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A L-RNA aptamer chiral stationary phase for the resolution of target and related compounds

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Abstract

In this paper, we report for the first time an aptamer-based chiral stationary phase (CSP) able to resolve racemates of both target and various related compounds. The enantiomers of tyrosine and analogues (11 enantiomeric pairs) were separated using an immobilized tyrosine-specific L-RNA aptamer as CSP and an aqueous buffer (8 mM Tris–HCl buffer, 25 mM NaCl, 5 mM $MgCl_2$; pH 7.4) as mobile phase, at a column temperature of 10 °C. It appeared that the carboxylic and amino groups as well as the aromatic side chain of amino acid controlled the stereospecific recognition. Modifications on the polar groups were strongly detrimental for enantioselectivity while the replacement of the phenolic group by some bicyclic aromatic residues of different polarity, size or shape did not impair the enantioselective interaction. In addition, the effects of the mobile phase composition and column temperature upon the retention and stereoselective properties of the CSP were assessed. Finally, it was found that the immobilized RNA aptamer could be used under hydro-organic mobile phase conditions without alteration of the stationary phase stability.

Keywords: Chiral stationary phase; L-RNA aptamer; Enantiomeric separation

1. Introduction

Three strategies can be described for the enantioselective chromatography on chiral stationary phases (CSPs) [1]. The first one is to adapt the solute to a particular CSP, for example, via the pre-column derivatization of the racemate. The second one is to select the CSP for a particular racemate. Such a strategy is the most used and involves classically the random screening of CSPs or a selection based on experience or from a database [2]. The third one, the most recent approach, concerns the selection of a CSP in relation to the analyzed racemate. Such target-specific CSPs can be obtained by molecular imprinting, production of monoclonal antibodies or combinatorial strategy. This latter approach implies the use of a small library of various low-molecular weight selectors [3–5].

Recently, single-stranded oligonucleotides obtained from very large combinatorial libraries (DNA or RNA aptamers) have been used successfully as affinity stationary phases in liquid chromatography (LC) or capillary electrochromatography (CEC) for the purification/separation of a variety of molecules, from compounds as large as proteins [6] to compounds as small as aminoacids, adenosine and derivatives or flavin mononucleotide [7–9]. More specifically, the enantioselective properties of DNA or RNA aptamers selected against a target enantiomer were accounted by our group to create a new class of target-specific chiral stationary phases (CSPs). These aptamer CSPs were developed for the chromatographic resolution of the racemates of an oligopeptide [10], a nucleoside [11], an amino acid [12] and an amino acid derivative [11]. At ambient temperature, the apparent enantioseparation factor was around 3.5 for the anti-D-adenosine aptamer CSP [11] while a very high enantioselectivity was obtained with the anti-D-vasopressin [10], anti-L-tyrosinamide [11] and anti-L-arginine [12] aptamer CSPs. Aptamer chiral se-

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lectors offer several advantages such as their production by chemical synthesis at a relative high degree of purity, the possibility to change their sequence in order to modulate their binding selectivity, the possibility to modify at precise locations in order to allow attachment to the chromatographic surface and the pre-determined elution order of enantiomers. However, as reported previously, various major drawbacks could prevent a practical use of these target-specific CSPs [11,12]. Notably, the oligonucleotidic stationary phase stability can be limited over the time, especially for the RNA CSP [12]. For the RNA aptamers, this problem has been recently resolved by applying the mirror-image strategy [13,14] which allows to create a CSP based on L-RNA aptamer intrinsically resistant to the enzymatic degradation [12]. Another major drawback is related to the too high target-specificity of the aptamers generated by the SELEX procedure. In most cases, even small structural changes in the target structure were responsible for a strong reduction of the binding ability of the aptamer. As an example, the theophylline-binding RNA aptamer exhibited a 10,000-fold discrimination between theophylline and caffeine [15]. For a broad application in the chiral separation field, such a high specificity is assumed to be of poor practical interest as each aptamer CSP is expected to resolve only one racemate [11].

In this paper, we report for the first time an aptamer CSP able to separate the enantiomers of both target and some related compounds. The sequence of a 63-mer RNA aptamer (Fig. 1), specifically selected against L-tyrosine was used [16]. The mirror-image strategy previously described to design a biostable RNA CSP was applied [12]. The L-RNA aptamer was immobilized on a chromatographic support via a biotin–streptavidin bridge. The enantioselective properties of this L-RNA CSP were described by injecting more than 20 racemates and some structure-binding features were assessed. Furthermore, the influence of some chromatographic condition changes on the solute retention and enantioselectivity was evaluated by varying the mobile phase composition (addition of organic modifier in the eluent, variation of the ionic strength of the mobile phase) and the column temperature.

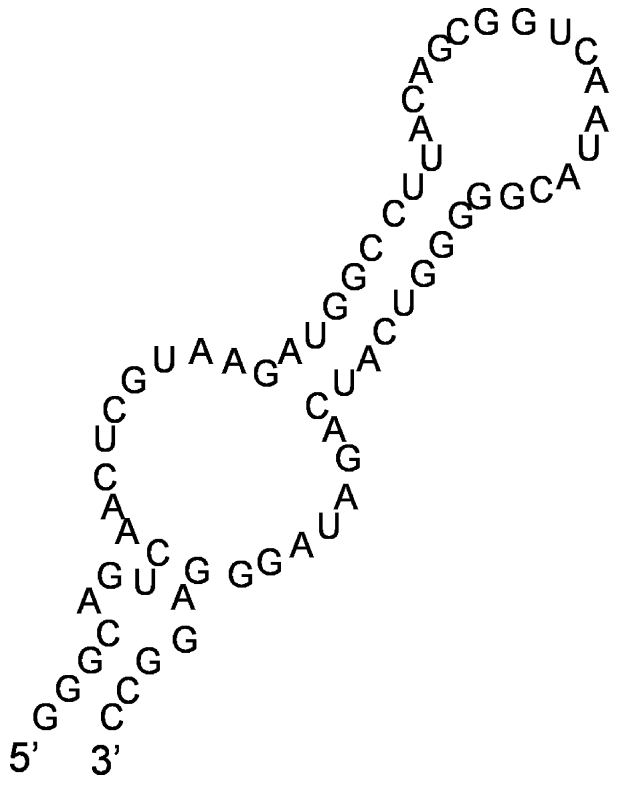


Fig. 1. Sequence and secondary structure of the minimal 63-mer RNA aptamer used as chiral selector; the active binding site is located in single-stranded regions, loops and internal bulges [10].

2. Experimental and methods

2.1. Reagents and materials

All the racemates and enantiomers analyzed in this study were obtained from Sigma–Aldrich (Saint-Quentin, France) or Bachem (Weil am Rhein, Germany). Tris–HCl, $NaNO_3$, NaCl and $MgCl_2$ were supplied by Sigma–Aldrich. Acetonitrile HPLC grade (ACN) was purchased from Fisher Scientific (Leicestershine, UK). Water was obtained from an Elgastat option water purification system (Odil, Talant, France) fitted with a reverse osmosis cartridge. L-RNA oligonucleotide was synthesized and 5′-biotinylated by CureVac (Tubingen, Germany). Biotin phosphoramidite containing a 16 atom spacer arm based on triethylene glycol was used for the aptamer biotinylation. The oligoribonucleotide was purified by HPLC and its identity was confirmed by MALDI-TOF mass spectrometry. The streptavidin POROS bulk media (20 μm polystyrene particles) was purchased from Applied Biosystems (Courtaboeuf, France). PEEK tubing (0.76 mm), PEEK end fittings (unions) and microbore frits were obtained from CIL Cluzeau Info Labo (Sainte-Foy-La-Grande, France) or Applied Biosystems.

2.2. Stationary phase preparation

Prior to immobilization, the biotinylated aptamer was renaturated by heating oligonucleotide at 85 °C for 5 min in an aqueous buffer (8 mM Tris–HCl buffer, 25 mM NaCl, 5 mM $MgCl_2$ adjusted at pH 7.4) and left to stand at room temperature for 30 min. The immobilization of the aptamer was attained by mixing around 60 nmol of oligonucleotide (in the aqueous buffer) per 1000 μl of the streptavidin media slurry during 3 h at ambient temperature. Around 20 nmol of biotinylated oligonucleotide were bound per 100 μL of support media.

2.3. Column packing

The RNA modified particles were packed in-house into a PEEK microbore column (350 mm × 0.76 mm) using a

slurry-packing procedure [11,12]. Non-modified streptavidin particles were also packed into a PEEK microbore column (350 mm × 0.76 mm) as a control stationary phase following the procedure reported previously. When not in use for an extended period of time, the columns were stored in the aqueous buffer containing sodium azide (0.05%).

2.4. Apparatus

The HPLC system consisted of a LC Shimadzu pump 10AT (Sarreguemines, France), a Shimadzu SIL-10AD auto injector, a Shimadzu SPD-10A UV–vis detector (detection at 208 or 220 nm, cell volume: 140 nl), a Shimadzu SCL-10A system controller with Class-VP software (Shimadzu) and an oven Igloocil (Interchim).

2.5. Chromatographic parameters

Solute samples were prepared in the mobile phase and injected (100 nl) in triplicate. The injected racemate concentration was 0.50 mM for all the solutes analyzed. The mobile phase flow rate was 15 μl/min for the L-RNA CSP and the control streptavidin column (non-modified streptavidin particles). The apparent retention factor k was determined using the following relation:

$$k = \frac{t_R - t_0}{t_0} \quad (1)$$

where t_R is the retention time of the enantiomer and t_0 the retention time of the unretained species. Although this is not the most accurate approach for estimating the retention factor, t_R was determined through the solute peak position. This simplification was justified because no thermodynamic or kinetic data were extracted from this chromatographic parameter. The column void time was determined using the sodium nitrate or mobile phase peak. The retention times and column void time were corrected for the extra-column void time. They were assessed by injections of solute onto the chromatographic system when no column was present. The apparent enantioselectivity α was calculated as follows:

$$\alpha = \frac{k_2}{k_1} \quad (2)$$

where k_2 is the retention factor for the more retained enantiomer (D-enantiomer) and k_1 the retention factor for the less retained enantiomer (L-enantiomer). The efficiency of the column was characterized by estimating the reduced plate height h:

$$h = \frac{L}{d_p N} \quad (3)$$

$$\text{with } N = 5.54\left(\frac{t_R}{W_{50}}\right)^2 \quad (4)$$

where N is the number of theoretical plates (W_{50} the peak width at half-height), L the column length and d_p is the average particle diameter. The asymmetry factor A_s was determined for the lowest injected solute concentration by calculating the ratio of the second (or right part) of the peak over the first (or early part) of the peak at 10% of the peak height.

3. Results and discussion

3.1. The oligonucleotidic sequence used in this study

The minimal sequence of the 63-mer RNA aptamer used in the present work was obtained via a SELEX procedure directed against L-tyrosine (see Fig. 1 for the secondary structure). This oligonucleotide is known to discriminate between L- and D-tyrosine [16]. The dissociation constants, determined by isocratic competitive affinity chromatography, are in the micromolar range: $K_d = \sim 25$ μM for the L-tyrosine-aptamer association and ∼250 μM for the D-tyrosine-aptamer association, corresponding to a "true" enantioselectivity of around 10. Such anti-L-tyrosine RNA aptamer was chosen because the sequence was selected from a degenerate pool derived from a previously selected dopamine aptamer [16]. As expected by the authors, the tyrosine-binding site of the aptamer could have some remembrance of the modalities of recognition of dopamine, explaining its ability to bind with relative high affinity some structurally related analogues such as L-DOPA.

3.2. The mirror-image approach to create a biostable RNA aptamer CSP

In order to use such aptamer in a routine chromatographic context, it was fundamental to develop a RNA molecule intrinsically resistant to the classical cleaving RNases. Recently, a very interesting strategy involving the mirror-image approach has been successfully developed by some researchers to design biostable peptide or nucleic acid ligands for potential therapeutic or diagnostic applications [13,14]. This concept was successfully applied to create a biostable RNA CSP. It was demonstrated that a CSP based on L-RNA, that is the mirror-image of the "natural" D-RNA aptamer, was stable for an extended period of time under usual chromatographic conditions of storage and experiments [12]. As the structure of nucleases is inherently chiral, the RNases accept only a substrate in the correct chiral configuration, i.e. the "natural" D-oligonucleotide. So, L-oligonucleotides are unsusceptible to the naturally occurring enzymes. In the present study, the L-RNA CSP was found to be stable over the time since the solute retention factors were not modified after the passage of more than 2000 column volumes of mobile phase (about 3 months of experiments). It further confirms the usefulness of such mirror-image approach for the generation of biostable RNA aptamer CSPs.

3.3. No interactions between the streptavidin chromatographic support and the various solutes tested

It was shown previously that streptavidin was able to discriminate the enantiomers of some analytes such as warfarin [17], trimipramine [17] or adenosine [18]. In order to evaluate possible enantioselective properties of the immobilized streptavidin toward solutes, a racemic mixture of the compounds enantioresolved on the RNA CSP (see above) was injected onto a microbore column packed with non-modified streptavidin POROS particles (eluent: Tris–HCl buffer 8 mM, NaCl 25 mM, $MgCl_2$ 5 mM, pH 7.4). All the enantiomers were not separated and were weakly retained by the column ($k < 0.10$). As an example, the chromatogram obtained when a racemic mixture of tyrosine was injected onto the non-modified streptavidin column is presented in Fig. 2a. This result indicates that the streptavidin chromatographic support was roughly inert toward the various compounds and did not affect the chiral recognition.

3.4. Retention, enantioselectivity and efficiency on the *anti*-D-tyrosine L-RNA CSP

In the first stage of this work, the chromatographic properties of the L-RNA aptamer CSP were analyzed using an aqueous buffer as eluent (Tris–HCl buffer 8 mM, NaCl 25 mM, $MgCl_2$ 5 mM, pH 7.4) at a column temperature of 10 °C. Racemates of tyrosine and various related compounds were injected onto the column. The chromatographic data (k_D and α) as well as the compound structure are summarized in Table 1. As expected from the data reported by Mannironi et al. [16], the L-RNA CSP was able to discriminate the enantiomers of tyrosine, the D-enantiomer being more retained than the L-enantiomer (Fig. 2). This is consistent with the principle of chiral inversion, i.e. the mirror-image of the "natural" aptamer recognizes with the same affinity and specificity the mirror image of the target [12]. More, L-tyrosine eluted roughly in the void volume, due probably to a low binding capacity of the RNA CSP [12]. In such case, it is important to note that it remains a major uncertainty in discussing quantitatively the value of the apparent separation factor due to the very low value of k_L. As can be seen in Table 1, the L-RNA aptamer CSP exhibited also discriminating properties for various other compounds (10 enantiomer pairs). Representative chromatograms are shown in Fig. 3, illustrating the separation of the enantiomers of tryptophan, *N*-acetyl-tryptophan, 1-methyl-tryptophan and 2-quinolyl-alanine. In all cases of enantioresolution, the D-enantiomers were more retained by the stationary phase than the L-enantiomers, as observed for tyrosine. The reduced plate height *h* was estimated to be between ~25 and ~120 in relation to the enantiomers analyzed. These values are consistent with the data obtained previously on a DNA or RNA aptamer CSP [10,12] and are similar to the reduced plate height values observed with imprinted or antibody-based chiral stationary phases [19,20]. In addition, the asymmetry factor A_s for the last eluting enantiomers was found to be comprised between ~1.5 and ~3.5. The high *h* value and the pronounced peak tailing can be attributed to slow homogeneous or heterogeneous (see below) mass transfer kinetics as well as some possible non-linear effects due to the limited binding capacity of the column [19,21].

From the data reported in Table 1, some important observations can be made about the structure-enantioselectivity relationships on the RNA CSP. Firstly, it was found that modifications on the side chain of tyrosine were highly critical for the binding and chiral recognition. For example, the phenylalanine racemate was not resolved (no retention), indicating that the *p*-hydroxyl group was fundamental for both the retention and enantioselectivity. Moreover, the methylation of the *p*-hydroxyl group (*O*-methyl tyrosine) or the *p*-amino substitution (*p*-amino-phenylalanine) also led to the loss of the enantioresolution. Furthermore, when the phenolic group was attached directly to the asymmetric carbon atom (at the α position, 4-hydroxy-phenylglycine), both retention and enantioselectivity were abolished. As shown previously [16], the aptamer can accommodate the *m*-hydroxyl substitution of the phenolic group (DOPA), with an affinity comparable to that obtained for tyrosine. This was associated to the resolution of the racemate. However, the introduction of two iodide atoms on the phenolic moiety (3,5-diiodo-tyrosine) was responsible for the loss of the binding and chiral recognition processes.

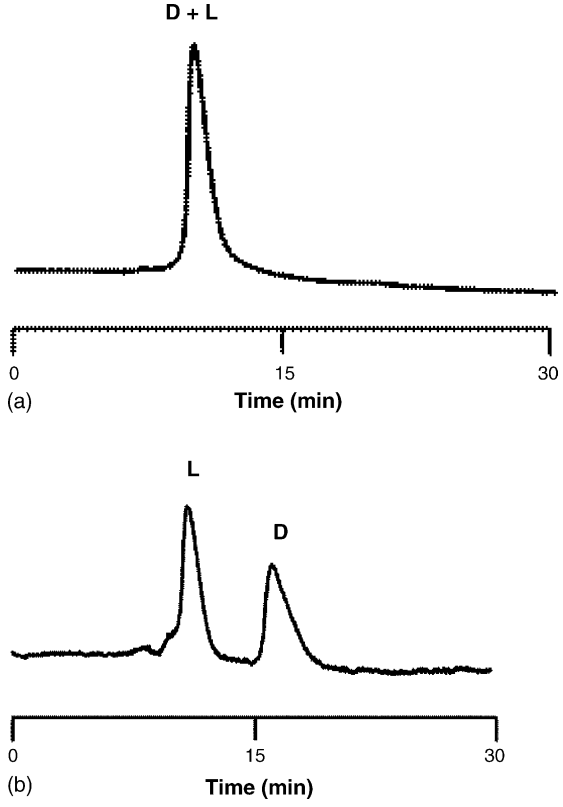


Fig. 2. Chromatograms obtained when a racemic mixture of tyrosine was injected onto the non-modified streptavidin (a) and the tyrosine-specific L-RNA aptamer (b) columns. Columns: 350 mm × 0.76 mm (i.d.); mobile phase: Tris–HCl buffer 8 mM, NaCl 25 mM, $MgCl_2$ 5 mM, pH 7.4; column temperature: 10 °C; injected concentration: 0.50 mM; injection volume: 100 nl; flow rate: 15 μl/min; detection at 220 nm.

Table 1
Structure, retention and enantioselectivity for the compounds analyzed in this study (see text for the experimental conditions)

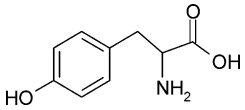
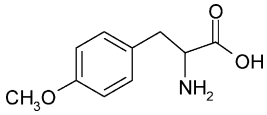
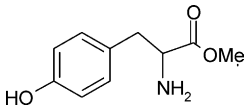
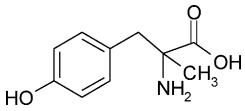
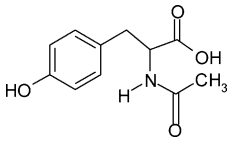
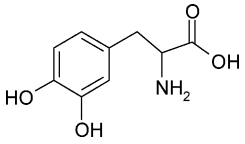
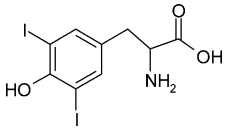
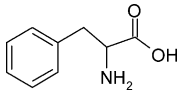
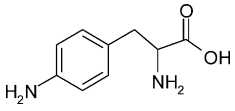
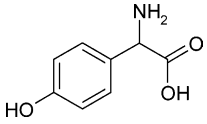
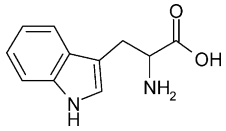
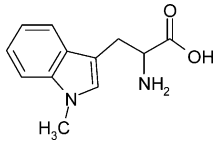
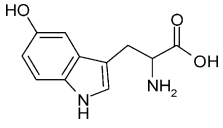
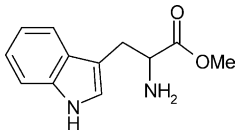
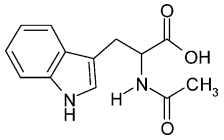
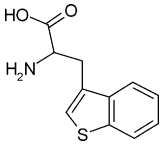
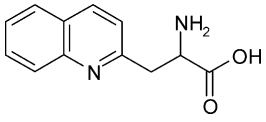
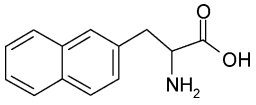
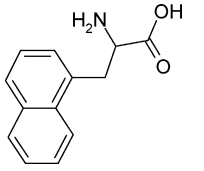
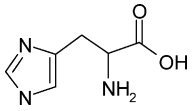
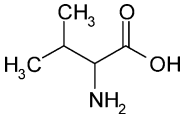
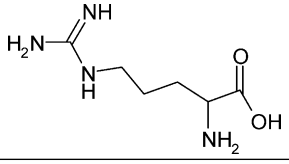
Compound	Structure	k_D[a]	α
Tyrosine		0.57	28.06[b]
O-Methyl-tyrosine		–[c]	–[d]
Tyrosine methyl ester		–	–
α-Methyl-tyrosine		0.59	21.91[b]
N-Acetyl-tyrosine		–	–
DOPA		0.83	7.20[b]
3,5-Diiodo-tyrosine		–	–
Phenylalanine		–	–
p-Amino-phenylalanine		–	–
4-Hydroxyl-phenylglycine		–	–
Tryptophan		1.42	4.09
1-Methyl-tryptophan		1.50	6.98
5-Hydroxyl-tryptophan		1.09	3.62

Table 1 (*Continued*)

Compound	Structure	k_D[a]	α
Tryptophan methyl ester		–	–
N-Acetyl-tryptophan		0.49	9.48[b]
3-Benzothienyl-alanine		2.13	1.73
2-Quinolyl-alanine		1.43	1.88
2-Naphtyl-alanine		5.29	3.06
1-Naphtyl-alanine		3.03	1.32
Histidine		–	–
Valine		–	–
Arginine		–	–

[a] Relative standard deviation of the D-enantiomer retention factor was less than 4%.
[b] Major uncertainty in the α value is expected due to the very low value of k_L.
[c] Elution roughly in the void volume.
[d] No apparent enantioselectivity.

Secondly, no binding was observed when the R moiety of tyrosine was replaced by a polar (arginine), aliphatic (valine) or heterocyclic (histidine) side chain. On the other hand, compounds possessing a bicyclic aromatic side chain were able to bind enantioselectively to the L-RNA CSP. The enantioseparation was obtained for substitutions by benzothienyl, naphtyl, quinolyl or indolyl residues (see Fig. 3a and b). In such case, the aptamer CSP was able to accommodate some substitutions on the ring. As an example, enantioresolution was observed when the tryptophan indolyl group was substituted by hydroxyl (position 5) or methyl (position 1) groups (see Fig. 3c). In all instances, the k_D values increased relatively to the retention factor of D-tyrosine or D-DOPA. The overall retention depended, at least in part, on the overall hydrophobic character of the substitutent. For the D-enantiomers, the compound substituted by the most hydrophilic group (5-hydroxyl-tryptophan, log $P = 0.97$) exhibited the lowest retention factor while the compounds having the most hydrophobic substitutents (benzothienyl-alanine, napthyl-alanine) displayed the higher k_D values. It appears that besides the hydrophobic character, the size or shape of the R substitutent participates also to the overall retention mech-

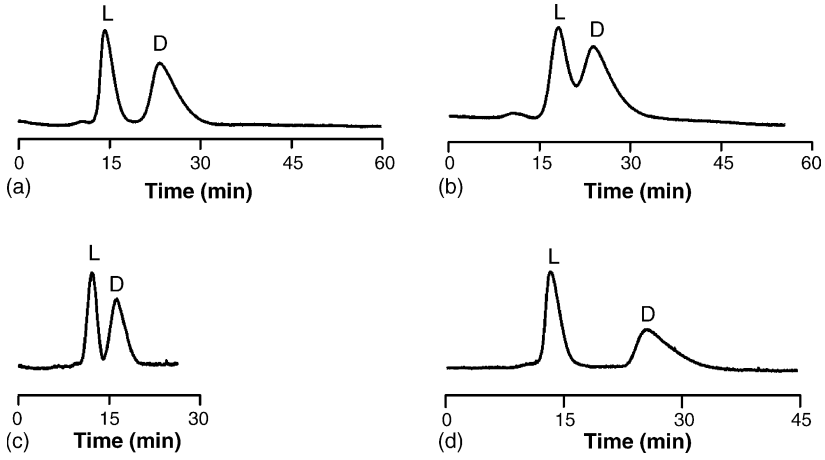


Fig. 3. Chromatographic resolution of (a) tryptophan, (b) 2-quinolyl-alanine, (c) *N*-acetyl-tryptophan and (d) 1-methyl-tryptophan using the tyrosine-specific L-RNA aptamer CSP. Column: 350 mm × 0.76 mm (i.d.); mobile phase: Tris–HCl buffer 8 mM, NaCl 25 mM, $MgCl_2$ 5 mM, pH 7.4; column temperature: 10 °C; injected concentration: 0.50 mM; injection volume: 100 nl; flow rate: 15 μl/min; detection at 220 nm.

anism. This is exemplified by the fact that the naphtyl derivatives ($\log P = 3.45$) were more bound to the stationary phase than 3-benzothienyl-alanine ($\log P = 4.38$). Furthermore, the attachment position of the alaninyl residue on the naphtalene ring affected significantly the solute retention (k_D equal to 5.29 for 2-naphtyl-alanine and 3.03 for 1-naphtyl-alanine). From a chiral discrimination point of view, tryptophan and derivatives exhibited a higher enantioselectivity (from 3.62 to 6.98) than that observed for the other bicyclic aromatic ring-substituted compounds (from 1.32 to 3.06). This can be explained by a better enantioselective fit into the RNA active site and/or by the weaker influence of possible interactions with non-specific regions of the immobilized aptamer. As the retention factor of the first eluting enantiomer was small for the indole derivatives (varying from 0.21 to 0.35), the solutes are expected to interact weakly, if any, with non-specific regions of the CSP. This was responsible for the high apparent enantioselectivity. In addition, the enantiomers of 2-naphtyl-alanine were baseline separated ($\alpha = 3.06$) while the 1-naphtyl-alanine racemate was incompletely resolved ($\alpha = 1.32$), demonstrating that a suitable R chain orientation is required for enantioselective binding to the active site.

Finally, the role of the other substitutents bonded to asymmetric carbon atom was also evaluated. The carboxylic group was found to play a preponderant role in the binding and chiral recognition process as the methyl esters of tyrosine and tryptophan were not resolved on the L-RNA CSP (elution in the void volume). This is in accordance with the previous results of Mannironi et al. [16] who showed that the RNA aptamer did not bind tyramine (tyrosine without the carboxylic group) with high affinity. The amino group of tyrosine was also involved in the enantioselective interaction since the *N*-acetylation of tyrosine inhibited completely the binding and chiral discrimination. However, although the retention factor of *N*-acetyl-D-tryptophan was reduced relatively to that of D-tryptophan (0.49 versus 1.42), the racemate was still partially resolved (see Fig. 3d). On the other hand, the hydrogen atom on the chiral center did not seem to be involved in the enantioselective interaction since the k_D values were similar for α-methyl-tyrosine and tyrosine (0.57 versus 0.59).

Classically, a "three-point" interaction is requires to obtain a stereospecific recognition as initially reported by Dalgliesh [22]. On the basis of the data presented above, it is believed that carboxylic and amino groups as well as the aromatic side chain of solutes interact with the active site of the RNA aptamer. Modification on the polar groups (–COOH and $–NH_2$) or change of the insertion position of the aromatic moiety on the asymmetric carbon atom ($\beta \rightarrow \alpha$) seems to have strong detrimental effects. However, some side chains of different polarity, size and shape can be accommodated. Apparently, the size of the aromatic residue is an important criterion for a tight binding to the stereoselective site. Besides some phenolic derivatives, chiral recognition would be possible for molecules having a substitutent of approximately the size of naphtalene or a little smaller. In addition, it appears that a suitable orientation/presentation of such aromatic moiety to the active site is required to achieve a great chiral recognition. As previously reported for other ligand-aptamer complexes [23–25], both NMR-molecular dynamics studies and X-ray crystallographic analyses would provide important information about the three-dimensional structure of the specific binding site as well as the detailed molecular basis of the binding and chiral discrimination mechanisms.

3.5. Effects of chromatographic condition changes on the solute retention and enantioselectivity

The influence of chromatographic parameter changes on the retention and enantioselectivity of some solutes (1-methyl-tryptophan and 2-naphtyl-alanine) was studied. Firstly, the mobile phase composition was modified by addition of acetonitrile in the eluent in order to (i) evaluate the possibility to use a hydro-organic mobile phase with an aptamer CSP and (ii) modulate the interaction between the analyte and

the CSP in order to optimize the enantiomeric separation. The k and α values of 1-methyl-tryptophan and 2-naphtyl-alanine were plotted against the proportion of acetonitrile in the mobile phase for a column temperature of 10 °C (Fig. 4). By increasing the acetonitrile content in the eluent from 0 to 10%, the apparent retention factors were reduced, especially for the more retained solute (2-naphtyl-alanine). For 1-methyl-tryptophan, the overall retention factor variation reflected mainly the modification of the enantioselective binding strength since the non-specific contribution was expected to be weak or nil (see above). It explains why the apparent enantioselectivity decreased significantly ranging from 6.98 to 4.67 between 0 and 10% of acetonitrile. Such behavior suggests that the enantioselective binding is under the dependence of hydrophobic effects. Addition of acetonitrile could also modify the tertiary structure of the RNA aptamer which would be responsible for a change of the active site conformation. A different behavior was obtained for 2-naphtyl-alanine since the α value did not change over the acetonitrile range (from 3.06 at 0% ACN to 3.03 at 10% ACN, see Fig. 4b). Such data support a solute binding involving both enantioselective and non-enantioselective interactions (heterogeneous binding) with the immobilized RNA aptamer [21]. If the non-specific part of retention comes from hydrophobic effects, then a strong decrease of this contribution would be expected to occur when acetonitrile was added in the mobile phase. Such reduction of the non-specific part could counterbalance the concomitant reduction of the stereoselective part so that the apparent enantioselectivity remained unchanged. Although the exact reason for the retention behavior difference between 1-methyl-tryptophan and 2-naphtyl-alanine is not known, it may reflect the different capability of naphthalene and indole moieties to engage stacking interactions with the nucleobases of some non-specific regions of the immobilized RNA. This is in accordance with the previous work of Sartorius and Schneider [26] showing that the naphthalene residue interacted more strongly with the nucleobases of calf thymus DNA than the indole residue did [26]. This was by a factor comprised between 4 and 10. From a stability point of view, the use of a hydro-organic mobile phase did not affect the RNA CSP performances since identical solute retention factors were obtained before and after the addition of the organic modifier for several use cycles. Such observation is of particular interest for the analysis of compounds characterized by long retention times.

Secondly, the effects of the mobile phase ionic strength on the retention and enantioselectivity were assessed, at a column temperature of 10 °C. The NaCl concentration in the aqueous eluent varied from 25 to 200 mM. An increase in the mobile phase ionic strength resulted in a decrease in the solute retention, except for the 1-methyl-L-tryptophan (Fig. 5).

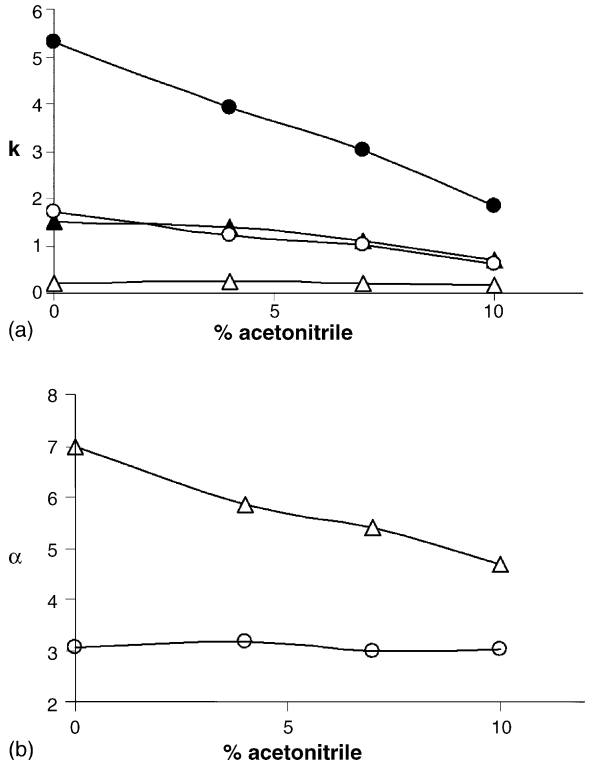


Fig. 4. Plots of the k (a) and α (b) values vs. the proportion of acetonitrile in the mobile phase (Tris–HCl buffer 8 mM, NaCl 25 mM, $MgCl_2$ 5 mM, pH 7.4). 1-Methyl-tryptophan: triangle; 2-naphtyl-alanine: circle. D-Enantiomer: filled symbol; L-enantiomer: open symbol. Column: 350 mm × 0.76 mm (i.d.); column temperature: 10 °C; injected concentration: 0.50 mM; injection volume: 100 nl; flow rate: 15 μl/min; detection at 220 nm.

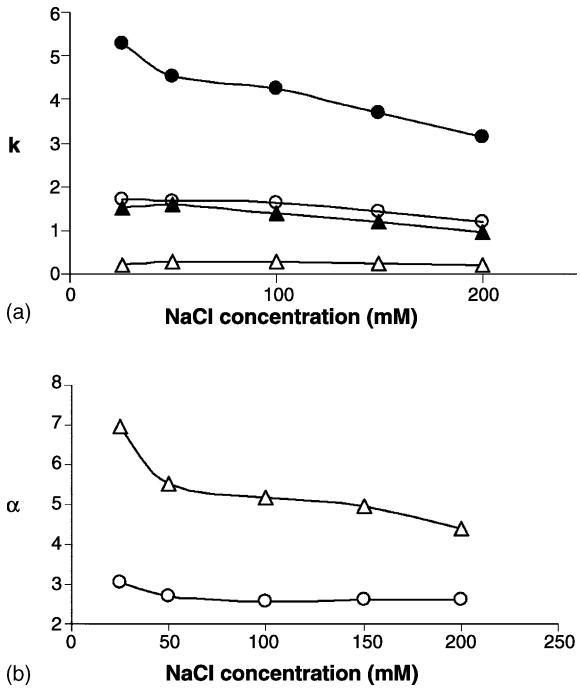


Fig. 5. Plots of the k (a) and α (b) values vs. the NaCl concentration in the mobile phase (Tris–HCl buffer 8 mM, $MgCl_2$ 5 mM, pH 7.4). 1-Methyl-tryptophan: triangle; 2-naphtyl-alanine: circle. D-Enantiomer: filled symbol; L-enantiomer: open symbol. Column: 350 mm × 0.76 mm (i.d.); injected concentration: 0.50 mM; injection volume: 100 nl; flow rate: 15 μl/min; detection at 220 nm.

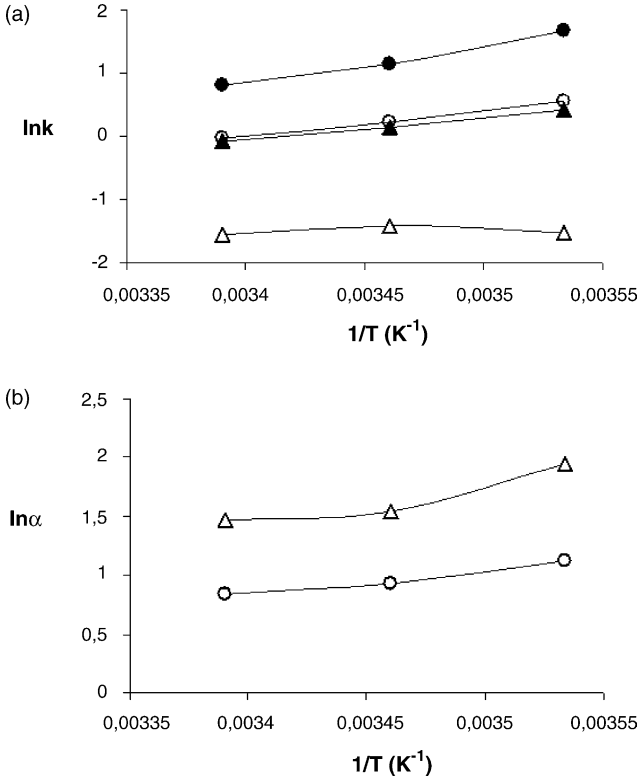


Fig. 6. Plots of $\ln k$ (a) and $\ln \alpha$ (b) vs. $1/T$. 1-Methyl-tryptophan: triangle; 2-naphtyl-alanine: circle. D-Enantiomer: filled symbol; L-enantiomer: open symbol. Column: 350 mm × 0.76 mm (i.d.); mobile phase: Tris–HCl buffer 8 mM, NaCl 25 mM, $MgCl_2$ 5 mM, pH 7.4; injected concentration: 0.50 mM; injection volume: 100 nl; flow rate: 15 μl/min; detection at 220 nm.

Between 25 and 200 mM of NaCl eluent concentration, a reduction of the apparent enantioselectivity was also observed, from 6.98 to 4.38 for 1-methyl-L-tryptophan and from 3.06 to 2.61 for 2-naphtyl-alanine. Such behavior seems to indicate that electrostatic interactions between the solutes and the aptamer CSP were involved in the binding mechanism. As evoked above for the acetonitrile effects, some changes in the tertiary structure of the RNA dependent on the eluent ionic strength could also explain these variations.

Finally, additional experiments were conducted at higher column temperatures (16 and 22 °C) using the aqueous buffer as mobile phase. The comparison of the retention factors and enantioselectivity for the two solutes revealed that an increase in the temperature was responsible for a decrease in the binding (except for the 1-methyl-L-tryptophan) and chiral discrimination processes (Fig. 6). Between 10 and 22 °C, the apparent enantioselectivity decreased from 6.98 to 4.37 for 1-methyl-tryptophan and from 3.06 to 2.30 for 2-naphtyl-alanine. This behavior confirms the results reported previously, i.e. both retention for the more retained enantiomer and enantioselectivity are dependent on enthalpically driven interactions [11].

4. Conclusion

This paper describes for the first time the usefulness of an aptamer CSP for the resolution of various racemates (target and analogues). From the structure-enantioselectivity relationship, it is expected that amino acid-related compounds with large aromatic side chain in β position of the asymmetric carbon atom would be able to be resolved on this CSP. The change of the mobile phase composition as well as the column temperature allows modulating the solute retention and enantioselectivity. Furthermore, it is shown that such stationary phase can be used with a hydro-organic mobile phase during an extended period of time without alteration of its stability.

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