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DNA-binding affinities and sequence selectivity of quaternary benzophenanthridine alkaloids sanguinarine, chelerythrine, and nitidine

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This paper is dedicated to Professor Irving H. Goldberg, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, on the occasion of his 80th birthday.

Abstract—A comparative study on the intercalating binding of sanguinarine, chelerythrine, and nitidine with CT DNA, poly(dG–dC)·poly(dG–dC), poly(dA–dT)·poly(dA–dT), and seven sequence-designed double-stranded oligodeoxynucleotides has been performed using fluorometric and spectrophotometric techniques, aiming at providing insights into their sequence selectivity for DNA-binding. The results show that both sanguinarine and nitidine bind preferentially to DNA containing alternating GC base pairs [d(TGCGCA)$_2$], while chelerythrine exhibits quite distinct sequence selectivity from sanguinarine, which shows a high specificity for DNA containing contiguous GC base pairs [5′-TGGGGA-3′/3′-ACCCCT-5′].
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1. Introduction

The anticancer activity of many small organic molecules is believed to be due to their interaction with DNA. The elucidation of the physico-chemical characteristics of such interactions is of considerable interest from viewpoints of bioorganic chemistry. Among the biologically active molecules from natural sources, alkaloids occupy an important position because of their extensive medicinal values, such as anticancer, antimicrobial and a host of other biological activities.[1–3]

Recently, the quaternary benzophenanthridine alkaloids, which are mainly distributed in Papaveraceae (*Chelidonium majus*, *Eomecon chionantha*, and *Macleaya cordata*) and Fumaraceae (*Corydalis ophiocarpa*, *Fumaria schrammii*) plants, have been the focus of increasing attention for their diverse biological activities. These botanical alkaloids possess potent anticancer,[4–7] promising antimicrobial,[8] apoptosis activities[6] as well as cytotoxic[6,9] and antiplaque effects.[10] In fact, the plants containing these alkaloids have been widely used as Chinese medicines and folk medicines for the treatment of human cancer, etc. In the UK and USA, sanguinarine has been in use in mouthwashes and toothpastes to inhibit dental plaque and improve gingival health.[11] Studies on the anticancer activities of sanguinarine, chelerythrine, and nitidine (Fig. 1) have suggested the development of them as potent anticancer drugs.[5–7,9]

The potential anticancer activities of these quaternary benzophenanthridine alkaloids may be attributed to their binding to chromosomal DNA. DNA-intercalating molecules have played an important role in the treatment of human cancer. As a typical quaternary benzophenanthridine alkaloid, sanguinarine was proved to be a strong DNA intercalator.[12] Recent studies have revealed that sanguinarine exhibits a remarkable pH-dependent structural equilibrium (Fig. 1) between iminium form (charged) and alkanolamine form (uncharged) with a pK_a value (in the absence of DNA) of 7.4 as revealed from spectrophotometric titration experiments in buffers of pH 5.2 and pH 10.4 where the physicochemical properties of DNA remain B-form structure.[13,14] It was shown that only iminium form of sanguinarine binds to DNA by intercalation,[14] with a higher specificity binding to GC-rich DNA,[15] while the

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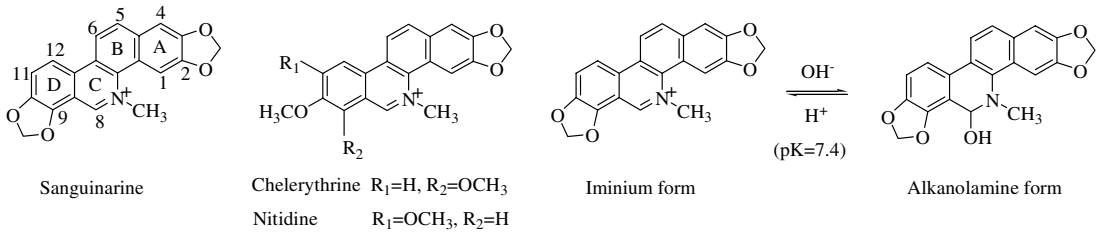

Figure 1. Chemical structures of three quaternary benzophenanthridine alkaloids and two forms of sanguinarine.

alkanolamine form does not bind to DNA. But alkanolamine form can be transformed to charged iminium form at a very high concentration of DNA. This conversion of alkanolamine form to iminium form induced by DNA can be explained by a shift of equilibrium.[14]

Chelerythrine and nitidine, which slightly differ from sanguinarine in the type and position of substituents in their aromatic D rings, are the active constituents of Chinese herbal medicines *Herba Chelidonii* ('Bai-Qu-Cai' in Chinese) and *Radix Zanthoxyli Nitidi* ('Ru-Di-Jin-Niu' in Chinese), respectively. Nitidine displays antileukemic activity and acts as topoisomerase I and topoisomerase II inhibitors.[16,17] Both nitidine and chelerythrine exhibit pronounced cytotoxicity[6,9,16,18] and anticancer activity,[6,7,9,18,19] which may result from their capacities to intercalate into DNA.[16] However, studies on their interactions with DNA are so far limited to calf thymus DNA (CT DNA), no work has been reported on the study of their interactions with other DNAs, such as poly(dG–dC)·poly(dG–dC), poly(dA–dT)·poly(dA–dT), and sequence-designed oligodeoxynucleotides. To the best of our knowledge, no sequence selectivity investigation has been previously reported on these three quaternary benzophenanthridine alkaloids. In addition, comprehensive study has been carried out on the binding of these three quaternary benzophenanthridine-type alkaloids to DNA. Consequently, their structure–activity relationships remain to be established.

In this paper, we describe the systematic study of the binding of three quaternary benzophenanthridine alkaloids, that is, sanguinarine, chelerythrine, and nitidine, to CT DNA, polydeoxynucleotides (poly(dG–dC)·poly(dG–dC) and poly(dA–dT)·poly(dA–dT)), and a series of sequence-designed double-stranded oligodeoxynucleotides using fluorescence spectrometric techniques. The results are expected to clarify their DNA-binding capacity, sequence selectivities, and structure–activity relationships. Furthermore, we present a detailed study on the determination of binding mode of these three alkaloids by using UV–vis absorption titration and competitive Ethidium Bromide (EB) displacement assays.

2. Results and discussion

2.1. DNA-binding affinities and sequence selectivities of three alkaloids

A wide variety of physical, chemical, and biochemical techniques have been proposed for determining binding mode, stoichiometry, affinity, and selectivity of noncovalent complexes between small organic molecules and biomacro-molecules. The methods include UV–vis absorption and fluorescence spectroscopies, circular and linear dichroism, mass spectrometry, NMR, X-ray crystallography, gel electrophoresis, viscosity titration, etc.[20] Among them, fluorescence spectrometry is an analytical technique with high sensitivity which has been widely used in the investigation of noncovalent binding of small organic molecules with biopolymers.[3] The binding is reflected in the change, either enhancement or quenching, of fluorescence intensities.

In our study, acidic buffer at pH 5.30 was used throughout the whole experiments to ensure that three alkaloids exist exclusively as iminium form. Fluorescence titrations of DNA with three alkaloids are performed with the excitation at 345 nm, and the emission wavelengths for sanguinarine, chelerythrine, and nitidine are 569, 550, and 445 nm, respectively. Upon the binding to DNA, the fluorescences of sanguinarine and nitidine are greatly quenched, while that of chelerythrine is sharply enhanced. Changes in fluorescence intensity ensure the spectrofluorometric determination of the binding constants (K_a) of three alkaloids toward DNA. A representative spectrofluorometric titration spectrum of sanguinarine with $d(TGCGCA)_2$ is shown in Figure 2. The fluorescence intensity of sanguinarine is quenched very rapidly with the addition of increasing concentrations of DNA, indicating its strong interaction with DNA. These significant changes make the association constants of three alkaloids with DNA available, from the analyses of the relationship between the fluorescence intensities and the DNA concentrations by nonlinear curve fitting methods.[2,3] The binding of three alkaloids–DNA complexes has been determined to be in a 1:1 stoichiometry by molar ratio methods as previously described.[3] The binding affinities of three alkaloids with CT DNA, poly(dA–dT)·poly(dA–dT), poly(dG–dC)·poly(dG–dC), and double-stranded oligodeoxynucleotides are listed in Table 1 and illustrated in Figure 3.

From Table 1, more predominant GC than AT preference is observed in sanguinarine, chelerythrine, and nitidine. They exhibit higher binding affinities with poly(dG–dC)·poly(dG–dC) than with poly(dA–dT)·poly(dA–dT), showing certain GC preference. Sanguinarine binds to poly(dG–dC)·poly(dG–dC) 5.3-fold, chelerythrine 2.3-fold and nitidine 1.7-fold more strongly than to poly(dA–dT)·poly(dA–dT), indicating the rank order of sanguinarine > chelerythrine ⩾ nitidine in terms of GC preference.

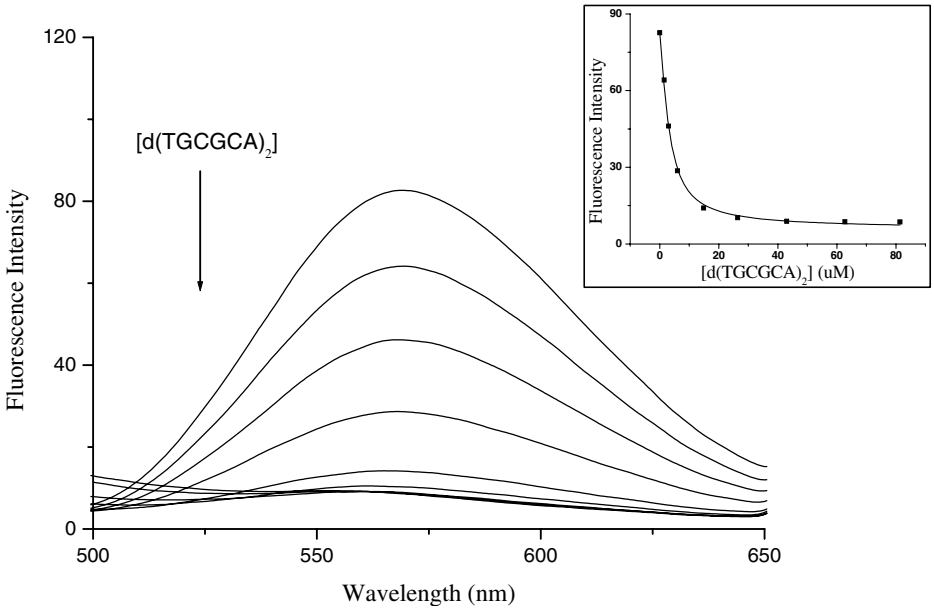

Figure 2. Fluorescence spectra of sanguinarine (3×10^{-6} M) with addition of d(TGCGCA)$_2$ in increasing concentrations (0–8.14×10^{-5} M) in 10 mM sodium phosphate (pH 5.30) at temperature (18 ± 1 °C), the excitation at 345 nm. The inset indicates the relationship between the fluorescence intensity (emission at 569 nm) and the concentration of d(TGCGCA)$_2$.

Table 1. Association constants (K_a, M^{-1}) of sanguinarine, chelerythrine, and nitidine with CT DNA, polydeoxynucleotides, and double-stranded oligodeoxynucleotides[a]

DNA	Sanguinarine		Chelerythrine		Nitidine	
	K_a	RA[b]	K_a	RA[b]	K_a	RA[b]
CT DNA	$(1.00 \pm 0.26) \times 10^6$	—	$(2.28 \pm 0.04) \times 10^5$	—	$(3.16 \pm 0.17) \times 10^5$	—
Poly(dA–dT)·poly(dA–dT)	$(4.22 \pm 0.50) \times 10^5$	1.0	$(3.08 \pm 0.14) \times 10^5$	1.0	$(6.01 \pm 0.29) \times 10^5$	1.0
Poly(dG–dC)·poly(dG–dC)	$(2.24 \pm 0.26) \times 10^6$	5.3	$(7.14 \pm 0.41) \times 10^5$	2.3	$(1.03 \pm 0.06) \times 10^6$	1.7
TGGGGA ACCCCT	$(1.01 \pm 0.21) \times 10^5$	1.0	$(1.04 \pm 0.11) \times 10^6$	20.7	$(1.37 \pm 0.06) \times 10^5$	3.3
TGGGCA ACCCGT	$(1.06 \pm 0.12) \times 10^5$	1.0	$(1.98 \pm 0.56) \times 10^5$	3.9	$(5.17 \pm 0.23) \times 10^4$	1.2
TGGCGA ACCGCT	$(1.40 \pm 0.19) \times 10^5$	1.4	$(5.54 \pm 0.64) \times 10^4$	1.1	$(4.13 \pm 0.30) \times 10^4$	1.0
TGCGGA ACGCCT	$(1.75 \pm 0.24) \times 10^5$	1.7	$(1.24 \pm 0.15) \times 10^5$	2.5	$(8.13 \pm 0.80) \times 10^4$	2.0
TGCCGA ACGGCT	$(1.99 \pm 0.19) \times 10^5$	2.0	$(1.82 \pm 0.19) \times 10^5$	3.6	$(7.01 \pm 0.36) \times 10^4$	1.7
TGGCCA ACCGGT	$(1.47 \pm 0.21) \times 10^5$	1.4	$(5.03 \pm 0.42) \times 10^4$	1.0	$(5.75 \pm 0.42) \times 10^4$	1.4
TGCGCA ACGCGT	$(5.67 \pm 0.46) \times 10^5$	5.6	$(1.34 \pm 0.36) \times 10^5$	2.7	$(2.03 \pm 0.11) \times 10^5$	4.9
ATGCGCAT TACGCGTA	$(1.01 \pm 0.17) \times 10^7$	—	$(2.86 \pm 0.58) \times 10^6$	—	$(1.10 \pm 0.22) \times 10^6$	—

[a] In 10 mM sodium phosphate buffer (pH 5.30) at temperature (18 ± 1 °C).
[b] RA denotes relative affinity.

In order to provide further insight into the sequence selectivities of DNA-binding of three alkaloids, their association constants with seven sequence-designed double-stranded oligodeoxynucleotides containing all possible four GC base pair sequences, that is, 5′-TGGGGA-3′/3′-ACCCCT-5′, 5′-TGGGCA-3′/3′-ACCCGT-5′, 5′-TGGCGA-3′/3′-ACCGCT-5′, 5′-TGCGGA-3′/3′-ACGCCT-5′, 5′-TGCCGA-3′/3′-ACGGCT-5′, d(TGGCCA)$_2$, and d(TGCGCA)$_2$, were measured by using fluorometric titration methods (Table 1 and Fig. 3). Some interesting observations can be extracted by analyzing the association constants derived from oligodeoxynucleotides with different base pairs. First, precise GC base pair sequence selectivities are observed in sanguinarine, chelerythrine, and nitidine. It can be seen that sanguinarine binds to seven DNAs in the order of d(TGCGCA)$_2$ > 5′-TGCCGA-3′/3′-ACGGCT-5′ ⩾ 5′-TGCGGA-3′/3′-ACGCCT-5′ ⩾ d(TGGCCA)$_2$ ⩾ 5′-

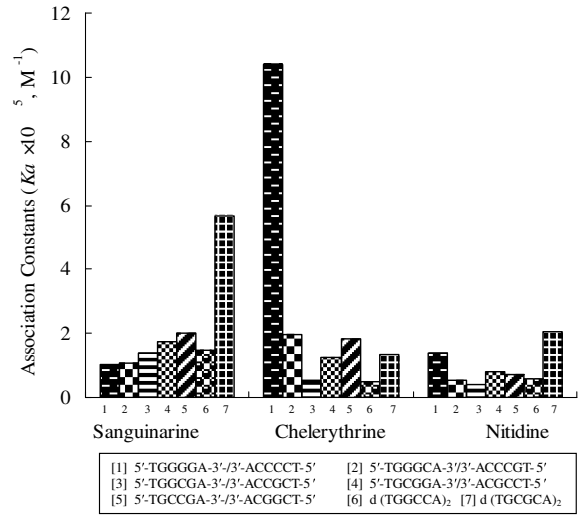

Figure 3. Binding constants of sanguinarine, chelerythrine, and nitidine with seven double-stranded oligodeoxynucleotides in 10 mM sodium phosphate buffer (pH 5.30) at temperature (18 ± 1 °C).

TGGCGA-3′/3′-ACCGCT-5′ ⩾ 5′-TGGGCA-3′/3′-ACCCGT-5′ ⩾ 5′-TGGGGA-3′/3′-ACCCCT-5′ and nitidine in the order of d(TGCGCA)$_2$ > 5′-TGGGGA-3′/3′-ACCCCT-5′ > 5′-TGCGGA-3′/3′-ACGCCT-5′ ⩾ 5′-TGCCGA-3′/3′-ACGGCT-5′ ⩾ d(TGGCCA)$_2$ ⩾ 5′-TGGGCA-3′/3′-ACCCGT-5′ ⩾ 5′-TGGCGA- 3′/3′-ACCGCT-5′. The greatest relative affinity (RA) value among seven DNA sequences is 5.6-fold for sanguinarine and 4.9-fold for nitidine, suggesting both sanguinarine and nitidine bind preferentially to d(TGCGCA)$_2$ (alternating GC base pairs). It can be also found that sanguinarine shows the smallest association constant to 5′-TGGGGA-3′/3′-ACCCCT-5′ (contiguous GC base pairs), which is in sharp contrast to chelerythrine which exhibits the highest binding affinity to this sequence. For the remaining six double-stranded DNAs, chelerythrine's binding affinities are in the order of 5′-TGGGCA-3′/3′-ACCCGT-5′ ⩾ 5′-TGCCGA-3′/3′-ACGGCT-5′ > d(TGCGCA)$_2$ ⩾ 5′-TGCGGA-3′/3′-ACGCCT-5′ > 5′-TGGCGA-3′/3′-ACCGCT-3′ ⩾ d(TGGCCA)$_2$. The greatest RA value of chelerythrine exceeds more than 20-fold. This result obviously indicates that chelerythrine has a high specificity for 5′-TGGGGA-3′/3′-ACCCCT-5′, exhibiting quite distinct sequence selectivity from sanguinarine and nitidine. In order to further confirm the GC selectivity of sanguinarine, sequence mutations have been made to d(TGCGCA)$_2$ by modifying one of its C-G base pairs to T-A and A-T base pairs, namely 5′-TG**T**GCA-3′/3′-AC**A**CGT-5′ and 5′-TG**A**GCA-3′/3′-AC**T**CGT-5′, to see whether replacement of C-G with T-A or A-T can lead to remarkable loss in the binding constants. As expected, the K_a values of sanguinarine for 5′-TG**T**GCA-3′/3′-AC**A**CGT-5′ and 5′-TG**A**GCA-3′/3′-AC**T**CGT-5′ are 6.96×10^4 and $1.71 \times 10^4 M^{-1}$, respectively, which are much smaller than that for d(TGCGCA)$_2$ by 7.2-fold and 32.3-fold, respectively. Additionally, these two binding constants of sanguinarine with 5′-TG**T**GCA-3′/3′-AC**A**CGT-5′ and 5′-TG**A**GCA-3′/3′-AC**T**CGT-5′ are even smaller than the smallest one (with 5′-TGGGGA-3′/3′-ACCCCT-5′) among all K_a values of sanguinarine with seven sequence-designed double-stranded oligodeoxynucleotides by an order of magnitude. These results provide more evidences for the conclusion of the strong GC selectivity of sanguinarine.

Second, sanguinarine, chelerythrine, and nitidine show a remarkable structure–activity relationship. Both chelerythrine and nitidine have two methoxyl substituents (C-9 methoxyl group for chelerythrine, C-11 methoxyl group for nitidine) in the aromatic D ring, while sanguinarine has a dioxymethylene group forming an additional planar ring neighboring to the D ring, which makes sanguinarine own higher degree of molecular planarity than chelerythrine and nitidine. Compared with chelerythrine and nitidine, sanguinarine exhibits much larger binding abilities with CT DNA, poly(dA–dT)·poly(dA–dT) (except for nitidine), and poly(dG–dC)·poly(dG–dC). This suggests that molecular planarity plays an important role for the DNA-binding activities of quaternary benzophenanthridine alkaloids. In addition, sanguinarine binds to d(TGCGCA)$_2$ 4.2-fold and 2.8-fold more strongly than chelerythrine and nitidine, respectively, while chelerythrine and nitidine bind to 5′-TGGGGA-3′/3′-ACCCCT-5′ 10.3-fold and 1.4-fold, respectively, more tightly than sanguinarine. This result indicates that the slight structural differences of sanguinarine, chelerythrine, and nitidine have significant impact on their sequence selectivity toward DNA.

In order to further evaluate their binding capacity to ds-DNA sequence with more base pairs, the association constants of the alkaloids toward d(ATGCGCAT)$_2$ have been measured (Table 1). The result reveals that these alkaloids interact with this 8-mer oligodeoxynucleotides at submicromolar concentration, indicating their large potential in DNA-binding capacity.

2.2. DNA-binding mode of sanguinarine, chelerythrine, and nitidine

The mode of interactions of sanguinarine, chelerythrine, and nitidine with CT DNA was investigated by employing spectrophotometric technique. A representative UV–vis spectrum for nitidine is shown in Figure 4, in which spectra a and b correspond to its UV–vis spectra before and after the addition of CT DNA, respectively. It is observed that the addition of CT DNA to the alkaloid solutions at the DNA/alkaloid molar ratios of 2.80 induces bathochromic shifts (3–8 nm) and strong hypochromicities (11.0–23.7%) in the spectra (Table 2 and Fig. 4). Furthermore, during the course of titration, three obvious isosbestic points at 332, 347, and 399 nm for nitidine, 352, 368, and 497 nm for sanguinarine, 346, 359, and 455 nm for chelerythrine are observed, demonstrating the existence of an equilibrium between bound and free alkaloids. Since the hypochromism and bathochromic shift are decisive characteristics of intercalative binding,[3] the binding mode of three alkaloids with CT DNA can be unambiguously established to be intercalation.

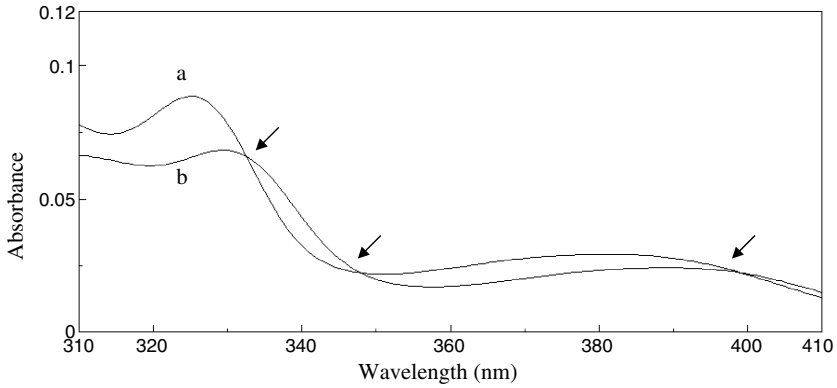

Figure 4. UV–vis absorption spectra of nitidine (6.25×10^{-6} M) in 10 mM sodium phosphate buffer (pH 5.30) before (a) and after (b) the addition of CT DNA. The concentration ratio of DNA-phosphate/nitidine is 2.80:1. The arrows indicate the isosbestic points.

Table 2. Spectrophotometric and EB displacement properties of sanguinarine, chelerythrine, and nitidine with CT DNA[a]

Alkaloids	Red shift[b] (nm)	Hypochromicity[b] (%)	Percent fluorescence decrease[c] (%)
Sanguinarine	8	23.7	19.3
Chelerythrine	3	11.0	15.4
Nitidine	5	13.5	5.1

[a] In 10 mM sodium phosphate buffer (pH 5.30) at temperature (18 ± 1 °C).

[b] Measured at the DNA/alkaloid molar ratio 2.80 at the absorption maximum at 326 nm for sanguinarine and nitidine, and 338 nm for chelerythrine, respectively.

[c] Obtained from EB displacement experiment at the maximum emission wavelength of EB at 593 nm at the alkaloid/EB molar ratio 0.24.

In order to confirm the DNA-binding modes of sanguinarine, chelerythrine, and nitidine, we further carried out competitive EB displacement assay. EB, a strong polyaromatic intercalating dye, can intercalate nonspecifically into double-stranded DNA and strongly fluoresces upon complexation. Addition of a DNA-intercalating molecule will result in a decrease in fluorescence intensity due to the displacement of the bound EB. This fluorescence-based competition technique can provide indirect evidence for the binding mode of intercalation. The percent fluorescence decrease[21,22] of EB caused by three alkaloids at the alkaloid/EB molar ratio 0.24 is shown in Table 2. It is obvious that three alkaloids notably decrease the fluorescence intensity of the EB-CT DNA complex, suggesting that three alkaloids can replace EB bound to CT DNA by intercalating with double-stranded DNA.

These EB displacement results, together with the bathochromic shifts, hypochromicities, and isosbestic points observed in the spectrophotometric titration experiments confirm that all three alkaloids bind to double-stranded DNA via the intercalative binding mechanism.

3. Conclusions

The noncovalent binding affinities of three quaternary benzophenanthridine alkaloids, that is, sanguinarine, chelerythrine, and nitidine, which are pharmacologically active constituents of some Chinese herbal medicines, with CT DNA, polydeoxynucleotides (poly(dG–dC)·poly(dG–dC) and poly(dA–dT)·poly(dA–dT)), and sequence-designed double-stranded oligodeoxynucleotides containing all possible four GC base pair sequences, have been systematically investigated by using fluorescence spectrometry. The binding constants of three alkaloids with CT DNA are in the order of sanguinarine > nitidine ⩾ chelerythrine. Sanguinarine showed much higher selectivity toward poly(dG–dC)· poly(dG–dC) than chelerythrine and nitidine. For the sequence selectivity, both sanguinarine and nitidine bind preferentially to $d(TGCGCA)_2$ (alternating GC base pairs). However, chelerythrine shows a high specificity for 5′-TGGGGA-3′/3′-ACCCCT-5′ (contiguous GC base pairs), exhibiting quite distinct sequence selectivity from sanguinarine and nitidine. Sanguinarine's GC selectivity has been further proved by measuring its K_a values with 5′-TG**T**GCA-3′/3′-AC**A**CGT-5′ and 5′-TG**A**GCA-3′/3′-AC**T**CGT-5′ obtained by mutating one C-G base pair in $d(TGCGCA)_2$ to T-A and A-T, respectively. Comparison of the binding constants also suggests that sanguinarine, chelerythrine, and nitidine show a remarkable structure–activity correlation when bound to double-stranded DNAs. The more planar molecule sanguinarine in general has much larger binding constants than chelerythrine and nitidine toward CT DNA, poly(dG–dC)·poly(dG–dC), and poly(dA–dT)·poly(dA–dT). This result indicates that molecular planarity plays an important role for DNA-binding activities of quaternary benzophenanthridine alkaloids. Moreover, three alkaloids interact even with 8-mer double-stranded oligodeoxynucleotide $d(ATGCGCAT)_2$ at submicromolar concentration, showing that they are extremely strong DNA-binding agents.

In addition to DNA-binding affinity and sequence selectivity, we have unambiguously verified that three alkaloids bind to duplex DNA in an intercalating mode by using spectrophotometric titration and competitive EB replacement assays.

This paper described the DNA-binding properties of sanguinarine, chelerythrine, and nitidine in acidic buffer

in which they are in iminium state. In the physiological condition, both iminium and alkanolamine forms of these alkaloids exist because they all exhibit pH-dependent structural equilibrium between these two forms. Alkanolamine form was reported to have larger cellular availability than iminium form due to its greater lipophilicity.[23] While, the iminium form of these alkaloids is expected to play an important role in their biological activities, such as anticancer and topoisomerase inhibitory activity, due to a positive charge on the polyaromatic nucleus, which facilitates the intercalative binding with DNA.[3]

The DNA-binding results of sanguinarine, chelerythrine and nitidine described in this paper shed light on the molecular mechanism of their anticancer activities. The structure–activity relationship of sanguinarine, chelerythrine, and nitidine may provide some important guidance for the quaternary benzophenanthridine alkaloid-based anticancer drug design.[24]

4. Experimental section

4.1. General

UV–vis absorption and fluorescence spectral measurements were performed on Jasco UV-530 ultraviolet–visible spectrophotometer and Perkin-Elmer Luminescence Spectrometer LS55, respectively. A mini circulator (Torrey Pines Scientific, USA) was employed to control the temperature (ca. 18 °C) for spectrofluorometric titration experiments. The pH measurement was made with a Sartorius electronic pH meter with an accuracy of ±0.005.

Sanguinarine, chelerythrine, and nitidine (purity greater than 98%, checked by HPLC) were obtained from China Chengdu Tianyin Industrial Co., Ltd., Chengdu, China. The stock solutions of three alkaloids were prepared in 10 mM sodium phosphate buffer (pH 5.30). CT DNA and polydeoxynucleotides (poly(dG–dC)·poly(dG–dC) and poly(dA–dT)·poly(dA–dT)) were products of Amersham Biosciences and Sigma Chemicals Co., respectively. Oligodeoxynucleotides (PAGE purified) were purchased from Takara Biotechnology (Dalian) Co., Ltd., China. Calf thymus DNA, polydeoxynucleotides, and oligodeoxynucleotides were used without further purification. The concentrations of single-stranded oligodeoxynucleotides were determined from UV absorbance at 260 nm, supposing the molar extinction coefficients (ε) of A, T, G, and C to be 16,000, 9600, 12,000, and 7000 M^{-1} cm^{-1}, respectively. Molar extinction coefficients of 13,200 M^{-1} cm^{-1} at 260 nm for calf thymus DNA and poly(dA–dT)·poly(dA–dT), and 16,800 M^{-1} cm^{-1} for poly(dG–dC)·poly(dG–dC) in base pair were used for determining their concentrations. Annealing of strands to form duplex DNA was accomplished by heating the solutions of single-stranded oligodeoxynucleotides in 10 mM sodium phosphate buffer (pH 5.30) at 95 °C in water bath for 4 min and then slowly cooling to room temperature. Milli-Q water (Millipore Co., USA) was used throughout the whole experiments. All experiments were conducted in 10 mM sodium phosphate buffer (pH 5.30). All other chemicals were analytical grade reagents.

4.2. Spectrofluorometric titration experiments

The fluorescence titration experiments were conducted in the following procedures: The solution of duplex DNA was titrated into the solution of alkaloids of the fixed concentrations in 10 mM sodium phosphate buffer (pH 5.30) and the corresponding fluorescence spectra (excitation at 345 nm for three alkaloids) were recorded under the temperature controlled by circulator. The concentrations of alkaloids and DNA were ca. 3.0×10^{-6} and 0–8.1×10^{-5} M in 10 mM sodium phosphate buffer (pH 5.30), respectively. An equilibrium period of 5 min (standing for 1 min after constant stirring of the sample for 4 min) was allowed before recording each spectrum. The association constants (K_a) were derived from the analysis of the relationship between relative fluorescence intensity (I/I_0) and the DNA concentrations by nonlinear curve fitting to the equation $I/I_0 = 1 + ((I_\infty - I_0)/2I_0) \times \{([DNA]_0 + [alkaloid]_0 + 1/K_a) - (([DNA]_0+[alkaloid]_0+1/K_a)^2 - 4[DNA]_0[alkaloid]_0)^{1/2}\}$, wherein I_0, I, and I_∞ represent the fluorescence intensities of alkaloid alone, the sample, and alkaloid totally bound, respectively. $[DNA]_0$ and $[alkaloid]_0$ are the initial analytical concentrations of DNA and alkaloids, respectively. KaleidaGraph (version 3.6, Synergy Software, USA) was applied for the nonlinear curve fitting.[3]

4.3. Spectrophotometric titration experiments

The spectrophotometric titrations were performed by keeping the concentration of alkaloids constant while gradually increasing the DNA concentration. To a solution of alkaloid (1.0×10^{-5} M) in 10 mM sodium phosphate buffer (pH 5.30) were added aliquots of CT DNA (1.7758×10^{-3} M) solution containing the same concentration of alkaloid (1.0×10^{-5} M) in 10 mM sodium phosphate buffer (pH 5.30). An equilibrium period of 5 min for constant stirring of the mixed solution was allowed before recording each spectrum. The spectrophotometric titrations of sanguinarine and nitidine were conducted in the similar way.

4.4. EB displacement experiments

Competitive EB displacement experiments were carried out according to the methods described in our previous paper.[3]

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References and notes

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