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Study on noncovalent complexes of cytotoxic protoberberine alkaloids with double-stranded DNA by using electrospray ionization mass spectrometry

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Abstract—The noncovalent complexes of four cytotoxic protoberberine alkaloids that is, berberine, palmatine, jatrorrhizine, and coptisine with double-stranded oligodeoxynucleotides d(AAGAATTCTT)$_2$ were investigated by electrospray ionization mass spectrometry. These four active components from Chinese herbal medicines showed both 1:1 and 1:2 binding stoichiometries, independent on the alkaloid-to-DNA ratios. Binding affinities in the order of palmatine $\geqslant$ jatrorrhizine > coptisine > berberine with d(AAGAATTCTT)$_2$ were obtained. Additionally, the preliminary results indicated that berberine had some sequence selectivities.

The specific and noncovalent interactions of small organic molecules with DNA and RNA are a topic of increasing interest in modern bioorganic chemistry.[1] Such interactions provide the molecular basis for the rational design of antitumor, antiviral, and antibiotic drugs, as well as better elucidation of their bioactivity mechanism. Significant attention has being paid to the noncovalent interactions of cytotoxic protoberberine alkaloids, the active constituents of some Chinese herbal medicines such as *Rhizoma Coptidis* ('Huang-Lian'), *Cortex Phellodendri* ('Huang-Bo'), with double-stranded DNA.[2] As a typical protoberberine-type alkaloid, berberine was reported to bind to DNA with high affinities, which was elucidated by several analytical techniques including absorption,[2a] fluorescence,[2b,c] and NMR[2d,e] spectroscopies. Recent results from NMR suggest that berberine acts as a minor groove binder with preferential AT-rich sequences.[2e] However, these studies are so far limited to berberine, and no work has been reported on the systematic study of the complexes of protoberberine-type alkaloids with DNA. Therefore, their structure–activity relationships have not yet established. Electrospray ionization mass (ESI-MS) spectrometry has been used as a sensitive and effective analytical technique for the detection of specific drug-oligonucleotide duplex noncovalent complexes.[3] However, to the best of our knowledge, no mass spectrometric investigation has been previously reported on cytotoxic protoberberine alkaloid−DNA complexes.

In this report, we describe the systematic ESI-MS spectrometric investigation of the complexes of four protoberberine alkaloids that is, berberine (B), palmatine (P), jatrorrhizine (J), and coptisine (C) (Scheme 1) which are active constituents of some Chinese herbal medicines, with double-stranded oligodeoxynucleotides d(AAGAATTCTT)$_2$, aiming at clarifying their structure–activity relationships. Recent investigation on the complex of berberine with d(AAGAATTCTT)$_2$ by NMR suggests that berberine binds to the minor groove site of this double helix at the A_4–T_7 and A_5–T_6 base pairs.[2e] Thus, the binding of berberine to other two double-stranded oligodeoxynucleotides, d(AAG*GATC*CTT)$_2$ and d(AAG*CATG*CTT)$_2$, was also investigated to examine whether berberine may show sequence selectivity.

Berberine, palmatine, and jatrorrhizine were obtained from Chinese National Institute for the Control of Pharmaceutical and Biological Products, and coptisine was a product from ChromaDex, Inc. Self-complementary

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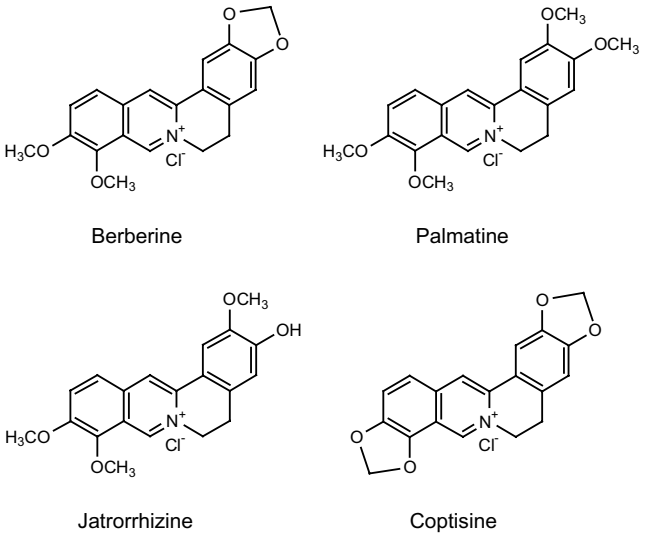

Scheme 1. Structures of the cytotoxic protoberberine alkaloids.

oligodeoxynucleotides 5′-AAGAATTCTT-3′ (monoisotopic mass = 3024.6 Da), 5′-AAGGATCCTT-3′, and 5′-AAGCATGCTT-3′ (monoisotopic mass = 3025.6 Da) were purchased from Invitrogen and purified by reversed-phase ODS column chromatography before use. Solutions of oligodeoxy-nucleotide stock solutions (1 mM) were annealed in 1 M ammonium acetate by heating at 90 °C for 5 min and then chilling slowly to room temperature to form double-stranded oligodeoxy-nucleotides (0.5 mM). Then the annealed solution (25 μL) of duplex oligodeoxy-nucleotides was mixed with the 10% methanol aqueous solution of berberine, palmatine, jatrorrhizine, and coptisine (each 2 mM), respectively, to make the individual complex. The resulting mixtures were diluted with a solution containing MeOH and 100 mM aqueous ammonium acetate (50/50, v/v) to 100 μL and subject to negative ion ESI-MS spectrometric analysis. ESI-MS analyses were conducted according to the reported protocols.[4]

ESI-MS spectra of d(AAGAATTCTT)$_2$, d(AAGGATCCTT)$_2$, and d(AAGCATGCTT)$_2$. In order to establish the optimum conditions for the observation of the specific duplex, ESI-MS experiments of the three double-stranded DNA were conducted. Figure 1 shows the obtained negative ESI-MS spectrum of d(AAGAATTCTT)$_2$. The most intense ion peak at *m/z* 1511.9 was assigned to the quadruply charged deprotonated molecular ion ($[ds-4H]^{4-}$) of the double-stranded oligodeoxynucleotide. The assignment was supported by the detection of the sodium adduct ion ($[ds+Na-5H]^{4-}$) at *m/z* 1516.9 (the inset in Fig. 1) and the three-charged molecular ion ($[ds-3H]^{3-}$) at *m/z* 2016.1. Under the same analytical conditions, similar ESI-MS spectra were obtained for the other two double-stranded DNA, d(AAGGATCCTT)$_2$, and d(AAGCATGCTT)$_2$.

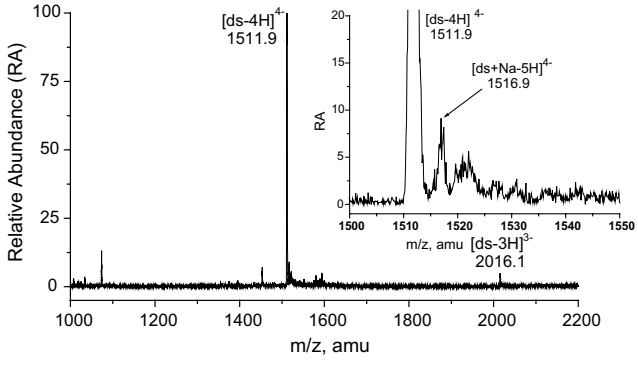

Figure 1. Negative ESI-MS spectrum of d(AAGAATTCTT)$_2$.

Observation of the noncovalent complexes of four individual alkaloids with d(AAGAATTCTT)$_2$. Firstly, binding stoichiometries were investigated by changing the molar concentration ratio of alkaloid-to-DNA from 0.5 to 2, taking the binding of berberine to d(AAGAATTCTT)$_2$ as an example (Fig. 2). Except the ion peak at *m/z* 1511.8 from four-charged unbound d(AAGAATTCTT)$_2$ ($[ds-4H]^{4-}$), two new ion peaks at *m/z* 1595.5 and 1679.3 were observed in Figure 2. These two ion peaks could be assigned to four-charged 1:1 ($[ds+B-5H]^{4-}$) and 1:2 ($[ds+2B-6H]^{4-}$) complexes formed by berberine and d(AAGAATTCTT)$_2$, respectively. Though the latter peak could be also assigned to two-charged 1:1 berberine-single-stranded DNA complex ($[ss+B-3H]^{2-}$), the assignment to $[ds+2B-6H]^{4-}$ is more favorable because single-stranded DNA was reported to bind with berberine with much lower affinities than double-stranded DNA.[2e] The result indicated that berberine had 1:1 and 1:2 binding stoichiometries. Moreover, the 1:1 and 1:2 complexes were consistently detected with comparable relative abundance (Fig. 2) even when the alkaloid/DNA concentration ratio decreased to 0.5, suggesting that the formation of 1:1 and 1:2 complexes was independent on the alkaloid-to-DNA concentration ratio.

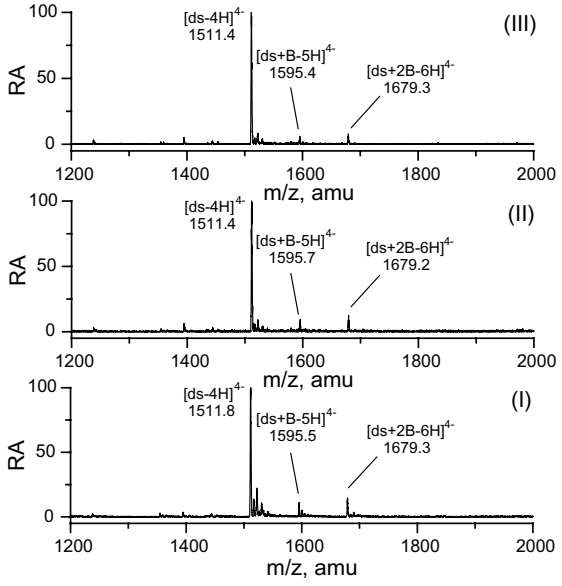

Figure 2. Negative ESI-MS spectra of the complex from berberine and d(AAGAATTCTT)$_2$ in a 2:1 (I), 1:1 (II), and 0.5:1 (III) berberine-to-DNA molar ratios.

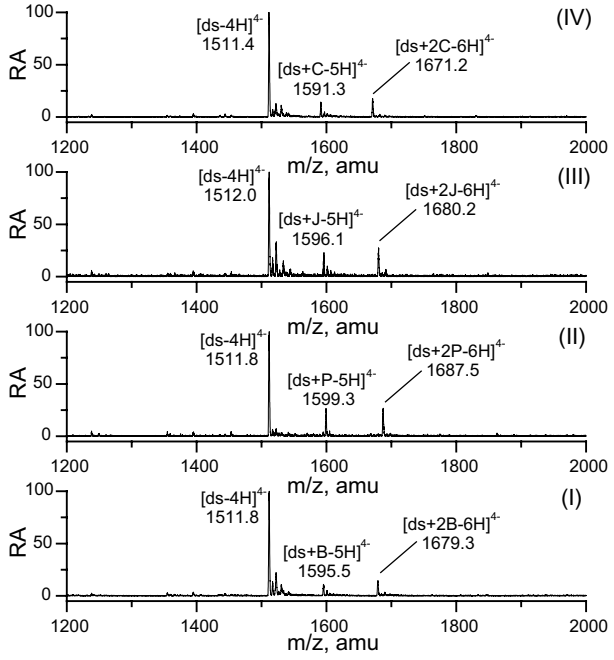

Figure 3. Negative ESI-MS spectra of the complexes of d(AAGAATTCTT)$_2$ with berberine (I), palmatine (II), jatrorrhizine (III), and coptisine (IV) in a 1:2 DNA-to-alkaloid molar ratio.

The complexes of the four alkaloids with d(AAGAATTCTT)$_2$ under 2:1 molar concentration of alkaloid-to-DNA were investigated. The obtained negative ESI-MS spectra of these complexes were shown in Figure 3. It was found that all four alkaloids exhibited similar binding behaviors with d(AAGAATTCTT)$_2$ that is, they all had both 1:1 ([ds+alkaloid$-5H]^{4-}$) and 1:2 ([ds+ 2alkaloid$-6H]^{4-}$) binding stoichiometries. To the best of our knowledge, this is the first time to demonstrate that jatrorrhizine and coptisine, two protoberberine-type alkaloids from Chinese herbal medicines, can form noncovalent complexes with DNA. Protoberberine alkaloids commonly exhibit self-association process in solution, and higher than 1:1 complexes with DNA were also observed in the binding of some minor groove binders, such as distimycin to the minor groove sites of duplex oligodeoxynucleotide.[5] However, how the two protoberberine molecules bound to d(AAGAATTCTT)$_2$ is currently unclear.

Relative binding affinities of the four alkaloids with d(AAGAATTCTT)$_2$. The relative binding affinities of the four alkaloids toward d(AAGAATTCTT)$_2$ were evaluated by measuring the ratios of the complex signals ([ds+alkaloid$-5H]^{4-}$+[ds+2alkaloid$-6H]^{4-}$) to those of the free duplex ([ds$-4H]^{4-}$). The results are illustrated in Figure 4. The data indicated that the alkaloids bind to d(AAGAATTCTT)$_2$ in the order of palmatine > jatrorrhizine > coptisine > berberine. Thus, palmatine exhibits the greatest affinity, while berberine has the lowest affinity. Meanwhile, it should be noted that the differences in the affinities of four alkaloids were not too significant. This result suggests that the slight differences in the structures of these four alkaloids have no great effect on their binding activities toward DNA.[6]

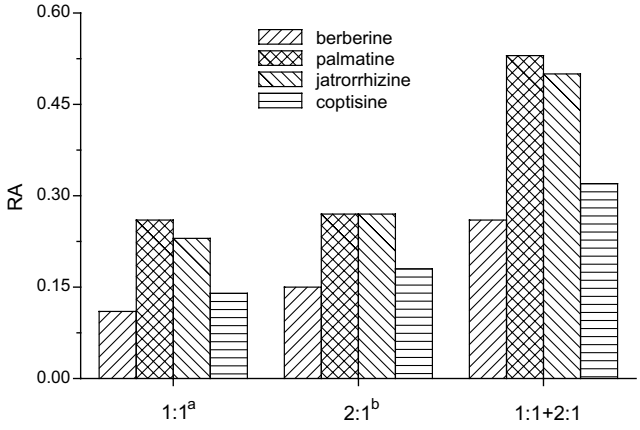

Figure 4. Relative binding affinities of four alkaloids toward d(AAGAATTCTT)$_2$. [a]The ratio of [ds+alkaloid$-5H]^{4-}$/[ds$-4H]^{4-}$; [b]The ratio of [ds+2alkaloid$-6H]^{4-}$/[ds$-4H]^{4-}$.

The relative binding affinities of the four alkaloids with d(AAGAATTCTT)$_2$ were further investigated by competitive binding experiments, in which d(AAGAATTCTT)$_2$ at a fixed concentration was mixed with two alkaloids at equimolar amounts. The obtained ESI-MS spectra are shown in Figure 5. These competitive experiments allowed us to determine, which alkaloid

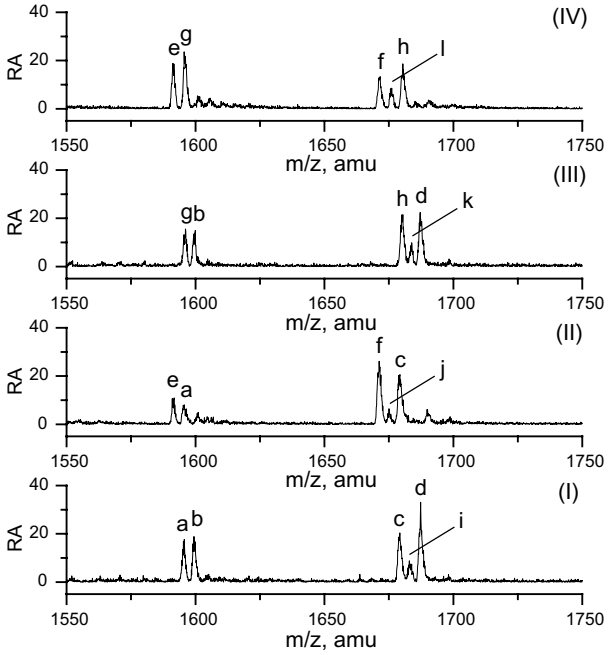

Figure 5. Competitive binding of the four alkaloids to d(AAGAATTCTT)$_2$ in a 1:2:2 DNA–alkaloid–alkaloid molar ratio: (I) berberine–palmatine, (II) berberine–coptisine, (III) palmatine–jatrorrhizine, and (IV) jatrorrhizine–coptisine. The letter a stands for [ds+B$-5H]^{4-}$ (*m/z* 1595.6), b for [ds+P$-5H]^{4-}$ (*m/z* 1599.4), c for [ds+2B$-6H]^{4-}$ (*m/z* 1679.2), d for [ds+2P$-6H]^{4-}$ (*m/z* 1687.3), e for [ds+C$-5H]^{4-}$ (*m/z* 1591.7), f for [ds+2C$-6H]^{4-}$ (*m/z* 1671.2), g for [ds+J$-5H]^{4-}$ (*m/z* 1596.1), h for [ds+2J$-6H]^{4-}$ (*m/z* 1680.4), i for [ds+B+P$-6H]^{4-}$ (*m/z* 1682.9), j for [ds+B+C$-6H]^{4-}$ (*m/z* 1675.0), k for [ds+P+J$-6H]^{4-}$ (*m/z* 1683.8), and l for [ds+P+C$-6H]^{4-}$ (*m/z* 1676.1), respectively.

in the mixture could favorably bind with d(AAGAATTCTT)$_2$. From the obtained total relative abundance of the 1:1 and 1:2 noncovalent complexes, we could establish the following order of the pairs of the complexes: palmatine > berberine (Fig. 5I), coptisine > berberine (Fig. 5II), palmatine ⩾ jatrorrhizine (Fig. 5III), and jatrorrhizine > coptisine (Fig. 5IV). Thus, the order of binding preference with d(AAGAATTCTT)$_2$ could be summarized as palmatine ⩾ jatrorrhizine > coptisine > berberine, which is in agreement with that described in the previous section.

Additionally, during the competitive binding experiments, we consistently observed the mixed complexes (ion peaks i–l in Fig. 5) in which two different alkaloids were bound to d(AAGAATTCTT)$_2$.[7] For example, when berberine competitively bound with palmatine (Fig. 5I), one new ion peak was present at *m*/*z* 1682.9 that could be unambiguously assigned to a four-charged mixed complex ([ds+B+P−6H]$^{4-}$) in which one molecule of berberine and one molecule of palmatine were bound to d(AAGAATTCTT)$_2$. Their lower abundances than the two homogeneous 2:1 complexes indicated the mixed complexes could not be formed randomly. This phenomenon was also observed in the literature.[7]

Sequence selectivities of berberine. It was reported that berberine could be noncovalently bound in the minor groove site of the AATT duplex regions of d(AAGAATTCTT)$_2$.[2e] The consecutive AATT in d(AAGAATTCTT)$_2$ was changed to GATC and CATG to examine whether berberine has some sequence selectivities. Figure 6 shows the ESI-MS spectra of the complexes of berberine with d(AAGAATTCTT)$_2$, d(AAGGATCCTT)$_2$, and d(AAGCATGCTT)$_2$ in a 2:1 alkaloid-to-DNA concentration ratio. Similarly, four-charged molecular ions of both 1:1 ([ds+B−5H]$^{4-}$) and 2:1 ([ds+2B−6H]$^{4-}$) complexes were observed in each case. The abundance ratios of the complex signals ([ds+B−5H]$^{4-}$+[ds+2B−6H]$^{4-}$) to those of the unbound duplex ([ds−4H]$^{4-}$) were 0.26 with d(AAGAATTCTT)$_2$, 0.50 with d(AAGCATGCTT)$_2$ and 0.54 with d(AAGCATGCTT)$_2$, respectively. Thus, berberine bound to three DNA in the order of d(AAGCATGCTT)$_2$ ⩾ d(AAGCATGCTT)$_2$ > d(AAGAATTCTT)$_2$, but noteworthily its sequence selectivity was not remarkable. Additionally, berberine bound to the former two DNA with binding affinities comparable to that of Hoechst 33258, a typical DNA minor groove binder (data not shown).

In conclusion, we systematically studied for the first time the noncovalent complexes of four protoberberine alkaloids from Chinese herbal medicines with double-stranded DNA by using ESI-MS spectrometry. The results indicated that palmatine among the four alkaloids exhibited the greatest binding affinity with the double-stranded DNA, while berberine had the lowest affinity. Our preliminary results indicated that berberine showed some sequence selectivities. Specific efforts are currently being made to modify these alkaloids through organic synthesis, with the aim to improve their DNA-binding affinities and sequence selectivities.

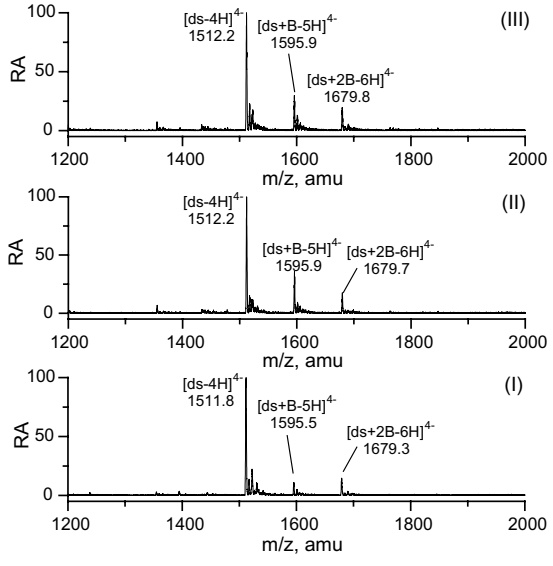

Figure 6. Negative ESI-MS spectra of the complexes of berberine with d(AAGAATTCTT)$_2$ (I), d(AAGGATCCTT)$_2$ (II), and d(AAGCATGCTT)$_2$ (III) in a 1:2 DNA-to-alkaloid molar ratio.

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6. This conclusion is also supported by the small differences in the association constants of $d(AAGAATTCTT)_2$ with these alkaloids, obtained by fluorospectroscopic methods, for example, $1.24 \times 10^4\,M^{-1}$ for berberine, $1.78 \times 10^4\,M^{-1}$ for palmatine, and $1.26 \times 10^4\,M^{-1}$ for coptisine in pH 6.35 50 mM Tris–HCl, respectively.
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