which in turn results in the improved electrophoretic performance of dsDNA.

3.2. Novel approach to check the annealing extent of oligodeoxynucleotide

Two groups of non-self-complementary oligodeoxynucleotides d (CTGAGACTAATTGACTG)·d (GACTCTGATTAACTGAC) (AATT 17-mer) and d (CTGAGACTGGCCGACTG)·d (GACTCTGACCGGTGAC) (GGCC 17-mer) were used to represent two kinds of sequences with the central base tracts of AATT and GGCC, respectively. These two dsDNA were designed according to the following rules: (1) base sequences were designed to make sure the least possibility of the formation of hairpin loops or interstrand base pairings during the annealing process; (2) the sequence lengths were all longer than 16-mer to ensure an integral double helix in case of the existence of grooving binding mode. Longer strands also have a higher melting temperature T_m (the temperature at which half of the DNA strands are single stranded and half are double-stranded). In our model, T_m was 42 °C for $dsDNA_{AB}$ and 52 °C for $dsDNA_{CD}$. The likely melting of shorter dsDNA into the corresponding ssDNA during analysis is undesirable as it increases the complexity of the measurement. In solution of low ionic strength, dsDNA could maintain the double helix B-form geometry and the result therefore reflect the interaction between berberine and dsDNA.

The electropherograms of the dsDNA and ssDNA in a coated capillary are shown in Fig. 3. The concentration of single strand C ($ssDNA_C$), single strand D ($ssDNA_D$) and $dsDNA_{CD}$ formed by annealing these two complementary strands were 30, 30 and 15 μM, respectively. The migration time of the $dsDNA_{CD}$ was 3.079 min, which was shorter than those of $ssDNA_C$ (3.167 min) and $ssDNA_D$ (3.192 min). The mobilities of the 17-mer dsDNA and ssDNA were quite different from the result reported previously [45], which suggests that differential mobilities between ss- and dsDNA molecules begin to be observed when the size of the oligomer increases beyond ~20 nucleotides. Our results show that even under the size of 20-mer, differences are observed in the electrophoretic mobilities of dsDNA and ssDNA, given that the T_m was higher enough to ensure the stability of dsDNA. These results suggest that CZE is a novel method to determine the extent of DNA annealing as an alternative to time-consuming slab electrophoresis.

3.3. CZE study of the interaction between berberine and $dsDNA_{AB}$/$dsDNA_{CD}$

Changes in the free $dsDNA_{AB}$ peaks for a series of samples containing 15 μM $dsDNA_{AB}$ mixed with various concentrations of berberine are shown in Fig. 4a. The concentrations of berberine in each sample were 0, 4, 15, 40 and 110 μM, respectively. According to the results presented in Section 3.2, the peak named #1 was likely to be one of the $ssDNA_{A/B}$, which was in stoichiometric excess in the annealing mixture. However, the existence of ssDNA in turn proved the possibility of separating ssDNA from dsDNA under same conditions. The peak named #2 indicated the free $dsDNA_{AB}$ in the equilibrium system. As each run was carried out under a reverse voltage, the positively charged berberine was not able to reach the detection window where $\mu_{eo} \approx 0$ (mesityl oxide was added to the sample both in coated and uncoated capillary electrophoresis as an indication of EOF). No other peaks were detected in this experiment or in similar experiments carried out in an uncoated capillary. The fluctuation in the migration time of the peak #2 in these five runs was very small with 0.25% R.S.D. A relatively small decrease in the peak height for $dsDNA_{AB}$ with increasing berberine concentration suggested a weak-binding affinity between $dsDNA_{AB}$ and berberine.

Similarly, the changes in the free $dsDNA_{CD}$ peaks for a series of samples containing 15 μM $dsDNA_{CD}$ mixed with various concentrations of berberine ranging from 0 to 110 μM, are shown in Fig. 4b. The concentrations of berberine in each sample were 0, 4, 15, 30, 60 and 110 μM, respectively. Exact equal molar amounts of the complementary strands gave complete DNA annealing with no peaks attributable to single stranded DNA appearing in the electropherograms on the coated capillary. The fluctuation in the migration time for $dsDNA_{CD}$ in these six runs was 0.20% R.S.D. Compared with the analysis of $dsDNA_{AB}$ berberine mixtures, there was no obvious trend of change in peak height of $dsDNA_{CD}$, indicating a much weaker binding affinity.

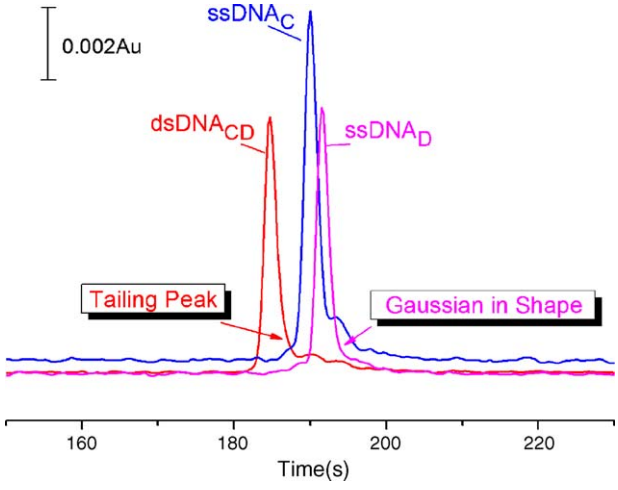

Fig. 3. Comparison of the mobilities of ssDNA and dsDNA in a coated capillary. The concentrations of the $ssDNA_C$ (strand C) and $ssDNA_D$ (strand D) and $dsDNA_{CD}$ were 30, 30 and 15 μM, respectively. The electrophoretic conditions were described in the Section 2.4.

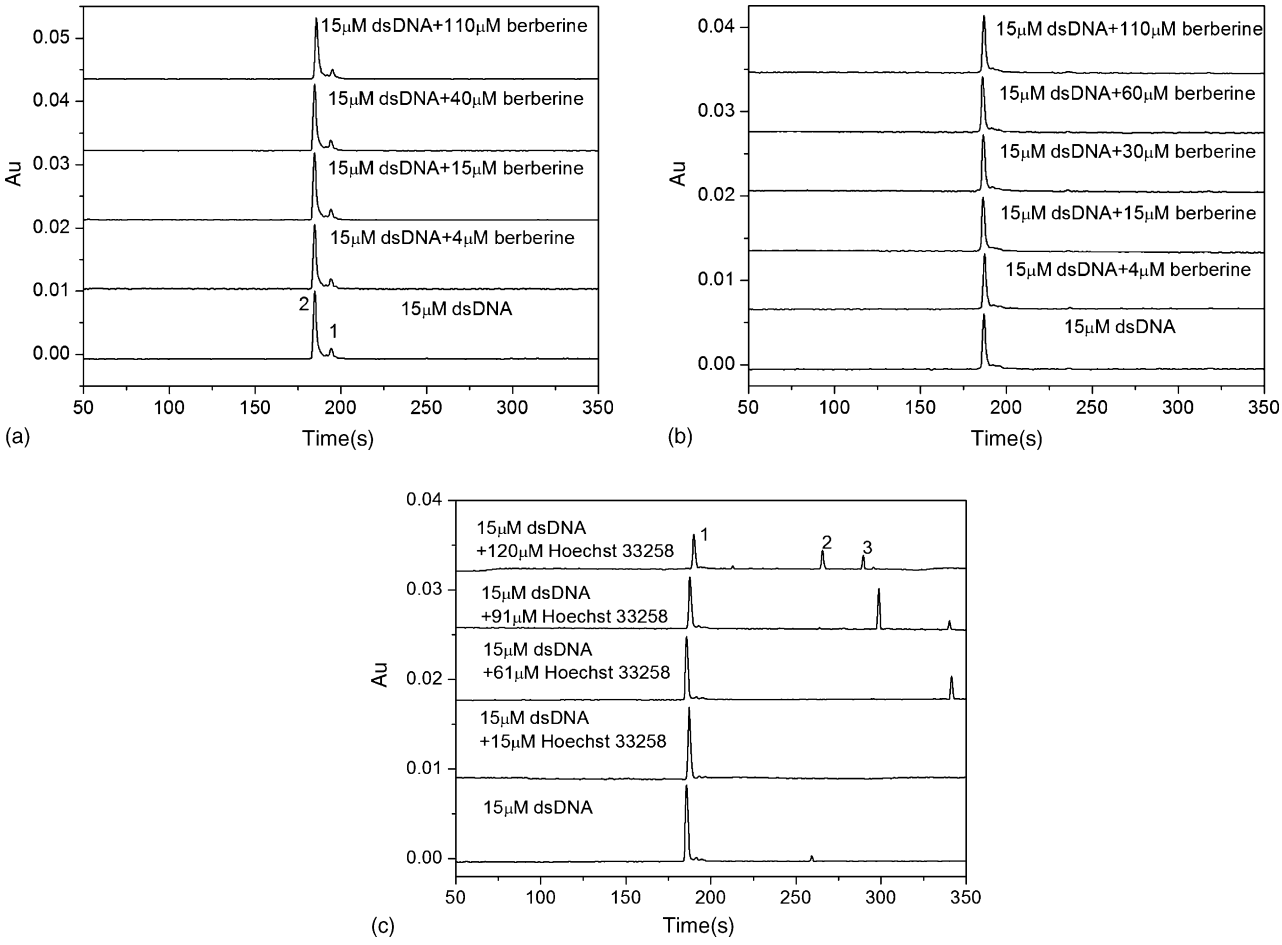

Fig. 4. (a) Electropherograms observed for a series of samples containing 15 μM $dsDNA_{AB}$ mixed with various concentrations of berberine. (b) Electropherograms observed for a series of samples containing 15 μM $dsDNA_{CD}$ mixed with various concentrations of berberine. (c) Electropherograms observed for a series of samples containing 15 μM $dsDNA_{AB}$ mixed with various concentrations of Hoechst 33258. The running conditions were described in the Section 2.4.

3.4. CZE study of the interaction between Hoechst 33258 and $dsDNA_{AB}$

Electropherograms observed for a series of samples containing 15 μM $dsDNA_{AB}$ mixed with various concentrations of Hoechst 33258 are shown in the Fig. 4c. The concentrations of Hoechst 33258 in each sample were 0, 15, 61, 91 and 120 μM, respectively. Compared with Fig. 4a and b, the change in the peak height of the free dsDNA (peak #1) in the equilibrium system was more distinct. Peaks #2 and #3 indicated the formation of the associate complexes with different stoichiometry or associative configuration between Hoechst 33258 and $dsDNA_{AB}$ when excessive dose of drug was added into the sample.

3.5. Quantitative evaluation of the interaction of berberine with $dsDNA_{AB/CD}$ using the Kenndler analysis

As neither of the reactants was added into the background electrolyte (BGE), the dissociation of berberine from dsDNA might occur on column. Our previous studies have already demonstrated the existence of non-covalent complexes formed by berberine and dsDNA using negative ESI-MS and fluorescence spectrometric methods [21]. Changes to the profile of the dsDNA peaks are usually attributed to some dissociation of the berberine–DNA complex when passing through the column and are most apparent with changes in the concentration of ligand. Such techniques are widely used to calculate the binding constants [28–30], and may be used to reflect the magnitude of the binding affinity.

The R.S.D. ($n \geq 4$) of the dsDNA peak heights for multiple analyses of the same sample was calculated as a measure of the precision of the method and was less than 2.5%. As a comparison, in the interaction study between berberine and $dsDNA_{AB}$, the R.S.D. of the fluctuation in the peak height due to the concentration change of the berberine was 4.0%, while for the study between berberine and $dsDNA_{CD}$, the value was only 2.1%, even lower than that of the precision test. Therefore, the interaction between berberine and $dsDNA_{CD}$ was insignificant in this study.

The dissociation constant (K_d) was deduced using the Kenndler model, where $K_d = K_a^{-1}$ and K_a is the binding constant of the systems. To simplify the quantification, it was assumed that the formation of the associate complex had 1:1 stoichiometry between the ligands and dsDNA. In the experiment, the average peak height of dsDNA obtained from repeated CE runs was proportional to the concentration of the free dsDNA in the equilibrium system. According to the Kenndler model, the

Table 1
Comparison of the association constants (K_a) for the interactions between berberine and oligonucleotide/nucleoside obtained from different methods

Oligonucleotide or nucleoside	K_a (M^{-1})	Methods	Buffer solution
5′-d(CTGAGACTAATTGACTG)$_2$-3′	$(1.0 \pm 0.7) \times 10^3$	CZE	Tris-acetic acid (pH 7.4)
5′-d(ACATCAAAAAGGT)$_2$-3′ [20]	$(1 \pm 0.1) \times 10^3$	UV spectrum	H_2O/D_2O (pH 5.8)
5′-d(ACATCAAAAAGGT)$_2$-3′ [20]	$(1 \pm 0.1) \times 10^3$	^{1}H NMR	H_2O/D_2O (pH 5.8)
5′-d(AAGAATTCTT)$_2$-3′ [21]	$(1.24 \pm 0.07) \times 10^4$	Fluorescence spectrum	Tris–HCl (pH 6.35)
Adenosine [22]	37 (2)	ACE	Sodium phosphate (pH 7.4)
Cytidine [22]	10 (4)	ACE	Sodium phosphate (pH 7.4)
Thymidine [22]	5 (1)	ACE	Sodium phosphate (pH 7.4)

equilibrium concentration of dsDNA, $[dsDNA]_f$ is as a function of the initial concentration of the ligands, $[Ligand]_0$:

$$[dsDNA]_f = [dsDNA]_0 - \frac{([dsDNA]_0 + [Ligand]_0 + K_d)}{2} \pm \sqrt{\left\{\frac{([dsDNA]_0 + [Ligand]_0 + K_d)}{2}\right\}^2 - [dsDNA]_0[Ligand]_0} \quad (1)$$

where $[dsDNA]_0$ is the initial concentration of the dsDNA. The result of nonlinear least-squares data fitting by the Gauss-Newton method (solved by using MATLAB v.6.5) gives the value of K_d, coefficient of the nonlinear function Eq. (1). Fig. 5 is the Kenndler plot of $dsDNA_{AB}$ and berberine showing the dependence of the equilibrium concentration of $dsDNA_{AB}$, $[dsDNA]_f$, on the initial concentration of berberine, $[Ligand]_0$, respectively. For the interaction study between berberine and $dsDNA_{AB,}$ nonlinear regression showed $K_d = (1.0 \pm 0.7) \times 10^{-3}$ M and $K_a = (1.0 \pm 0.7) \times 10^3$ M^{-1}. The value of $\lg K_d/[dsDNA_{AB}]_0$ was ~1.8, which indicated the interaction belonged to the low affinity range [31]. Meanwhile, the results were quite comparable with those presented using UV, ^{1}H NMR, Fluorescence titration, etc. [20–22] (Table 1). The differences between these constants are likely to be the result of using different methods of analysis as well as differences in the reactants. It must be pointed out that the systematic error of this estimation of K_d using the Kenndler model might increase if there existed more complicated binding stoichiometry or associative configurations at high ligand concentrations.

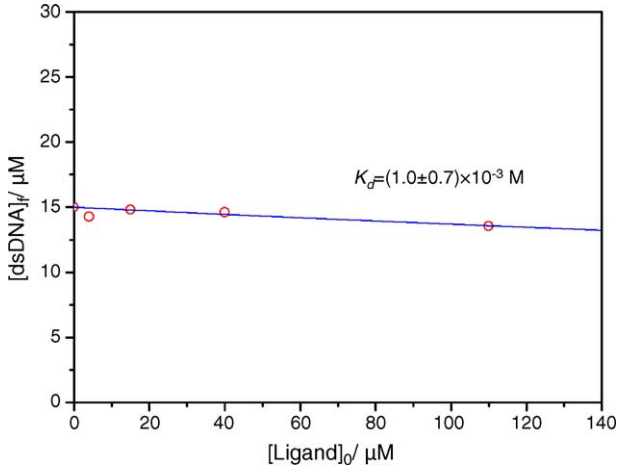

Fig. 5. Kenndler plot of $dsDNA_{AB}$ with berberine, showing the dependence of the equilibrium concentration of $dsDNA_{AB}$, $[dsDNA]_f$, on the initial concentration of ligands, $[ligand]_0$, respectively. The values of K_d was calculated by nonlinear regression for the system of $dsDNA_{AB}$ and berberine.

Changes in the profile of the receptor peak usually represent the characteristics of many model DNA-intercalators and groove binders in CE studies [28,29], which is reaffirmed by the interaction study between Hoechst 33258 and $dsDNA_{AB}$. However, berberine showed no obvious effects on the profile of the free dsDNA peaks. The data presented in this study suggests that berberine shows greater preference to bind the $dsDNA_{AB}$ with the central base tracts of AATT than to bind the $dsDNA_{CD}$ with GGCC. It might be partially because the $dsDNA_{AB}$ had more negative electrostatic potential than $dsDNA_{CD}$ and therefore enhanced affinity for positively charged molecules. Alternative influences may include factors such as steric hindrance or dimension of these two kinds of sequences [20]. Other factors, such as the effect of the electric field on the interaction process, the self-assembly of the drug molecules under high concentration, have not been considered here. The results were enough to reveal the characteristics of berberine-dsDNA interaction and to provide an approach for further studies on weak affinity systems. The concentration range for which the binding constant between dsDNA and berberine were calculated was limited both by the solubility of the ligands in the buffer solution and by the limits of sensitivity.

Nonlinear least-squares data fitting gave binding constants that compared well with similar data derived from alternative techniques. Further studies are being carried out in our lab for exploring the binding performance in a larger concentration range of the reactants and will be summarized in our next study.

4. Conclusion

A simple and rapid method for separating the 17-mer ssDNA and dsDNA by CZE in linear polyacrylamide coated capillary without adding any sieving matrix in BGE is described as an alternative to slab electrophoresis in checking the annealing extent of DNA. The Kenndler model was used to explore the interaction of berberine and dsDNA with different central base sequences. A weak binding affinity between berberine with dsDNA and a weak-binding preference for AATT-containing rather than GGCC-containing dsDNA was found. In the present paper, CE has shown its superiority in studying the interactions between macrobiomolecules and their ligands.

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