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Non-covalent complexes between bis-β-carbolines and double-stranded DNA: A study by electrospray ionization FT-ICR mass spectrometry (I)

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Abstract—The non-covalent complexes of five bis-β-carbolines alkaloids with three different double-stranded oligodeoxynucleotides d(GCGCGATCGCGC)$_2$, d(GCGCAATTGCGC)$_2$, and d(GCGAAATTTCGC)$_2$ were investigated by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. These five antitumor compounds all showed DNA-binding abilities. Binding affinities in the order of **2** > **3**, **4** > **5**, and **1** with double-stranded DNA were obtained, which mean that the length of the linkage chain between two β-carbolines has a remarkable effect on the formation of the non-covalent complexes. Additionally, the preliminary results indicated that bis-β-carbolines had no notable sequence selectivities.
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The specific, non-covalent interaction of small organic molecules with duplex DNA is the molecular basis of many antitumor, antiviral, and antibiotic drugs. Compounds that bind to DNA with high affinity can influence gene expression and, therefore, affect cell proliferation and differentiation.[1]

Among the various DNA-binding small organic molecules, natural products exert their activities through site-specific non-covalent binding to DNA with well established binding affinities and modes. They are attractive as versatile platforms for the development of DNA ligands because the structural modification of natural compounds may lead to more efficient DNA-binding agents.[2] Some alkaloids have been shown to work against cancer, such as γ-carboline.[3] The planar polycyclic aromatic residues in these molecules may be act as intercalating moieties like some DNA-binding drugs.[4] Some studies showed that dimerization of various intercalating moieties can lead to a very large increase in the affinity by bis-intercalation mechanism.[5] Several years ago, a series of bis-β-carbolines were designed and synthesized by our group (Fig. 1). The preliminary in vitro antitumor test indicated that they were active against L1210 and that the activity could be modulated by varying the linker chain of the bis-β-carboline system.[6]

Researches on the non-covalent interaction of small molecules with duplex DNA are helpful to elucidate their structure–activity relationships and to better understand their mechanisms, which can guide the rational design of better sequence specific DNA-binding drugs. A wide variety of physical and chemical

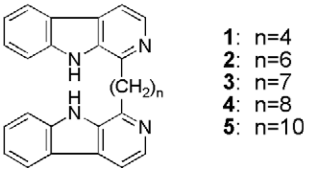

Figure 1. Chemical structures of bis-β-carbolines **1**–**5**.

Keywords: Bis-β-carbolines; DNA; Non-covalent interaction; Mass spectrometry.

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techniques such as NMR, X-ray crystallography, gel footprinting, fluorescence spectroscopy, and mass spectrometry have been developed to study the non-covalent complexes of small organic molecules and duplex DNA.[1] Among them, mass spectrometry has assumed a more active role in investigations of non-covalent complexes owing to the gentle nature of the electrospray ionization process, which allows a wide range of non-covalent complexes to be introduced intact into the gas phase. Some known non-covalent DNA-binding drugs have been investigated by ESI mass spectrometry to study their antitumor activity and the corresponding specific non-covalent interaction with DNA.[7–9]

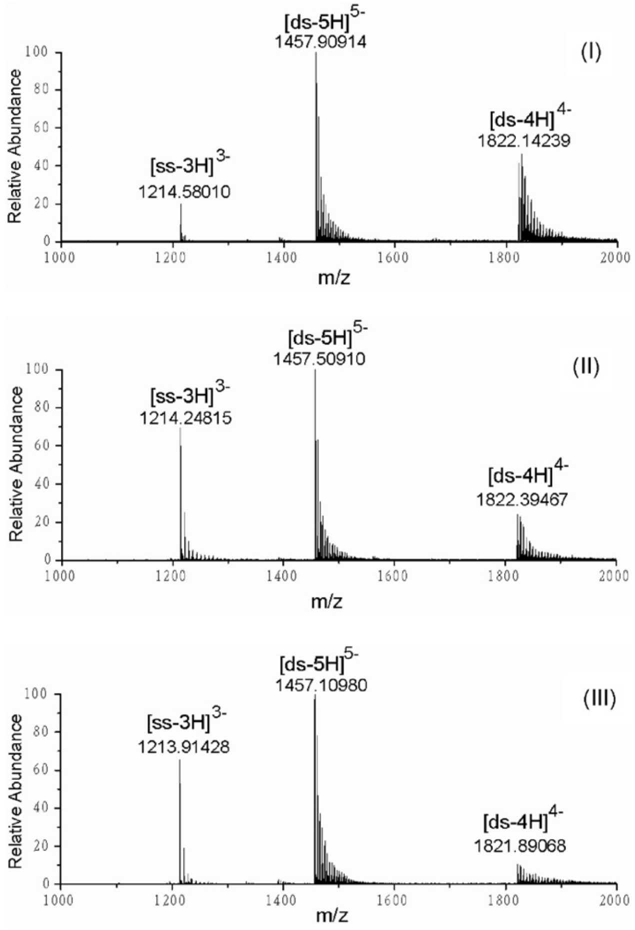

Figure 2. Negative ESI-FT-ICR MS spectrum of double-stranded DNA. (I): d(GCGCGATCGCGC)$_2$, (II): d(GCGCAATTGCGC)$_2$, (III): d(GCGAAATTTCGC)$_2$.

Fourier transform (FT)-ion cyclotron resonance (ICR) mass spectrometry (MS), in some respects, is the highest performance MS method currently available. The high mass accuracy and resolution of FT-ICR MS are making it an increasingly useful tool in drug discovery and development. ESI-FT-ICR has proven to be a powerful tool in the study of non-covalent complexes.[10]

In this study, ESI-FT-ICR mass spectrometry was used to investigate the complexes of five bis-β-carbolines **1**–**5** (Fig. 1) with double-stranded oligodeoxynucleotides of different sequences, aiming at clarifying their structure–activity relationships and sequence selectivities, especially the influence of the linker chain of the bis-β-carboline system on the non-covalent interaction with duplex DNA.[11] Fluorescence spectrometric experiments were also carried out to compare with the MS results.[12]

The synthetic bis-β-carbolines derivatives **1**–**5** were prepared as previously reported.[6] Three different 12-mer self-complementary oligonucleotide single-strands: d(GCGCGATCGCGC), d(GCGCAATTGCGC), and d(GCGAAATTTCGC) were chosen for this study.[13] Oligodeoxynucleotide stock solutions were annealed in 1 M ammonium acetate by heating at 80 °C for 10 min and cooling to room temperature slowly to form double-stranded oligodeoxynucleotides.

ESI-FT-ICR MS spectra of double-stranded DNA. ESI-FT-ICR experiments of the three double-stranded DNAs were conducted firstly to establish the optimum conditions for the observation of the specific duplex. We employed self-complementary oligonucleotides in this study because less sample preparation is involved and their mass spectra are simple and easy to interpret. If using a normal ESI-MS instrument, peak assignment is sometimes problematic because single-stranded and double-stranded oligonucleotides with an even number of charges may have the same mass-to-charge ratio.[1] But the high resolution of FT-ICR MS we used can easily get the accurate mass difference between two isotopic peaks and the correct charge state was then obtained.

Figure 2 shows the obtained negative ESI-FT-ICR spectra of d(GCGCGATCGCGC)$_2$, d(GCGCAATTGCGC)$_2$, and d(GCGAAATTTCGC)$_2$. In the spectrum of d(GCGCGATCGCGC)$_2$ (I), the most intense ion peak at *m*/*z* 1457.90914 was assigned to the five-charged deprotonated molecular ion ([ds-5H]$^{5-}$) of the double-stranded oligodeoxynucleotides. The four-charged molecular ion ([ds-4H]$^{4-}$) at *m*/*z* 1822.14239 and three-charged molecular ion ([ss-3H]$^{3-}$) of the single-stranded oligodeoxynucleotide at *m*/*z* 1214.58010 were also observed. Furthermore, the sodium adducts of these three molecular ions were detected. Under the same analytical conditions, similar ESI-FT-ICR spectra were obtained for the other two double-stranded DNA d(GCGCAA TTGCGC)$_2$ (II) and d(GCGAAATTTCGC)$_2$ (III).

Observation of the non-covalent complexes and relative binding affinities of five individual bis-β-carbolines with double-stranded DNA. The annealed solution of duplex oligodeoxynucleotides with a final concentration of 12.5 μmol/L was mixed with 1 equivalent of the stock solution of bis-β-carbolines **1**–**5** (1 mg/mL in $CHCl_3$/MeOH, 1:9), respectively, to make the individual complex. The resulting mixtures were diluted with spray solvent (50/50 v/v MeOH/100 mM aq ammonium acetate) to 200 μL and subject to negative ion ESI-FT-ICR MS spectrometric analysis. Figure 3 shows the obtained negative ESI-FT-ICR spectra of the complexes between d(GCGCAATTGCGC)$_2$ and bis-β-carbolines **1** (I), **2** (II), **3** (III), **4** (IV), and **5** (V). In these spectra, ions [ds+alkaloid−5H]$^{5-}$ were observed as the non-covalent

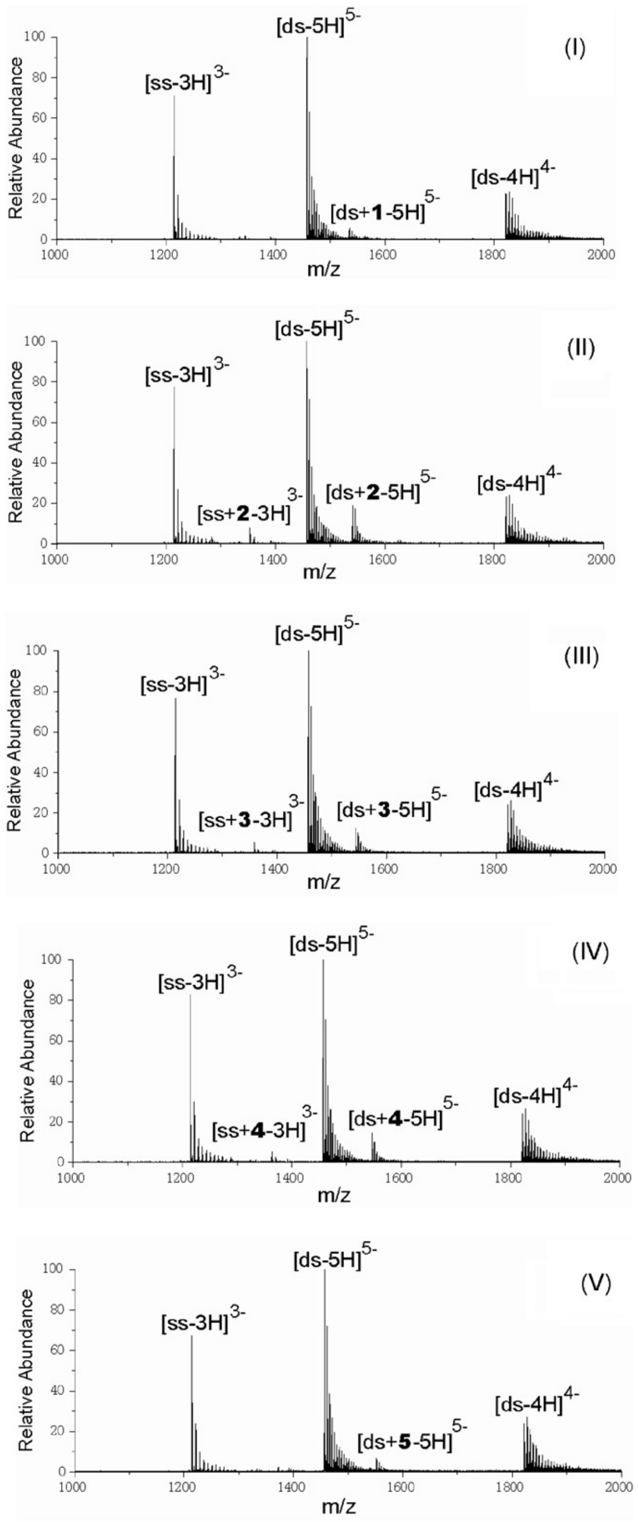

Figure 3. Negative ESI-FT-ICR MS spectra of the complexes of d(GCGCAATTGCGC)$_2$ with bis-β-carbolines **1** (I), **2** (II), **3** (III), **4** (IV), and **5** (IV) in 1:1 DNA-to-alkaloid molar ratio.

complexes between the bis-β-carbolines and the double-strands DNA. In the same time, ions [ss+alkaloid−3H]$^{3-}$ were found as the non-covalent complexes between the bis-β-carbolines and single-strand DNA. No 1:2 complexes were detected, which indicated that bis-β-carbolines only had 1:1 binding stoichiometries. Among the five compounds, bis-β-carbolines **2** showed the highest relative abundance for forming oligonucleotide duplex/drug complexes. The similar ESI-FT-ICR spectra were obtained for the binding with the other two double-stranded DNA d(GCGCGATCGCGC)$_2$ and d(GCGAAATTTCGC)$_2$.

The relative binding affinities of the five alkaloids toward d(GCGCGATCGCGC)$_2$, d(GCGCAATTG CGC)$_2$, and d(GCGAAATTTCGC)$_2$ were evaluated by measuring the ratios of the complex signals [ds+alkaloid−5H]$^{5-}$ to those of the free duplex [ds−5H]$^{5-}$. The results are shown in Table 1. The data indicated that the alkaloids bind in the order of $\mathbf{2} > \mathbf{3} \approx \mathbf{4} \approx \mathbf{5} > \mathbf{1}$ to d(GCGCGATCGCGC)$_2$ and bind in the order of $\mathbf{2} > \mathbf{3} \approx \mathbf{4} > \mathbf{5} > \mathbf{1}$ to d(GCGCAATTGCGC)$_2$ and d(GCGAAATTTCGC)$_2$. Bis-β-carboline **2** which has a six-carbon linkage chain between two β-carbolines exhibits the greatest affinity. When the carbon linkage chain is extended or shortened, the affinity toward the double-stranded DNA will decrease. This phenomenon is more clear in the binding experiment with d(GCGCAATTGCGC)$_2$ and d(GCGAAATTTCGC)$_2$. This result suggests that the length of linkage chain between two β-carbolines has a great effect on their binding activities toward DNA.

Competition binding experiment. Competition binding experiments were further carried out for five bis-β-carbolines with d(GCGCAATTGCGC)$_2$ to test whether the extent of compound binding as determined by relative ion abundances matches solution affinities. MS competitive binding experiments have proven to be very promising in terms of sensitivity and specificity, and allow the easy determination of which alkaloid of the two alkaloids in the mixture binds to DNA duplexes preferentially, because the preferential binding of the alkaloid usually leads to greater relative abundance in the MS spectra.[14] In each competition experiment, solutions of the oligodeoxynucleotide duplexes of fixed concentration were mixed with equimolar amounts of two alkaloids to give final concentrations of each [alkaloid] = 12.5 μmol/L, [duplex] = 12.5 μmol/L. All the reactions were interrogated by ESI-FT-ICR MS by using the same spray solvent under identical ESI interface conditions.

On the basis of relative abundances of the non-covalent complexes, we established the following ordering (Fig. 4): of pairs of complexes: $\mathbf{2} > \mathbf{3}$ (I), $\mathbf{3} \approx \mathbf{4}$ (II), $\mathbf{4} > \mathbf{1}$ (III), $\mathbf{1} \approx \mathbf{5}$ (IV). From such a series of pairwise

Table 1. Relative binding affinities (the ratio of [ds+compound−5H]$^{5-}$/[ds−5H]$^{5-}$) of five alkaloids toward d(GCGCGATC GCGC)$_2$, d(GCGCAATTGCGC)$_2$, and d(GCGAAATTTCGC)$_2$

Compound	AT	AATT	A_3T_3
1	5.82	5.56	5.10
2	19.16	18.78	15.29
3	14.82	13.84	12.44
4	14.84	14.13	12.36
5	12.21	6.09	9.20

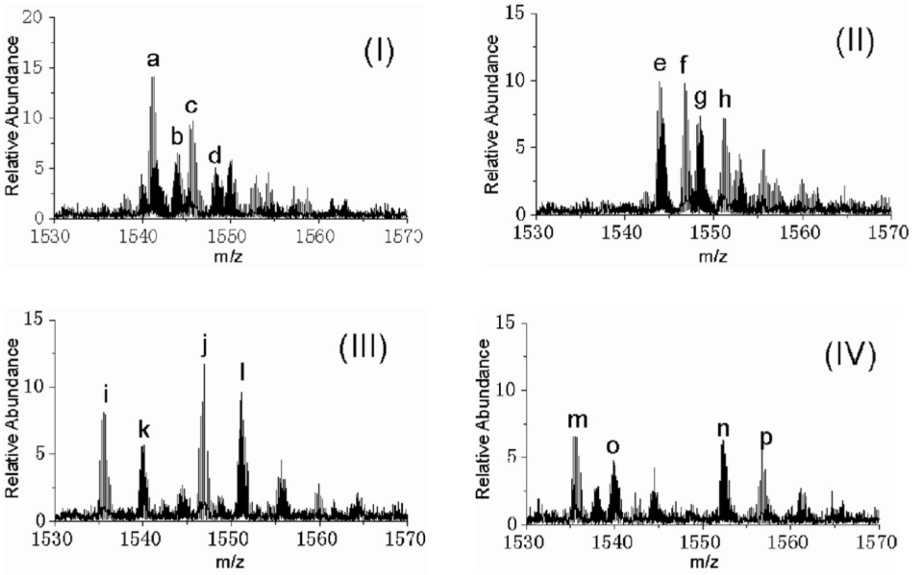

Figure 4. Competitive binding of the five alkaloids to d(GCGCAATTGCGC)$_2$ in a 1:1:1 DNA/alkaloid/alkaloid molar ratio: (I) bis-β-carbolines **2**–bis-β-carbolines **3**, (II) **3**, and **4**, (III) **1**–**4**, and (IV) **1**–**5**. (a) Stands for [ds+**2**−5H]$^{5-}$ (*m*/*z* 1541.33953), (b) for [ds+**3**−5H]$^{5-}$ (*m*/*z* 1543.93682), (c) for [ds+**2**+Na−5H]$^{5-}$ (*m*/*z* 1545.73937), (d) for [ds+**3**+Na−5H]$^{5-}$ (*m*/*z* 1548.34285), (e) for [ds+**3**−5H]$^{5-}$ (*m*/*z* 1543.94402), (f) for [ds+**4**−5H]$^{5-}$ (*m*/*z* 1546.75813), g for [ds+**3**+Na-5H]$^{5-}$ (*m*/it z 1548.35474), (h) for [ds+**4**+Na−5H]$^{5-}$ (*m*/it z 1551.14890), (i) for [ds+**1**−5H]$^{5-}$ (*m*/*z* 1535.52763), (j) for [ds+**4**−5H]$^{5-}$ (*m*/*z* 1546.75203), (k) for [ds+**1**+Na−5H]$^{5-}$ (*m*/*z* 1539.92442), and (l) for [ds+**4**+Na−5H]$^{5-}$ (*m*/*z* 1551.14630), (m) for [ds+**1**−5H]$^{5-}$ (*m*/*z* 1535.52738), (n) for [ds+**5**−5H]$^{5-}$ (*m*/*z* 1552.35285), (o) for [ds+**1**+Na−5H]$^{5-}$ (*m*/*z* 1539.92218), and (p) for [ds+**5**+Na−5H]$^{5-}$ (*m*/*z* 1556.74443), respectively.

comparisons, we determined that the overall order of binding preferences for bis-β-carbolines is **2** > **3** ≈ **4** > **5** ≈ **1**. This result is almost the same as that determined by relative ion abundances. The competition binding experiments with d(GCGCGATCGCGC)$_2$ and d(GCGAAATTTCGC)$_2$ were also carried out and the similar result were obtained (data not shown).

Sequence selectivities of the five bis-β-carboline alkaloids. The mode of non-covalent binding is crucial for understanding the molecular basis of the drug action. There are two main binding modes: minor-groove binding at AT-rich regions and intercalation at GC-rich regions.[1] We used three 12-mer duplex DNA with different sequences in this research so that the AT/GC content in those duplexes varied from GC-rich to equal GC/AT proportion. From the relative binding affinities we obtained (Table 1), bis-β-carbolines **2**, **3**, and **4** bound to three DNAs in the order of d(GCGCGATCGCGC)$_2$ > d(GCGCAATTGCGC)$_2$ > d(GCGAAATTTCGC)$_2$. It indicated that the presence of G–C base pairs enhances the non-covalent interaction between duplex and bis-β-carbolines. But this sequence selectivity was not remarkable, and the sequence selectivity was not found in bis-β-carbolines **1** and **5** which possess lower binding affinity.

Fluorescence spectrometric investigation. Fluorescence spectrometry is a sensitive analytical technique and widely used in the investigation of non-covalent complexes of small organic molecules with biomolecules such as DNA.[15] To compare with the results obtained by mass spectrometry, the association constants (K_a) of the five bis-β-carbolines with calf thymus (ct) DNA were further measured in Tris–HCl buffer (pH 7.4) by means of fluorescence spectrometry.[16] The obtained values varied in the range from 4.25×10^4 to 3.50×10^5 M^{-1} which indicated that all the five bis-β-carbolines showed binding preferences for ct-DNA. Among them, the bis-β-carbolines **2** exhibits the greatest affinity ($K_a = 3.50 \times 10^5$ M^{-1}). This result was consistent with what we obtained from ESI-FT-ICR mass spectrometry.

In summary, we systematically studied for the first time the non-covalent complexes of five bis-β-carbolines derivatives with double-stranded DNA by ESI-FT-ICR MS. The results indicated that all the five bis-β-carbolines can form non-covalent complexes with three different 12-mer double-stranded oligodeoxynucleotides. The length of linkage chain between two β-carbolines has great effect on their binding activities. But no notable sequence selectivities were found in this research. Chemical modifications of these alkaloids are currently being made to improve their DNA-binding affinities and sequence selectivities.

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11. Mass spectra were obtained on a hydrid QqFT/ICR (ApexQe, Brucker) equipped with an ApolloII orthogonal ESI source. Solutions were infused at a rate of 120 μL/h. The image signal was amplified and digitized using 1M data points.
12. UV–vis absorption and fluorescence spectra were measured on Shimadzu UV-260 UV/vis Spectrophotometer and Hitachi Spectrofluorophotometer F-3000, respectively.
13. Single-strands oligodeoxynucleotide were purchased from DNA Technology A/S (Denmark).
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16. The calf thymus (ct) DNA sample was used without further purification. The purity of the DNA was checked by monitoring the absorption spectrum and the ratio of the absorbance at 260 and 280 nm. The sample was dissolved in 0.05 M Tris-buffer with HCl (pH 7.4). The DNA concentration per nucleotide (c(P)) was determined by absorption spectroscopy, using the molar extinction coefficients at 260 nm as 6600 M^{-1}.The fluorescence titration experiments were conducted in the following way: 0.1 mL solution of a compound in DMSO (10^{-3}–10^{-4} M) mixed with 50 mM Tris–HCl (pH 7.4) to 2 mL. Then, two groups of samples were prepared in the concentration of chemical at 2, 4, 6, 8, 10 μM, one contained calf-thymus DNA 10 μM, the other contained no DNA but had the same concentration of chemical as control. All the above solution was shaken for 10 min. Fluorescence wavelength and intensity area of samples were measured at room temperature. The association constants (K_a) were derived from the analysis of the relationship between the fluorescence intensity changes and the compound concentrations by Scatchard method.