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Short communication

Characterization of enantioselective binding of racemic natural tetrahydropalmatine to DNA by chromatographic methods

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Abstract

A racemate from natural product, tetrahydropalmatine (THP), was characterized on its enantioselective binding to DNA by the chromatographic methods including microdialysis/HPLC, centrifugal ultrifiltration/HPLC and immobilized DNA affinity chromatography. It was found that its (+)-enantiomer was preferential to binding on B-form duplex DNA including calf thymus DNA, AT and GC sequence oligo DNA, as well as triplex oligo DNA. The binding constants of the THP enantiomers to ct-DNA were determined with the methods of microdialysis/HPLC and frontal affinity chromatography. In addition, the DNA structural preference of either enantiomer was evaluated with the chromatographic methods.
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Keywords: Tetrahydropalmatine; Enantioselectivity; DNA binding; Natural products; Chromatographic methods

1. Introduction

DNA exists in a variety of distinct conformations including those of single stranded, duplex, triplex and tetraplex. The duplex DNA can adopt secondary structures that range from the canonical right-handed B through the left-handed Z conformation. All these unique conformations may play important functional roles in gene expression. Consequently, finding or synthesizing the enantiomers that recognize the conformation and sequence of DNA has been the subject of intense study because of its potential application to molecular biology and genetic engineering. Enantiomers of chiral metal complexes [1–3] constitute a big family of structural probes of DNA conformation since Norden and Tjerneld [4] first reported the selective binding of the Δ enantionmer of Tris (dipyridyl)Fe(II) for right-handed B-form DNA. Tatibouet et al. [5] first synthesized an acridine substituted Tröger's base with its (−)-isomer preferential binding to B-DNA. A few of other synthesis chiral drugs [5-8] were found as DNA structural probes. Methods including UV, fluorescence, circular and linear dichroism (CD and LD), calorimetry and NMR were adopted in these works.

Tetrahydropalmatine (THP) is a natural racemate occurring in the natural product *Corydalis yanhusuo W.T. Wang* [9]. During our investigation on the screening of DNA-binding active compounds from natural products with chromatographic methods, a number of alkaloids showed their binding activity to DNA [10–14]. Several binding modes of these compounds have been reported but mainly considered as groove binding. Here we report THP, as a DNA binder, which displays enanoselectivity to DNA. To the best of our knowledge, this is the first report of the natural racemate to have enantioselective binding to DNA. The characterization of the binding properties of this compound was performed by the chromatographic methods including the microdialysis and centrifugal ultrafiltration sampling coupling with HPLC as well as the immobilized DNA affinity chromatography. Some had been applied successfully [14,15] on the binding study of multiple compounds in natural products with DNA or other biomolecules. These chromatographic methods were relatively simple, easy and fast, and it showed to be an alternate to characterize the binding of racemic mixture with DNA.

2. Experimental

2.1. Reagents and chemicals

The calf thymus DNA (ct-DNA) was purchased from Sigma (St. Louis, MO, USA) and deproteinized with phenol/

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chloroform extraction. Briefly, the ct-DNA in solution was extracted with equal volumes of phenol three times, and then was extracted with an equal volume of chloroform/isoamyl alcohol (24/1) twice to remove the trace phenol. Finally, the DNA was precipitated with cold ethanol and re-dissolved in BPES buffer. The resulted DNA had A_{260}/A_{280} ratios of 1.8–2.0, indicating that they were free of protein. The synthetic oligo DNA was purchased from Takara Biotechnology Co. Ltd. (Dalian, China). They were all purified by polyacryamide gel electrophoresis (PAGE) with purity greater than 95% as commercial declaimed. Single-stranded purine dA_{12} and pyrimidine dT_{12} were used without treatment. Duplex DNA [5′-$(dAdT)_{12}$] and [5′-$(dGdC)_{12}$], triplex DNA [5′-$(dAdT_2)_{12}$] and tetraplex DNA [5′-$(T_2G_{20}T_2)_4$] were prepared as described by Ren and Chaires [13]. The duplex DNA [5′-$(dAdT)_{12}$] and [5′-$(dGdC)_{12}$] were generated from single stranded $d(AT)_6$ and $d(GC)_6$, respectively. Triplex were from mixture of dA_{12} and dT_{12} (molar ratio 1:2) and tetraplex from 5′-$[T_2G_{20}T_2]_4$. The duplex and triplex were prepared by heating the solution to 90 °C and slowly cooling to room temperature. Tetraplex was prepared by heating the solution to 90 °C for 2 min, slowly cooling to room temperature, and then equilibrating for 48 h at 4 °C. Concentration of all the DNA samples was determined by UV absorbance measurements at 260 nm [13]. The concentration of the bases in the solution was calculated from the following formula:

$$C_{\text{nucleotides}}(\text{mmol}) = \frac{A_{260}}{15200N_A + 7400N_C + 11500N_G + 8300N_T},$$

where $C_{\text{nucleotides}}$ is the concentration of the nucleotides, N is the number of bases (A, T, C and G) on the oligo DNA strand and A_{260} is the UV absorbance at 260 nm. The final concentration of DNA was expressed in terms of the monomeric unit that comprises the polymer, i.e., nucleotides, base pairs triplets, and tetrads. All the synthetic oligo DNA and ct-DNA for microdialysis and centrifugal ultrafiltration was dissolved in BPES buffer at pH 7.0 (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA, 185 mM NaCl). The standard of rac-THP was purchased from the National Institute for the Control of Pharmaceutical and Biological Products, and (−)-THP from Beijing Tianli Co. Ltd. (Beijing, China). (+)-THP was prepared from the racemic THP by HPLC with purity of more than 99% as the preparation conditions described in Section2.5. The aminopropyl silica (5 μm, 300 Å) were prepared in National Chromatographic R. & A. Center (Dalian, China). EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide) was purchased from Acros Organics (NJ, USA). Ultra pure grade tris was purchased from Amresco (Solon, OH, USA). The HPLC gradient grade CH_3CN was from Merck (Darmstadt, Germany). Distilled water was further purified by Milli-Q system (Millipore, Bedford, MA, USA); other chemicals were of analytical grade.

2.2. Microdialysis experiments

The microdialysis system comprises a Cole-Parmer 74900 microdialysis pump (Cole-Parmer, Chicago, USA) and a home-made microdialysis probe with a cellulose membrane (Spectrum, LA, USA) at a length of 10 mm and a molecular weight cut-off of 18 000 Da. The perfusion solution was BPES buffer and the perfusion rate was 1 μL/min. The microsyringe was filled with the perfusion solution before sampling.

The THP–DNA mixture in BPES buffer was incubated at 37.0 °C in a water bath over 10 min with the THP concentration ranging from 22.6 to 90.4 μM maintaining the DNA concentration at 40.2 μM. Then the probe was placed into the solution for microdialysis sampling. After proceeding for 20 min to reach an equilibration, the "interaction microdialysate" was collected for 40 min. The collected microdialysate was handled for HPLC analysis. The "blank microdialysate" was also collected by exactly the same procedure as the "interaction microdialysate" with the absence of DNA in sample solution. The "interaction microdialysate" and "blank microdialysate" represent the THP solutions after and before the interaction with DNA, respectively.

2.3. Centrifugal ultrafiltration experiments

The centrifugal ultrafiltration was performed on a Hermle Z300 centrifuge (Hermle, Gosheim, Germany). Microcon centrifugal filters with the molecule weight cut-off of 3000 were purchased from Millipore (Bedford, MA, USA). The experiments of DNA structural preference were carried by the following procedure. Twenty microlitre 0.25 mM THP in BPES buffer and 100 μL 1 mM oligo DNA solution with various structure or 100 μL BPES buffer was mixed in the centrifugal filters, and the final pH was adjusted to 7.0 by concentrated NaOH solution, and the final volume was 130 μL. The mixtures were incubated at 37.0 °C for over 10 min then centrifugalized simultaneously at 12 000/min for 30 min. The filtrate was stored at 4 °C for further HPLC analysis. The mixture of THP and BPES buffer with the absence of DNA was regarded as blank, which represents the THP solution before interaction with DNA.

2.4. Preparation of DNA immobilized stationary phase

The DNA-immobilized silica was prepared with the process described by Rasmussen et al. [16] with modification. Briefly, 25 mg ct-DNA in 20 mL 10 mM 1-methyl imidazole was disrupted for 30 min in an ice bath with a JY92-II sonicator (Scientz Biotechnique Co. Ltd, Ningbo, China). Then, the DNA solution was added in 1.8 g aminopropyl silica. After mixing homogeneously, 2.7 mL fresh-made 200 mM EDC in 10 mM 1-methyl imidazole was added. Then the mixture was allowed to react at 50 °C for 5 h. The DNA immobilized silica was packed in the stainless steel column, which was subsequently washed with the mobile phase until the absorption peak at 260 nm on the UV spectrum disappeared. The DNA loadability was estimated by subtracting the amount recovered in all of the washes of the support from the amount added. DNA was quantified through the high absorption of DNA at 260 nm. The final DNA loadability was 11.25 ± 0.93 mg/g silica. The column was allowed to equilibrate overnight prior to use.

2.5. HPLC analysis

The HPLC system consisted of two LC-10ATvp pumps (Shimadzu, Kyoto, Japan), a Rheodyne-type injector valve with a 10 μL loop, a WatersTM 996 photodiode array detector (Waters, Milford, MA, USA) and a Millennium 32 workstation (Waters, Milford, MA, USA). The chiral column packed with 5 μm covalently bonded cellulose tris-(3,5-dimethylphenyl carbamate) chiral stationary phase (CSP) was prepared with the procedure described previously [17]. On the chiral column, the analysis of racemic mixtures was performed under the aqueous mobile phase of $CH_3CN/NaClO_4$ (aq, 0.1M, pH 3.0). (+)-THP was prepared from the racemic THP on the chiral column under the nonaqueous mobile phase of hexane/isopropanol (60/40) at flow rate of 1.0 mL/min. The obtained (+)-THP was reanalysed on this column under the same conditions, and its purity was determined as its peak area percentage on the chromatogram. The mobile phase for immobilized DNA column was 20 mM tris–HCl buffer (pH 7.4) containing 20 mM NaCl and 2 mM EDTA.

3. Results and discussion

3.1. Chiral selectivity of rac-THP with ct-DNA

As an alternate of evaluating the binding properties between the complex system and DNA or HSA, microdialysis/HPLC method had been established and applied successfully in the case of extraction of natural products [14,15]. This method is based on the semi-permeability of the microdialysis probe that only allowed the free small molecules in the sample solution to pass through. With the microdiaysis sampling, the small molecules can be collected from the their interaction system with DNA without interrupting their equilibrium as proved previously [18]. The interaction information was obtained from the comparison of microdialysate of the drugs before and after the interaction at microdialysis conditions, thus, avoiding the effect of the possible extraneous drug binding and the adsorption onto the apparatus walls, membranes or other components on the determination of the binding degree. In this case for the interaction of the THP racemate with DNA, the interaction buffer was chosen as phosphate buffer with pH of 7.0 and ion strength of 200 mM, which is compatible to keep the DNA activity and avoid the static adsorption. In addition, this buffer can maintain the stability of all the DNA structures chosen for study especially the tetraplex DNA. The addition of the EDTA prevents the DNA degradation by inhibiting the Dnase. The whole interaction process must be avoided to introduce any organic solvents or other interfering substance to DNA.

Fig. 1 shows a chromatogram comparison of *rac*-THP before and after the interaction with ct-DNA obtained by microdialysis/HPLC method [18–23]. After the interaction, as shown in the figure, the peak areas of both enantiomers decreased, suggesting that their binding on DNA reduced the concentration of free enantiomers. The calculation of the peak-area decrease showed that 30.03% (R.S.D. = 1.6%, $n = 5$) of the peak area of (+)-THP was lost after interaction, but only 9.96% (R.S.D.=1.9%, $n = 5$) for (−)-THP. The results indicate that (+)-THP has a stronger binding to DNA than (−)-THP under the same conditions.

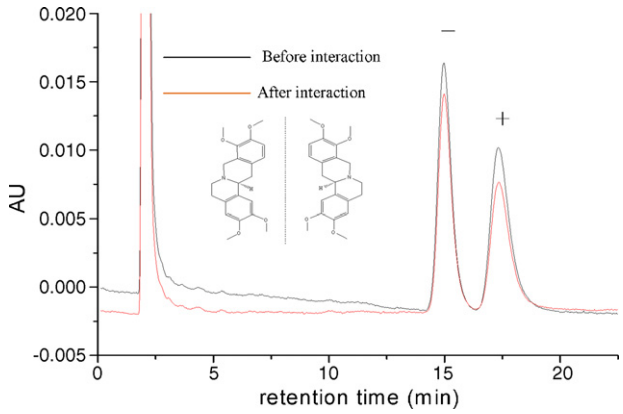

Fig. 1. Comparison of chromatograms of THP before and after the interaction with ct-DNA. Chromatograms for 10 μL microdialysate from the 0.10 mM racemic THP itself and the mixed solution of it with ct-DNA at a final concentration of 2.0×10^{-4} M base pairs are indicated as black and red curves, respectively. Chromatographic conditions: column: 150 mm × 4.6 mm I.D. packed with cellulose tris-(3,5-dimethylphenyl carbamate) chiral stationary phase; mobile phase: $CH_3CN/NaClO4$ (aq, 0.1M, pH 3.0) = 25/75; flow rate: 1.0 mL/min; detection wavelength: 207 nm.

Additional evidence comes from the chromatographic separation of rac-THP on the DNA immobilized column. A fundamental chromatographic process can be regarded as the inter-molecular interactions (hydrophobic, electrostatic, hydrogen bonding, etc.) of the analytes between the stationary phases and mobile phases, which are similar to the interactions between ligands and receptors in biological environments [24]. Therefore, the retention of the solutes on the affinity column was a factor to evaluate the interaction between the ligand and the acceptors. In this case, the retention times for (−) and (+) enantiomer were 4.427 min (R.S.D. = 2.0%, $n = 5$) and 5.444 min (R.S.D. = 2.9%, $n = 5$), respectively as shown in Fig. 2. The stronger retention of (+)-enantiomer than (−)-enantiomer sug-

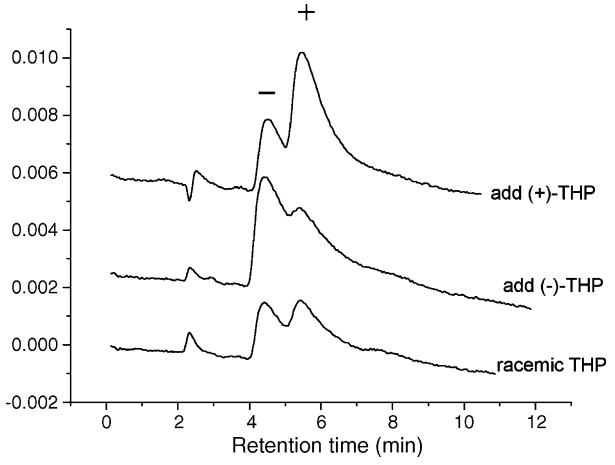

Fig. 2. Chromatograms of racemic THP on the DNA immobilized column. Chromatographic conditions: column: 100 mm × 4.6 mm I.D. packed with 5 μm, 300 Å silica immobilized with DNA; mobile phase: 20 mM tris–HCl at pH 7.4 containing 20 mM NaCl and 2mM EDTA; flow rate: 0.6 mL/min; detection wavelength: 280 nm; ambient temperature.

gests its stronger binding to DNA. This result, together with that from microdialysis/HPLC experiment, supports the conclusion that (+)-THP binds preferentially to ct-DNA, i.e., B-form duplex DNA as compared with (−)-THP.

3.2. Binding constants for the interaction of THP enantiomers with ct-DNA

The determination of the binding constants of THP enantiomers was performed by the methods of both microdialysis/HPLC and frontal chromatography. The former was based on the formula of McGhee and Von Hippel [25]:

$$\frac{r}{C_f} = K - nKr,$$

where r is the ratio of concentration of the binding analyte to that of the nucleotides. In this equation the association constant for binding to an isolated site (K) is given by the intercept on the ordinate of a Scatchard plot [26] (r/c axis) and n, the apparent site-size, represents the number of nucleotides occupied by a single analyte molecule [25]. The parameters r and C_f were calculated from the peak areas of the enantiomers before and after the interaction of various concentrations of THP ranging from 22.6 to 90.4 μM and DNA with a maintained concentration of 40.2 μM. The recoveries for (−) and (+) THP were 59.51% (R.S.D. = 1.2%, n = 5) and 59.32% (R.S.D. = 1.5%, n = 5), respectively; and the calculation curves were $A = 30220C + 28186$ ($r = 0.9995$) and $A = 29360C + 27007$ ($r = 0.9995$) for (−) and (+) THP ranging from 10 to 320 μM, respectively, where C is the concentration of isomers (μM) and A of the corresponding peak areas. The LOQs for (−) and (+) THP were 2.5 and 5.0 μM (S/N = 10), respectively. The Scatchard plots of (−) and (+) THP are shown in Fig. 3. As can be seen from the results in Table 1, the obtained apparent sizes (n) for the two enantiomers are similar, suggesting their same binding mode on ct-DNA.

Frontal chromatography was used to quantitatively evaluate column loading and the dissociation constant (K_d) of the analyte with immobilized molecules [27–29]. This method is based on the following relationship between K_d and chromatographic parameters:

$$\frac{1}{[A]_0(V - V_0)} = \frac{K_d}{B_t}\frac{1}{[A]_0} + \frac{1}{B_t},$$

where A is the analyte, B is the receptor (DNA here) and B_t is the total amount of immobilized DNA binding sites, V_0 is column void volume, and V is the volume required to elute a continuously applied concentration of A ($[A]_0$) from the column. According to the equation, a plot of $1/[A]_0(V - V_0)$ versus $1/[A]_0$, obtained

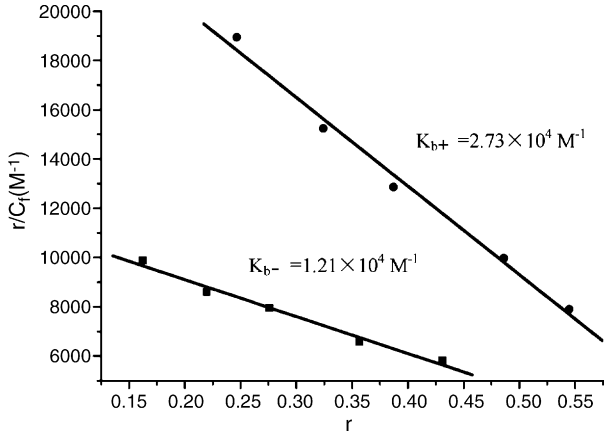

Fig. 3. Scatchard plots for interaction of (+)-THP-DNA and (−)-THP-DNA.

by measuring V from different $[A]_0$, should yield a straight line with the slope corresponding to K_d/B_t and ordinate intercept corresponding to $1/B_t$, whereas the binding constant K_b can be obtained from $1/K_d$. The void volumne of the column (V_0) is determined in the same way using an unretained solute acorbic acid in this case.

Data from concentration of 5–250 μM (−) and (+) THP allowed construction of the plots as shown in Fig. 4, and the binding constants obtained as compared with those from the method of microdialysis/HPLC are shown in Table 1. It can be seen that the binding constant of (+)-THP is greater than that of (−)-THP obtained from either method.

The K_b for both enantiomers obtained from the frontal chromatography are less than those obtained from the microdialysis/HPLC method, which may come from the following reasons. First, the ct-DNA for the experiment of microdiaysis/HPLC was intact whereas those immobilizing on the column was sonicated into fragments before use; thus, the effective binding sites on DNA may decrease for the increase of the molecular ends unavailable for binding. Secondly, the DNA molecules are free in the solution for microdialysis experiment whereas they are immobilized for the frontal chromatography. The reduced flexibility and the covered binding sites on the DNA molecules prevent the binding of the anlytes on them. In addition, the difference between the interaction buffers of the two systems may bring a slight variation into the binding process.

3.3. Structural preference of THP enantiomers to DNA

Studies of DNA sequence and structural selectivity of (−) and (+) THP were performed by the method of centrifugal ultrafiltration combined with HPLC by the procedure as details described

Table 1
Binding parameters for THP-DNA interaction determined by the methods of microdialysis/HPLC and frontal chromatography

Binding constant ($K_b/10^4$ M^{-1})		Apparent site-size (n/nucleotides)		Method
(+)-THP	(−)-THP	(+)-THP	(−)-THP	
2.73 (±0.18)	1.21 (±0.10)	1.32 (±0.01)	1.21 (±0.01)	Microdialysis/HPLC
0.863 (±0.026)	0.502 (±0.022)	–	–	Frontal chromatography

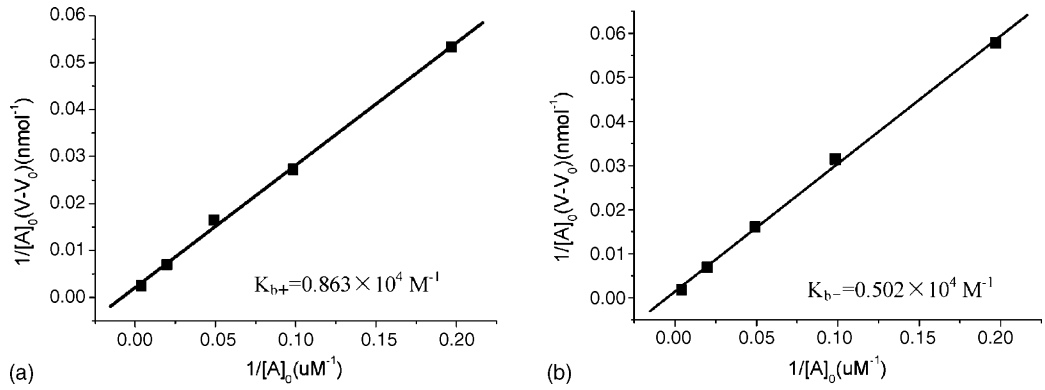

Fig. 4. $1/[A]_0(V - V_0)$ vs. $1/[A]_0$ plots based on the frontal affinity chromatography of (a) (+) and (b) (−)-THP with concentration ranging from 5 to 250 μM on the DNA immobilized column with chromatographic conditions as shown in Fig. 3.

in the experimental section. In this method, the centrifugal ultrafiltration serves as a sampling technique of the small molecules from the interaction mixture as mirodialysis does but is more suitable to the case of the samples with small volume and low concentration. The binding degrees of both enantiomers to a kind of target can be obtained simultaneously from the comparison of chiral chromatograms before and after the interaction.

Oligo DNA with six kinds of structure and sequence including single strand dA_{12} and dT_{12}, duplex conformations with GC and AT sequence, triplex and tetraplex conformation was adopted as targets. As seen from the results shown in Fig. 5, for the single stranded DNA, nearly no binding of THP enantiomers to both dA_{12} and dT_{12} sequence was observed. For the two duplex forms, either enantiomer showed a greater binding degree on DNA with AT than GC sequence. However, for both sequence, (+)-THP displayed stronger binding. The binding preference for GC sequence is the most considerable for (+)-THP that binds more than six times tightly than (−)-THP does, whereas only 2.6 for AT sequence. Together with the above results of ct-DNA, belonging to B-DNA as well, it can be concluded that the (+)-enantiomer of THP are preferential binding on B-DNA. Stronger binding was observed for the triplex than duplex conformation. Preference of the (+)-enantiomers can also be seen. Its binding degree is about 2.7 times greater than that of (−)-enantiomers, which is similar to the case of AT duplex. The strongest binding of both THP enantiomers to tetraplex conformation was observed, which is an important molecular target of the anti tumor agents. However, there is nearly no preference shown for the two enantiomers.

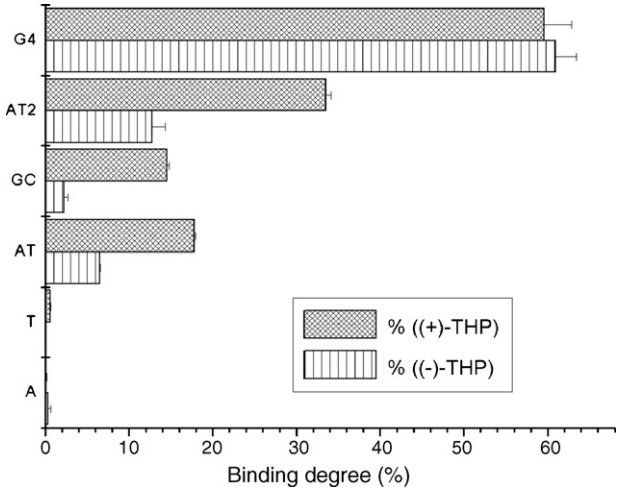

Fig. 5. Results of the DNA structural preference evaluation. The binding degree of the compound to each DNA structure is shown as a bar graph.

4. Conclusions

THP racemates naturally occurring in herb were firstly discovered to have enantio-selectivity to bind with DNA. Its (+)-enantiomer showed stronger binding to B-DNA and triplex DNA than the (−)-enantiomers. Chromatographic methods including the microdialysis and centrifugal ultrafiltration sampling coupled with HPLC as well as the immobilized DNA affinity chromatography played a major role in the enantio-selectivity estimation, binding constant determination and DNA structural preference evaluation of THP in binding to DNA. All of those chromatographic methods showed to be promising alternates in the characterization of enantio-selectivity of chiral compounds to DNA or other biopolymer targets.

Acknowledgments

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References

[1] J.K. Barton, J. Biomol. Struct. Dyn. 1 (1983) 621.
[2] J.K. Barton, Science 233 (1986) 727.
[3] C.S. Chow, J.K. Barton, Method Enzymol. 212 (1992) 219.
[4] B. Norden, F. Tjerneld, FEBS Lett. 67 (1976) 368.
[5] A. Tatibouet, M. Demeunynck, C. Arnaud, A. Collet, J. Lhomme, Chem. Commun. (1999) 161.
[6] E.J. Lee, J.A. Yeo, K. Jung, H.J. Hwangbo, G.J. Lee, S.K. Kim, Arch. Biochem. Biophys. 395 (2001) 21.
[7] X.G. Qu, J.O. Trent, I. Fokt, W. Priebe, J.B. Chaires, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 12032.
[8] Y. Xu, Y.X. Zhang, H. Sugiyama, T. Umano, H. Osuga, K. Tanaka, J. Am. Chem. Soc. 126 (2004) 6566.

[9] H.N. Kaneko, S. Naruto, J. Org. Chem. 34 (1969) 2803.
[10] W.H. Chen, Y. Qin, Z.W. Cai, C.L. Chan, G.A. Luo, Z.H. Jiang, Bioorg. Med. Chem. 13 (2005) 1859.
[11] T.K. Li, E. Bathory, E.J. LaVoie, A.R. Srinivasan, W.K. Olson, R.R. Sauers, L.F. Liu, D.S. Pilch, Biochemistry 39 (2000) 7107.
[12] D.S. Pilch, C. Yu, D. Makhey, E.J. LaVoie, A.R. Srinivasan, W.K. Olson, R.R. Sauers, K.J. Breslauer, N.E. Geacintov, L.F. Liu, Biochemistry 36 (1997) 12542.
[13] J.S. Ren, J.B. Chaires, Biochemistry 38 (1999) 16067.
[14] X.Y. Su, L. Kong, X. Li, X.G. Chen, M. Guo, H.F. Zou, J. Chromatogr. A 1076 (2005) 118.
[15] M. Guo, X.Y. Su, L. Kong, X. LI, H.F. Zou, Anal. Chim. Acta 556 (2006) 183.
[16] S.R. Rasmussen, M.R. Larsen, S.E. Rasmussen, Anal. Biochem. 198 (1991) 138.
[17] X.M. Chen, F. Qin, Y.Q. Liu, X.D. Huang, H.F. Zou, J. Chromatogr. A 1034 (2004) 109.
[18] M. Guo, H.F. Zou, H.L. Wang, L. Kong, J.Y. Ni, Anal. Chim. Acta 443 (2001) 91.
[19] H.L. Wang, H.F. Zou, Y.K. Zhang, Chromatographia 44 (1997) 205.
[20] H.L. Wang, H.F. Zou, Y.K. Zhang, Biomed. Chromatogr. 12 (1998) 4.
[21] H.L. Wang, H.F. Zou, Y.K. Zhang, Anal. Chem. 70 (1998) 373.
[22] H.F. Zou, H.L. Wang, Y.K. Zhang, J. Liq. Chromatogr. Relat. Technol. 21 (1998) 2663.
[23] H.F. Zou, H.L. Wang, Y.K. Zhang, Rev. Anal. Chem. 18 (1999) 383.
[24] R. Moaddel, L.L. Lu, M. Baynham, I.W. Wainer, J. Chromatogr. B 768 (2002) 41.
[25] J.D. McGhee, P.H. Von Hippel, J. Mol. Biol. 86 (1974) 469.
[26] G. Scatchard, Ann. NY Acad. Sci. 51 (1949) 660.
[27] F.H. Arnold, S.A. Schofield, H.W. Blanch, J. Chromatogr. 355 (1986) 1.
[28] S. Golshan-Shirazi, S. Ghodbane, G. Guiochon, Anal. Chem. 60 (1988) 2630.
[29] K.I. Kasai, Y. Oda, J. Chromatogr. 376 (1986) 33.