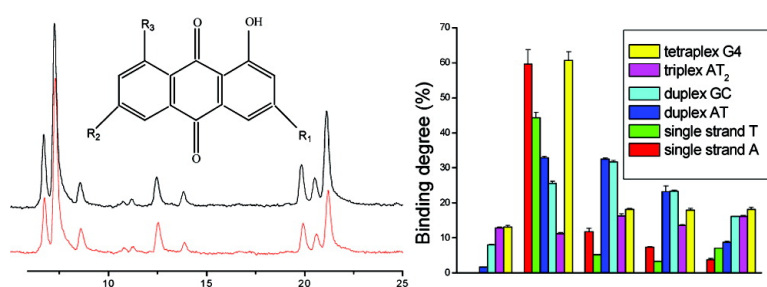## Article

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Biological Fingerprinting Analysis by Liquid Chromatography/Mass Spectrometry for Evaluation of DNA Structural Selectivity of Multiple Compounds in Natural Products

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A method by combination of centrifugal ultrafiltration (CUF) sampling with liquid chromatography−mass spectrometry (LC−MS) analysis was established to evaluate the DNA structure and sequence selectivity of the multiple compounds in a small molecule library. The developed method was applied to analyze the extracts of natural products *Coptis chinensis* Franch and *Rheum palmatum* (L.). From the obtained biological fingerprinting chromatograms, 7 compounds in *C. chinensis* Franch and 11 in *R. palmatum* (L.) were screened out as DNA binding agents. Most of these compounds were identified by standards and LC−MS analysis after the sample pretreatment with the DNA immobilized cartridge. DNA structural binding preference of the multiple active compounds in these two extracts was then evaluated simultaneously without purification.

Introduction

DNA is the molecular target of many antimicrobial, antiviral, and antitumor active drugs.[1] Knowing about the structural preferences may facilitate understanding of the binding mechanisms of the small molecules to DNA. On the other hand, some DNA with specific sequences or structures may represent attractive targets for small-molecule therapeutics. As but one possible example, tetraplex DNA appears to be an integral part of telomeres and is a substrate for telomerases involved in chromsome replication. Compounds that stabilize tetraplex DNA within telomeres might effectively block telomerase activity by locking the nucleic acid substrate into an unfavorable conformation for its replication. Such small molecules may be potentially valuable as therapeutic antitumor agents.[2]

The availability of a number of techniques, such as NMR, UV−visible, fluorescence, and surface plasma resonance have facilitated the studies of drug−DNA interactions. To evaluate the DNA structural preference of small molecules, Ren and Chaires[3] introduced the competition dialysis method, which has been adopted widely in drug−DNA interaction investigations;[4−7] however, all of these techniques mainly focus on studying the simple binding system in which one compound binds to one biomacromolecular receptor. With the development in combinatorial chemistry and the preparation of natural products, recognition studies based on screening of libraries turn into reality for the identification of interacting counterparts against known or unknown libraries, and the objectives of studying small-molecule−biomacromolecule interactions have been changing from simple objects to complex interaction systems.[8] Thus,

methods targeting the interaction of the complex system are accordingly required.

Biological fingerprinting analysis, which is defined as the comparison of the fingerprinting patterns of a small compound library, such as extracts of traditional Chinese medicines, before and after interaction with biological systems (DNA, protein, cell, etc.), was proposed previously[9] for screening and analysis of the multiple bioactive compounds in a library. On the basis of the method of microdialysis/HPLC, it has been proven to be effective for studying the complex interaction of a single target of calf thymus DNA with the multiple alkaloids in the extract of some natural products. With biological fingerprinting chromatogram analysis, biological interaction of the multiple components in the extract of natural products with DNA can be simultaneously discerned.[9,10] However, the microdialysis sampling fails to apply to samples with a low amount. Centrifugal ultrafiltration (CUF) is another filtrate selection method for a wide range of biomedical and clinical applications.[11,12] Because only minute volumes are needed for analysis, it is expected to be employed in the biological fingerprinting analysis.

In this work, biological fingerprinting chromatogram analysis was developed by utilizing the CUF/LC−MS method. On the basis of this method, the evaluation of the DNA structural preference of multiple compounds in the extracts of natural products, as an example of a small compound library, was performed.

Experimental Section

Reagents and Chemicals. Calf thymus DNA (ct-DNA) (type I, highly polymerized) purchased from Sigma (St. Louis, MO) was deproteinized and dissolved in BPES buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA, 185 mM NaCl) at pH 7.0. The synthetic oligo deoxyribonucleic acid

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(DNA) was purchased from Takara Biotechnology (Dalian, China) and dissolved in BPES buffer. Single-stranded purine dA_{12} and pyrimidine dT_{12} were used as received. Duplexes DNA [5′-(dAdT)$_6$] and [5′-(dGdC)$_6$], triplex DNA [5′-(dAdT$_2$)$_6$], and tetraplex DNA [5′-(T$_2$G$_{20}$T$_2$)$_4$] were prepared as described by Ren and Chaires.[3] Concentrations of all the DNA samples were determined by UV absorbance measurements[3] and expressed in terms of the monomeric unit that comprises the polymer, that is, nucleotides, base pairs, triplets, and tetrads. EDC (1-ethyl-3- (3-dimethylaminopropyl)-carbodiimide) was purchased from Acros Organics (NJ). The standards were purchased from the National Institute for the Control of Pharmaceutical and Biological Products. The HPLC grade CH$_3$CN was from Merck (Merck; Darmstadt, Germany), distilled water was further purified by Milli-Q system (Millipore; Milford, MA), and other chemicals were of analytical grade.

Preparation of the Extracts of Natural Products. The natural products *Coptis chinensis* Franch and *Rheum palmatum* (L.) were purchased from a local store (Dalian, China). A 15-g portion of those natural products was crushed into powder in a grinder and immersed in 150 mL of 95% ethanol overnight, then heated under reflux for 1.5 h. The extraction was repeated twice. After being combined, the extract was allowed to filter through a 0.45-μm membrane. The residues obtained upon evaporation of the solvents was dissolved in 25 mL of BPES buffer and stored for further experiments.

Microdialysis Sampling. The microdialysis system consists of a Cole-Parmer 74900 microdialysis pump (Cole-Parmer; Chicago, IL) and a homemade microdialysis probe with a cellulose membrane (Spectrum; LA) at a length of 10 mm and a molecular weight cutoff of 18 000 Da. The perfusion rate was 1 μL/min. The microsyringe was filled with the perfusion solution before sampling. A 200-μL portion of extract of the natural products and 1000 μL of ct-DNA solution (final concentration of base pairs was 100 μM) were mixed and incubated at 37.0 °C over a water bath for 10 min. The microdialysis procedure was preceded as described previously.[9]

CUF Sampling and DNA Structural Preference Experiment. The CUF was performed on a Hermle Z300 centrifuge (Hermle; Gosheim, Germany). A 20-μL portion of extract of natural products and 100 μL of ct-DNA solution were mixed in a Microcon centrifugal filter from Millipore (Bedford, MA) with molecular weight cutoff of 100 000 Da and incubated at 37.0 °C over a water bath for 10 min. A blank sample was prepared with the addition of 100 μL of BPES buffer instead of ct-DNA, then they were centrifuged at 8000 rpm for 1 min, and the filtrates were analyzed by HPLC.

The experiments of DNA structural preference were carried out using the following procedure. A 20-μL portion of extract of natural products in BPES buffer and 100 μL of 1 mM oligo DNA solution with various structures was mixed in the centrifugal filters with the molecular weight cutoff of 3000 Da. The final pH was adjusted to 7.0 by concentrated NaOH solution, and the volume was 130 μL. The mixtures were incubated at 37.0 °C for 10 min then centrifuged

simultaneously at 12 000 rpm for 10 min. The filtrate was stored at 4 °C until further HPLC analysis. The mixture of the extract of natural products and BPES buffer was regarded as the blank, which represents the extract of natural product solution before interaction with DNA.

HPLC and LC−MS Analysis. The HPLC system consisted of two LC-10ATvp pumps (Shimadzu, Kyoto, Japan), a Rheodyne-type injector valve with a 10-μL loop, a Waters 996 photodiode array detector (Waters; Milford, MA), and a Millennium 32 workstation (Waters; Milford, MA). The 250 × 4.6-mm-i.d. column packed with 5-μm Hypersil-BDS was from Elite Analytical Instruments Co. (Dalian, China) for the analysis of *C. chinensis* Franch. The mobile phase was acetonitrile/20 mM Britton-Robinson buffer (1350 μL of H$_3$PO$_4$, 1150 μL of HAc, 1.236 g of H$_3$BO$_3$ in 1000 mL water, pH 3.0) containing 5 mM sodium heptyl sulfate. For *R. palmatum* (L.) analysis, the 150 × 4.6-mm-i.d. column with 5-μm Hypersil-ODS packed in house was used. The mobile phase was CH$_3$CN/water with pH 3.0 adjusted with formic acid. Online LC−MS detection and analysis was performed on an APCI-MS detector (Shimadzu, Kyoto, Japan) in negative ion detection mode. The APCI probe voltage was set at 1800 V; the nebulizing gas flow was 2.5 L/min; and the APCI, CDL, and block temperatures were set at 400, 250, and 200 °C, respectively. The mass range (m/z) was from 100 to 800, and the scan rate was set at 2 s/scan.

SPE with the ct-DNA Column. The ct-DNA immobilized silica SPE cartridge was prepared in-house as the following procedure. A 2.5 mg portion of ct-DNA in 2.0 mL of 1-methylimidazole solution (10 mM) was sonicated for 30 min in an ice bath, and then 0.18 g of aminopropyl silica was added in the DNA solution. After being mixed homogeneously, 0.27 mL of freshly made EDC solution (200 mM) in 10 mM 1-methylimidazole was added, then the mixture was allowed to react at 50 °C for 5 h for immobilization of the ct-DNA onto the silica. After being washed by water thoroughly, the ct-DNA-immobilized silica was packed into the cartridge. Before use, the cartridge was conditioned by 0.9 mL of 20 mM Tris−HCl buffer (pH 7.4) containing 20 mM NaCl, then 100 μL of extract of *R. palmatum* (L.) was added. After being washed by another 0.9 mL of Tris−HCl buffer, the cartridge was eluted by 0.3 mL of CH$_3$CN/20 mM Tris−HCl (pH 7.4, containing 20 mM NaCl and 5 mM MgCl$_2$) (20/80), and the eluted solution was collected. Before LC−MS analysis, it was desalted by SPE using an ODS cartridge.

Results and Discussion

Biological Fingerprinting Analysis of Natural Products by CUF Sampling Combined with HPLC. *C. chinensis* Franch has been commonly used with the effects of clearing heat, drying up dampness, purging toxicosis, and detoxicification in a clinic in China. It has been proven previously[9] that seven compounds, including jatrorrhizine, palmatine, and berberine, in the extract of *C. chinensis* Franch are active in binding to ct-DNA. *R. palmatum* (L.) is another commonly used natural product for the treatment of hemorrhaging of the digestive system, acute hepatitis, gallstones, inflammation
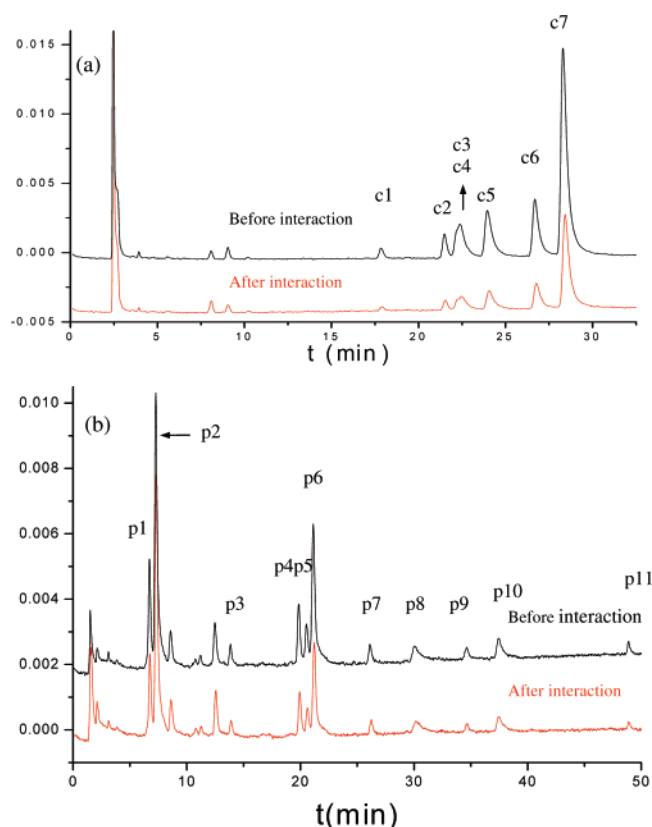
Figure 1. Biological fingerprinting chromatograms for the extract of (a) *C. chinensis* Franch and (b) *R. palmatum* (L.) by combing CUF sampling with HPLC. Chromatograms are for ultrafitrates from the extract of *C. chinensis* Franch or *R. palmatum* (L.) themselves and the mixed solution of their extract with ct-DNA, respectively. Chromatographic conditions: (a) column, 250 × 4.6-mm-i.d. packed with 5-μm Hypersil-BDS; mobile phase, CH_3CN/20 mM Britton-Robinson buffer (1350 μL of H_3PO_4, 1150 μL of HAc, 1.236 g of H_3BO_3 in 1000 mL of water, pH 3.0) containing 5 mM sodium heptenylsulfonate; ambient temperature; flow rate, 1 mL/min; linear gradient elution, 0−40 min for 20−45% CH_3CN. Peak identification: (c3) jatrorrhizine, (c6) palmatine, and (c7) berberine. (b) Column, 150 × 4.6-mm-i.d. packed with 5-μm Hypersil-ODS; mobile phase, CH_3CN/water (pH 3.0, adjusted by formic acid); linear gradient elutions from 17.6 to 48% CH_3CN in 40 min and from 48 to 80% CH_3CN in another 10 min; ambient temperature; flow rate, 1 mL/min; detection wavelength, 430 nm. Peak identification: (p4) chrysophanol 8-O-β-D-glucopyranoside (primarily identified), (p7) physcion 8-O-β-D-glucopyranoside (primarily identified), (p9) aloe-emodin, (p10) rhein, and (p11) emodin.

of the sweat glands, and for extermination of *Helicobacter pylori*.[13] It has been reported to contain anthraquinone derivatives, such as physcion, chrysophanol, aloe-emodin, emodin, rhein,[14−20] and anthraquinone glycosides.[16−20] Because emodin and aloe-emodin have been proven to have cytotoxic and DNA damage-inducing activities,[16,21] and some other natural and synthesized anthraquinone derivatives were also reported as DNA binding agents,[22−29] it can be expected that other compounds in the extract of *R. palmatum* (L.) may bind to DNA due to the structural similarity of the components.

The ultrafiltrate from the extract of the natural product itself and the mixed solution of the extract with DNA were collected by the procedure described in the Experimental Section and were analyzed by HPLC under identical conditions. Because the free concentration of the compounds

Table 1. Binding Degrees of the Active Components to ct-DNA Obtained by Microdialysis or Centrifugal Ultrafiltration Sampling Combined with HPLC

peak	solute	microdialysis	CUF
	Extract of *C. chinensis* Franch		
		Binding Degree (%, ±SD, $n = 3$)	
c1	not identified	36.50 (±1.10)	35.28 (±0.98)
c2	not identified	43.51 (±0.93)	41.11 (±0.95)
c3 +	jatrorrhizine +	64.65 (±1.85)	49.87 (±1.32)
c4	not identified		
c5	not identified	58.67 (±0.88)	48.80 (±0.95)
c6	palmatine	47.59 (±1.21)	41.93 (±0.75)
c7	berberine	51.82 (±0.99)	46.72 (±1.03)
	Extract of *R. palmatum* (L.)		
p1	not identified	21.3 (±0.13)	16.2 (±0.28)
p2	not identified	3.74 (±0.15)	3.00 (±0.56)
p3	not identified	35.4 (±0.43)	30.3 (±0.53)
p4	chrysophanol 8-O-β-D-glucopyranoside[a]	36.9 (±0.69)	30.3 (±0.68)
p5	not identified	43.0 (±0.72)	39.9 (±0.75)
p6	not identified	41.3 (±0.53)	32.1 (±0.62)
p7	physcion 8-O-β-D-glucopyranoside[a]	12.5 (±0.49)	9.58 (±0.53)
p8	not identified	13.2 (±0.43)	9.00 (±0.61)
p9	aloe-emodin	26.2 (±0.92)	24.5 (±0.76)
p10	rhein	4.58 (±0.11)	4.55 (±0.34)
p11	emodin	31.9 (±1.02)	26.2 (±0.99)

[a] Primarily identified compound.

binding on DNA decreased after the interaction, the interaction properties of compounds in the extract of natural product with DNA can be deduced from a comparison of the two obtained chromatograms, that is, a biological fingerprinting chromatogram. The biological fingerprinting chromatograms of *C. chinensis* Franch and *R. palmatum* (L.) before and after interaction with ct-DNA are shown in Figure 1.

As defined previously,[9,10] the binding degree of any component to DNA can be calculated as

$$\text{binding degree} = \frac{A_b - A_a}{A_b} \times 100\%$$

where A_a and A_b are the peak areas of a compound after and before the interaction with DNA in the biological fingerprinting chromatograms, respectively.

Obtained from the peak areas integrated using the UV wavelength of 345 and 430 nm, respectively, the binding degrees of the compounds in *C. chinensis* Franch and *R. palmatum* (L.) by biological fingerprinting chromatogram analysis with CUF sampling are shown in Table 1. It can be seen that seven peaks, c1−c7, in *C. chinensis* Franch and seven peaks, p1, p3, p4, p5, p6, p9 and p11, in *R. palmatum* (L.) bind on DNA obviously. In addition, there are four peaks, p2, p7, p8, and p10 in *R. palmatum* (L.), that are found to have weak binding.

Peak Identification of the Active Compounds to ct-DNA. The components in the extract of natural products are extremely complex. As seen in Figure 2a, there are so many components in *R. palmatum* (L.) that they cannot be well-separated in a limited time. The implication of the inactive peaks can be avoided by choosing a suitable detection wavelength; however, under the MS detection mode, the
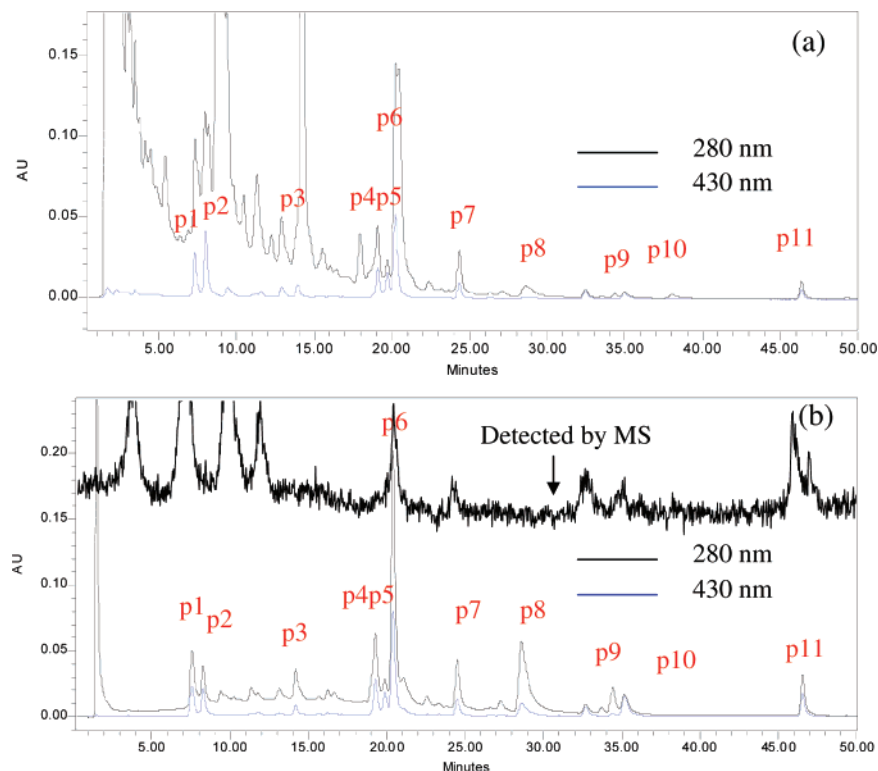
Figure 2. Chromatograms of the extract of *R. palmatum* (L.) (a) before and (b) after the SPE extraction with the immobilized ct-DNA cartridge. Detection wavelength, 280 and 430 nm. Other chromatographic conditions were the same as in Figure 1b. The upper chromatogram in part b is that of an MS trace.

implication of these compounds renders the detection of the active peak rather difficult. To identify the active peaks, SPE extraction with the immobilized DNA cartridge in combination with LC−MS analysis was performed with the *R. palmatum* (L.) extract. Since the active compounds have affinity interaction with DNA, they can be retained on the immobilized DNA SPE cartridge under a weak mobile phase while the inactive components are washed away. Then the active compounds can be eluted by washing the cartridge with a strong mobile phase. As shown in Figure 2b, after the treatment, most of the inactive compounds were removed.

The eluted fraction was desalted by the SPE cartridge using an ODS stationary phase before analyzing by LC−MS, and the obtained mass spectra of the peaks are shown in Figure 3. Peaks p9, p10, and p11 show molecular ion peaks at *m/z* 270, 283, and 269, matching those of aloe-emodin, rhein, and emodin as the MS spectra of the standard compounds shown in Figure 3. The other peaks can be primarily identified as anthraquinone glycosides, which are richly contained in *R. palmatum* (L.). Except for peaks p2 and p8 without mass signal, the spectra of all the other six peaks show two prominent peaks. One was the molecular ion peak and the other was the fragment ion by losing a molecular weight (M_r) of 162, which was generated by losing a side chain of glucose during the ionization process. By the comparison of the UV and mass spectrum with the results previously reported,[17,30] peak p4 can be primarily identified as chrysophanol 8-*O*-β-D-glucopyranoside. Peaks p1, p3, p5, and p6 have the same molecular ion signal at *m/z* 431. Their MS and UV spectra characteristics, which are similar to p4, suggest that they are also the glucopyranoside of anthraquinone. Peak p7 has a molecular ion peak at *m/z* 446,

a fragment ion resulting from loss of a glucose and a fragment ion resulting from loss of another methyl. According to its mass and UV spectrum, it can be primarily identified as physcion 8-*O*-β-D-glucopyranoside.[17,30] The structures of chrysophanol 8-*O*-β-D-glucopyranoside and physcion 8-*O*-β-D-glucopyranoside are shown in Figure 4.

By the comparison of retention times and UV−visible spectra with standards, three peaks in the chromatogram of *C. chinensis* Franch were identified as jatrorrhizine, palmatine, and berberine as structures shown in Figure 4, which is in agreement with our previous results.[9] In addition to the MS spectra, the identification of aloe-emodin, rhein, and emodin was also confirmed using the standards. With the addition of the standards in the sample in HPLC analysis, the peak that sharply increases in height was identified as the same compound with the standard. The identification was also confirmed by the comparison of UV spectra with the standards, as shown in Figure 3.

Comparison of the Sampling by CUF with Microdialysis. For the compounds in the extract of *C. chinensis* Franch, the recoveries of the microdialysis and CUF are 47.14−58.41% and 66.69−77.47%, respectively. And for those in *R. palmatum* (L.), the recoveries are 13.4−23.8% and 50.1−73.6%. For the compounds in these two kinds of natural products, the recoveries of CUF are much higher than those of microdialysis. In addition, the CUF is timesaving because centrifugation of the samples can proceed simultaneously in the batch.

As shown in Table 1, the binding degrees of most active compounds obtained from the sampling by CUF are slightly lower than those obtained by microdialysis under identical interaction conditions. It is probably caused by the shifting
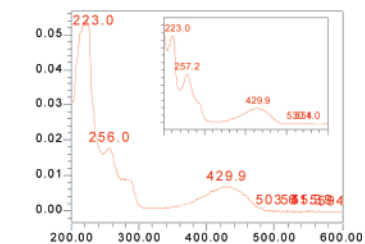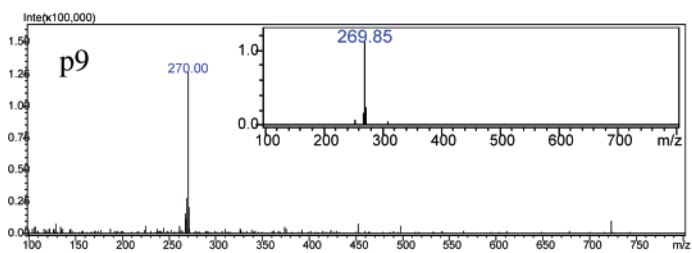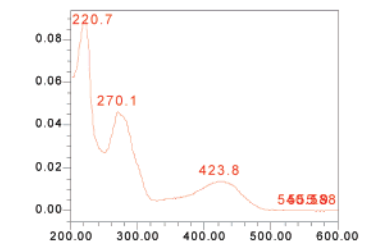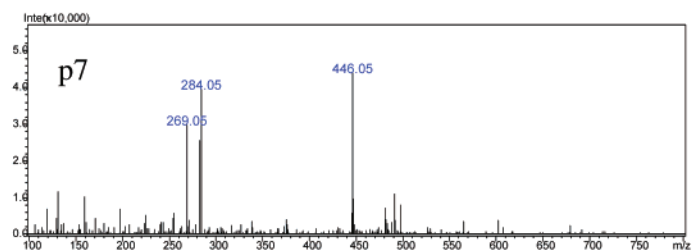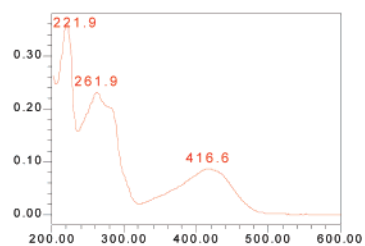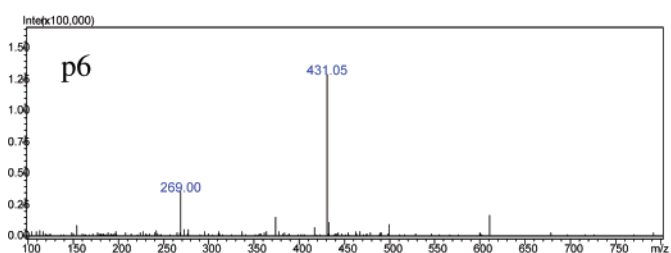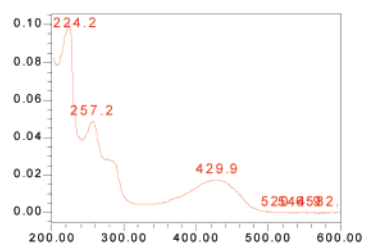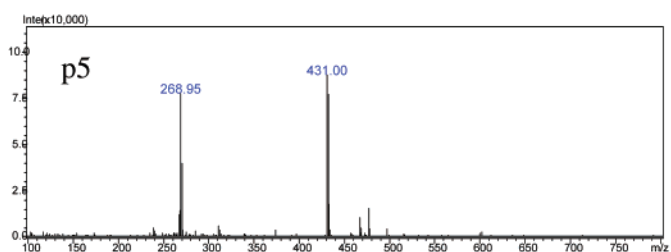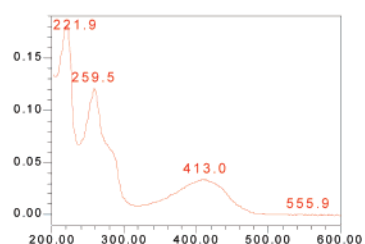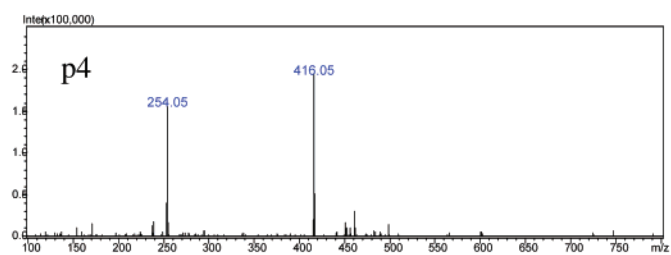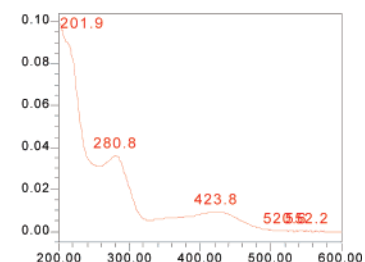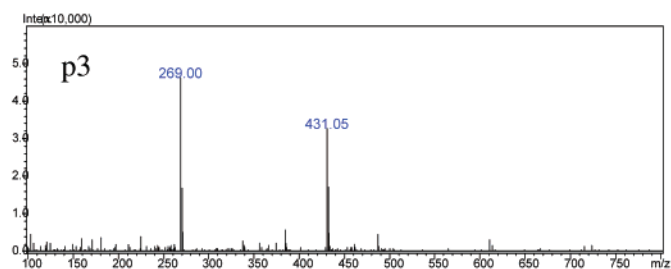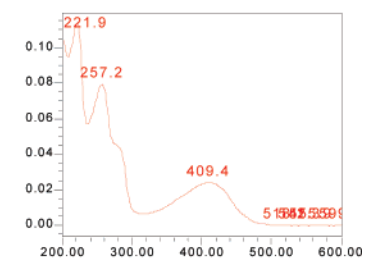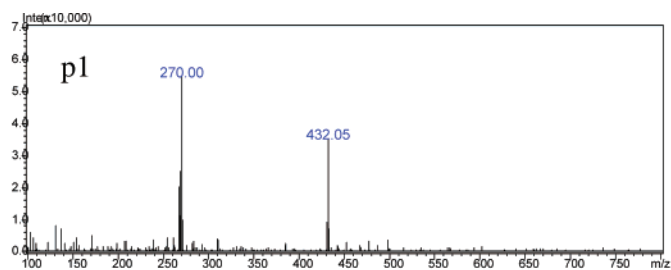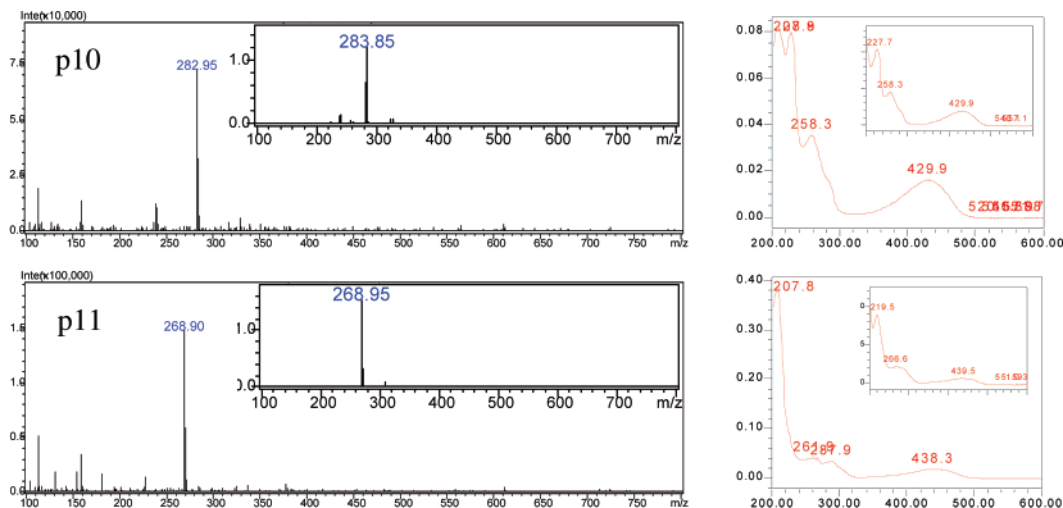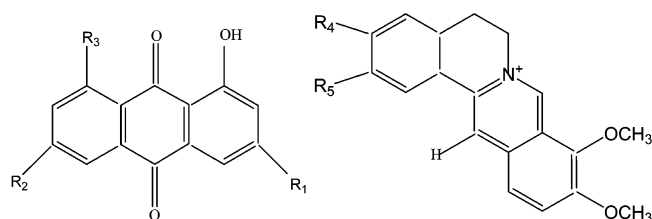
Figure 3. MS and UV spectra of the active compounds in the extract of *R. palmatum* (L.). Insets are those of standards.

Compound	R_1	R_2	R_3
Aloe-emodin	CH_2OH	H	OH
Rhein	H	COOH	OH
Emodin	OH	CH_3	OH
Chrysophanol 8-O-β-D-glucopyranoside	CH_3	H	$O\text{-}C_6H_{11}O_5$
Physcion 8-O-β-D-glucopyranoside	CH_3	OCH_3	$O\text{-}C_6H_{11}O_5$
Compound	R_4	R_5	
Jatrorrhizine	OH	OCH_3	
Palmatine	OCH_3	OCH_3	
Berberine	OCH_2O		

Figure 4. Molecular structures of the identified compounds in *R. palmatum* (L.) and *C. chinensis* Franch.

of the interaction equilibrium during CUF sampling. In the interaction solution, there exists an equilibration between the DNA and the free small molecules, described as the following:

$$M_f + DNA \rightleftharpoons [M_bDNA]$$
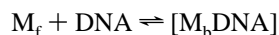
where M_f and M_b are the free and bound small molecules, respectively. As the CUF proceeded, 20–30 μL of the filtrate was removed from the total 130 μL of solution. As a result, the amount of the free molecules in the mixture reduced and the equilibration shifted left, which led to a decrease of the binding degree. The microdialysis sampling, however, as demonstrated in our previous work,[31] had little influence on the equilibration due to its comparatively small sampling portion of the whole mixture. In addition, CUF is not as friendly as microdialysis and, thus, may disturb the equilibra-

tion of the interaction. Therefore, the CUF sampling may not be suitable for the accurate determination of the binding parameters of the drugs to the biopolymers. However, information concerning the relative binding ability of a compound to various targets can still be provided, which allows it to be competent for the task of the DNA preference evaluation.

Sequence and Structural Selectivity in Binding of Active Compounds in Natural Products to DNA. Studies of sequence and structural selectivity of the DNA binding components in the extract of *C. chinensis* Franch and *R. palmatum* (L.) were performed by CUF sampling combined with HPLC. For one kind of the oligo DNA, competitive binding degrees of each compound can be discerned simultaneously from the biological fingerprinting chromatograms. Results obtained for the active compounds are shown in Figure 5 as bar graphs in which the binding degrees of the compounds are given for each DNA structure included in the assay. It can be seen in Figure 5 that all of the active compounds in *C. chinensis* Franch show similar binding preferences. They have the binding degrees in the order of tetraplex > triplex > duplex > single strand. For the duplex, they prefer the GC sequence. Their binding on single strand is very little. The similar structural and sequence selectivity suggests that the same mode is used for them to bind to DNA. For the active compounds with strong interaction to ct-DNA in *R. palmatum* (L.), p1 prefers triplex and tetraplex and has poor selectivity to single-stranded forms. For duplex forms, a moderately binding degree was observed on GC sequence, but much less was observed on the AT sequence, which indicates that the GC sequences are the main binding sites on natural DNA where the double strands are the major form. P2 has strong binding on single strand and tetrapex structures. p4 and p5 show no preference for either the GC or the AT sequence. They also bind to both single strands. The selectivity of aloe-emodin (p9) and emodin (p11) are similar. They have relatively strong binding to duplex, triplex, and tetraplex DNA. There was a preference on the T sequence between the single strands.

Conclusions

A simple and rapid method has been developed for determining the DNA structural and sequence selectivity of
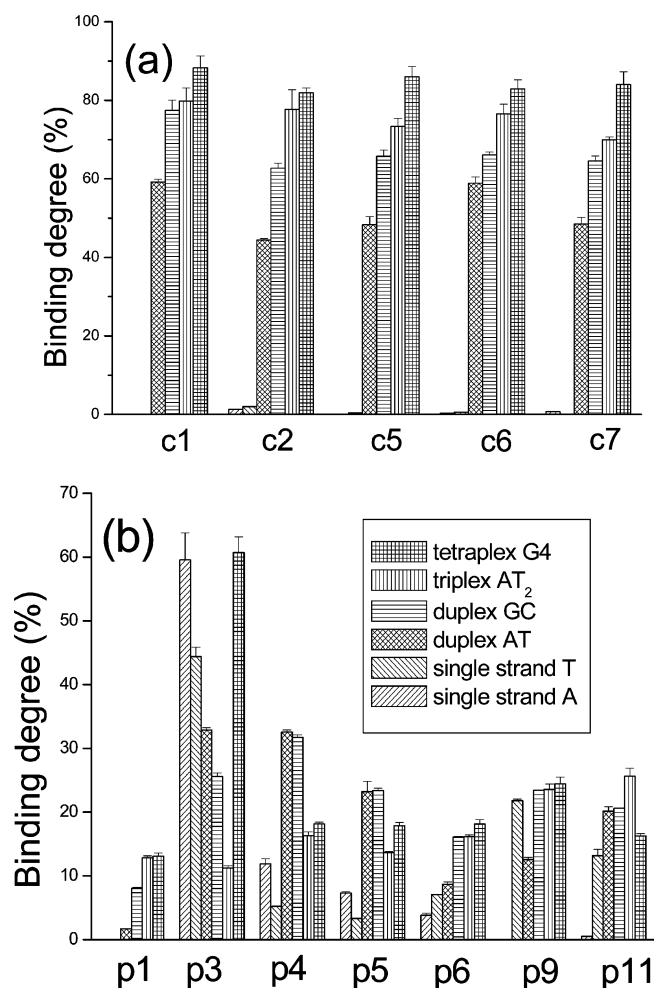
Figure 5. Evaluation for DNA structural preference of the interacted compounds in *C. chinensis* Franch and *R. Palmatum* (L.). The binding degree of the compound to each DNA structure is shown as a bar graph.

a multiple-compound library. An important advantage of this method is its applicability to a relatively small amount of a complex sample of small molecule libraries, such as natural products. The multiple compounds can be analyzed simultaneously without purification. The sampling of the interaction system with all the DNA structures can be accomplished in 30 min, which is much faster than the competitive dialysis assay, which requires at least 24 h. This method provides an alternative for the biomacromolecular recognition study of the small molecular combinatorial library.

References and Notes

(1) Pindur, U.; Fischer, G. *Curr. Med. Chem.* **1996**, *3*, 379−406.
(2) Mergny, J. L.; Helene, C. *Nat. Med.* **1998**, *4*, 1366−1367.
(3) Ren, J. S.; Chairs, J. B. *Biochemistry* **1999**, *38*, 16067−16075.
(4) Carrasco, C.; Rosu, F.; Gabelica, V.; Houssier, C.; De Pauw, E.; Garbay-Jaureguiberry, C.; Roques, B.; Wison, W.;

Chaires, J. B.; Waring, M.; Bailly, C. *ChemBioChem* **2002**, *3*, 1235−1241.
(5) Teulade-Fichou, M. P.; Carrasco, C.; Guittat, L.; Bailly, C.; Alberti, P.; Mergny, J. L.; David, A.; Lehn, J. M.; Wilson, W. D. *J. Am. Chem. Soc.* **2003**, *125*, 4732−4740.
(6) Arya, D. P.; Xue, L.; Willis, B. *J. Am. Chem. Soc.* **2003**, *125*, 10148−10149.
(7) Chaires, J. B.; Ren, J. S.; Hamelberg, D.; Kumar, A.; Pandya, V.; Boykin, D. W.; Wilson, W. D. *J. Med. Chem.* **2004**, *47*, 5729−5742
(8) Tian, R. J.; Xu, S. Y.; Lei, X. Y.; Jin, W. H.; Ye, M. L.; Zou, H. F. *TrAC, Trends Anal. Chem.* **2005**, *24*, 810−825.
(9) Su, X. Y.; Kong, L.; Li, X.; Chen, X. G.; Guo, M.; Zou, H. F. *J. Chromatogr., A* **2005**, *1076*, 118−126.
(10) Guo, M.; Su, X. Y.; Kong, L.; Li, X.; Zou, H. F. *Anal. Chim. Acta* **2006**, *556*, 183−188.
(11) Menguy, T.; Chenevois, S.; Guillain, F.; Maire, M.; Falson, P.; Champeil, P. *Anal. Biochem.* **1998**, *264*, 141−148.
(12) Ronco, C.; Clark, W. *Semin. Dial.* **2001**, *14*, 257−262.
(13) Yin, J., Guo, L. G., Eds. Zhongyao Xiandai Yanjiu Yu Linchuang Yingyong (Advanced Studies of Traditional Chinese Medicines and Its Clinical Applications), Xueyuan Press: Beijing, 1993, pp 61−86.
(14) Li, L. S.; Liu, M.; Da, S. L.; Feng, Y. Q. *Chin. J. Anal. Chem.* **2004**, *32*, 511−515.
(15) Liu, S. Y.; Sporer, F.; Wink, M.; Jourdane, J.; Henning, R.; Li, Y. L.; Ruppel, S. *Trop. Med. Int. Health* **1997**, *2*, 179−188.
(16) Shi, Y. Q.; Fukai, T.; Sakagami, H.; Kuroda, J.; Miyaoka, R.; Tamura, M.; Yoshida, N.; Nomura, T. *Anticancer Res.* **2001**, *21*, 2847−2853.
(17) Yang, X.; Gu, Z.; Ma, C.; Masao, H.; Tsuneo, N. *Zhongcaoyao* **1998**, *29*, 5−11.
(18) Okabe, H.; Kiyoshi, M.; Itsuo, N. *Chem. Pharm. Bull.* **1973**, *21*, 1254.
(19) Matsuda, H.; Kageura, T.; Morikawa, T.; Toguchida, I.; Harima, S.; Yoshikawa, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 323−327
(20) Yang, X.; Zhao, J.; Zhang, Y. *Zhongcaoyao* **1998**, *29*, 289−293.
(21) Mueller, S. O.; Lutz, W. K.; Stopper, H. *Mutat. Res.* **1998**, *414*, 125−129.
(22) Plumbridge, T. W.; Knight, V.; Patel, K. L.; Brown, J. R. *J. Pharm. Pharmacol.* **1980**, *32*, 78−80.
(23) Toshima, K.; Ouchi, H.; Okazaki, Y.; Kano, T.; Moriguchi, M.; Asai, A.; Matsumura, S. *Angew. Chem., Int. Ed.* **1997**, *36*, 2748−2750.
(24) Takenaka, S.; Itakura, Y.; Kondo, H. *Supramol. Chem.* **1998**, *9*, 69−73.
(25) Parker, B.; Buley, T.; Evison, B.; Gutts, S.; Neumann, G.; Iskander, M.; Phillips, D. *J. Biol. Chem.* **2004**, *279*, 18814−18823.
(26) Coltman, C. A.; McDaniel, T. M.; Balcerzak, S. P.; Morrison F. S.; Von Hoff, D. D. *Invest. New Drugs* **1983**, *1*, 65−70.
(27) Neldhart, J. A.; Gochnour, D.; Roach, R. A.; Hoth, D.; Young, D. *J. Clin. Oncol.* **1986**, *4*, 672−677
(28) Lawton, F.; Blackledge, G.; Mould, J.; Latief, T.; Watson, R.; Chetiyawardana, A. D. *Cancer Treat. Rep.* **1987**, *71*, 627−629.
(29) Birot-Bartapulle, F.; Catovsky, D.; Slocumbe, G.; Newland, A. C.; Marcus, R. E.; Goldman, J. M.; Galton, D. A. *Cancer Treat Rep.* **1987**, *71*, 161−163.
(30) Hu, L. H.; Li, X.; Feng, S.; Kong, L.; Su, X. Y.; Chen, X. G.; Qin, F.; Ye, M. L.; Zou, H. F. *J. Sep. Sci.* **2006**, *29*, in press.
(31) Wang, H. L.; Zou, H. F.; Zhang, Y. K. *Chromatographia* **1997**, *44*, 205−208.