Anal Bioanal Chem (2013) 405:8121–8129
DOI 10.1007/s00216-013-7207-4

RESEARCH PAPER

Dress-up chiral columns for the enantioseparation of amino acids based on fluorous separation

Kenichiro Todoroki · Yasuhiro Ishii · Koji Toyoda · Takashi Ikawa · Jun Zhe Min · Koichi Inoue · Shuji Akai · Toshimasa Toyo'oka

Received: 26 February 2013 / Revised: 28 June 2013 / Accepted: 2 July 2013 / Published online: 25 July 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract In this paper, we report a new type of chiral high-performance liquid chromatography (HPLC) column—a so-called dress-up chiral column—featuring a chiral stationary phase adsorbed reversibly in a commercial fluorous HPLC column through fluorous interactions. We synthesized perfluroalkylated proline derivatives as chiral stationary phase compounds and then adsorbed them reversibly in the fluorous HPLC column through the pumping of their solutions. By using this dress-up chiral column and fluorophobic elution of an aqueous copper(II) sulfate/MeOH mixture, we could enantioseparate seven racemic amino acids within 40 min. When we washed the dress-up chiral column with fluorophilic tetrahydrofuran or MeOH, the adsorbed chiral stationary phase compounds desorbed from the column, completely destroying its enantioseparation ability. The relative standard deviation of the retention times, the number of theoretical plates, and the resolution for each of four preparations of the dress-up columns were all less than or equal to 9.53 % in 20-times repeated analysis, and were all less than or equal to 18.7 % in four different preparations, respectively.

Keywords Amino acids · Peptides · HPLC · Chiral analysis

Published in the topical collection *Amino Acid Analysis* with guest editor Toshimasa Toyo'oka.

K. Todoroki · Y. Ishii · K. Toyoda · J. Z. Min · K. Inoue · T. Toyo'oka (✉)
Department of Analytical and Bio-Analytical Chemistry, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka, Shizuoka 422-8526, Japan
e-mail: toyooka@u-shizuoka-ken.ac.jp

T. Ikawa · S. Akai
Department of Synthetic Organic Chemistry, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka, Shizuoka 422-8526, Japan

Introduction

Amino acids, peptides, biogenic and food ingredients, and many of pharmaceutical drugs are chiral compounds with optically active enantiomers that generally exhibit different physiological functions in vivo. Accordingly, the analysis of chiral compounds is essential in many fields, including drug development, biochemical research, food analysis, and clinical diagnosis. At present, analyses of chiral compounds are performed most commonly using high-performance liquid chromatography (HPLC) and (1) chiral derivatization to form diastereoisomers, (2) the chiral stationary phase (CSP) of a chiral separation column, or (3) a chiral mobile phase to form diastereoisomeric complexes during the elution process. Among these approaches, the CSP method is most common because of its ease of operation and the robustness of the analytical system. Many chiral columns featuring different types of CSPs are widely available, including Pirkle-type CSPs [1], ligand exchange-type CSPs [2, 3], polysaccharide derivatives as CSPs [4, 5], and cinchona alkaloid derivatives as CSPs [6, 7]. Nevertheless, the separation behavior of analytes when using CSP methods can be quite complicated because small differences in energy for the interactions (e.g., hydrogen bonding, electrostatic, and π–π interactions) leading to complex formation between the CSP and the analytes are reflected in their separation. Accordingly, the ability to choose an appropriate commercial chiral column for a particular analyte requires extensive knowledge and experience. In addition, most chiral columns are more expensive than conventional reversed-phase (RP) columns, burdening users in terms of the time and cost required to purchase another column for every individual analysis.

In this present study, we developed a new type of chiral column, a so-called dress-up chiral column, that functions using fluorous separation techniques. The term "fluorous" refers to a system highly fluorinated or rich in fluorine atoms; short perfluoroalkyl chains (C_4–C_{10}) are often known as

fluorous tags. Compounds bound to fluorous tags can be trapped selectively by a fluorous silica gel HPLC column or a solid-phase extraction (F-SPE) cartridge [8, 9]. In most cases, fluorous methodologies have been employed in the fields of organic chemistry, combinatorial chemistry [10], and green sustainable chemistry [11], but we have also applied them successfully to biomedical analyses [12–19]; for instance, we have reported fluorous derivatization methods combining HPLC with fluorescence detection [13–15] or liquid chromatography (LC) with tandem mass spectrometry [16, 17]. We also have developed a novel pre-column fluorescence derivatization reagent, F-trap pyrene, for use in the HPLC analyses of biological amines [18]. Fluorous scavenging derivatization methods for reagent peak-free HPLC/fluorescence analyses of carboxylic acids have also been developed [19, 20].

Figure 1 illustrates the concept of the dress-up chiral column, which features a fluorous-tagged chiral stationary phase (F-CSP) adsorbed reversibly through fluorous interactions onto a commercial perfluoroalkyl modified silica gel HPLC column. Accordingly, various F-CSPs can be adsorbed onto a single column, much like a person changing clothes. Moreover, it is also possible to adsorb a mixture of various types of CSPs in a single column, thereby facilitating the separation of multiple chiral analytes. So far, many separation columns which are coated with various stationary phase compounds by hydrophobic interactions have been reported. For instance, zwitter-ionic surfactants coated columns have also been generally used for the separation of inorganic salts [21–23], theophylline and caffeine [24], and nucleosides [25]. However, to the best of our knowledge, this present report is the first to describe single chiral column with readily replaceable CSPs based on fluorous separation technique.

In this study, the F-CSPs adsorbed in the fluorous HPLC column were not eluted when employing fluorophobic eluents, but only when washed with fluorophilic eluents, namely MeOH, MeCN, and tetrahydrofuran (THF). Furthermore, the eluted F-CSPs could be reused after selective collection using an F-SPE.

Among the many available enantioseparation modes, for this study we adopted the method of ligand exchange chromatography (LEC) combining dynamic coating, as reported by Davankov et al. [2, 3]. In LEC, ternary complexes formed from a stationary chiral ligand (e.g., *N*-alkylated amino acid derivative), a central copper(II) ion, and the analytes can express high enantioselectivity. The combination of HPLC [26–30], capillary HPLC [31], and the dynamic coating method has been widely used for the chiral separation of amino acids, hydroxy acids, and pharmaceuticals. In dynamically coated chiral columns, CSP compounds are adsorbed on the hydrophilic stationary phases not through covalent bonding but through noncovalent coating. Therefore, leakage and

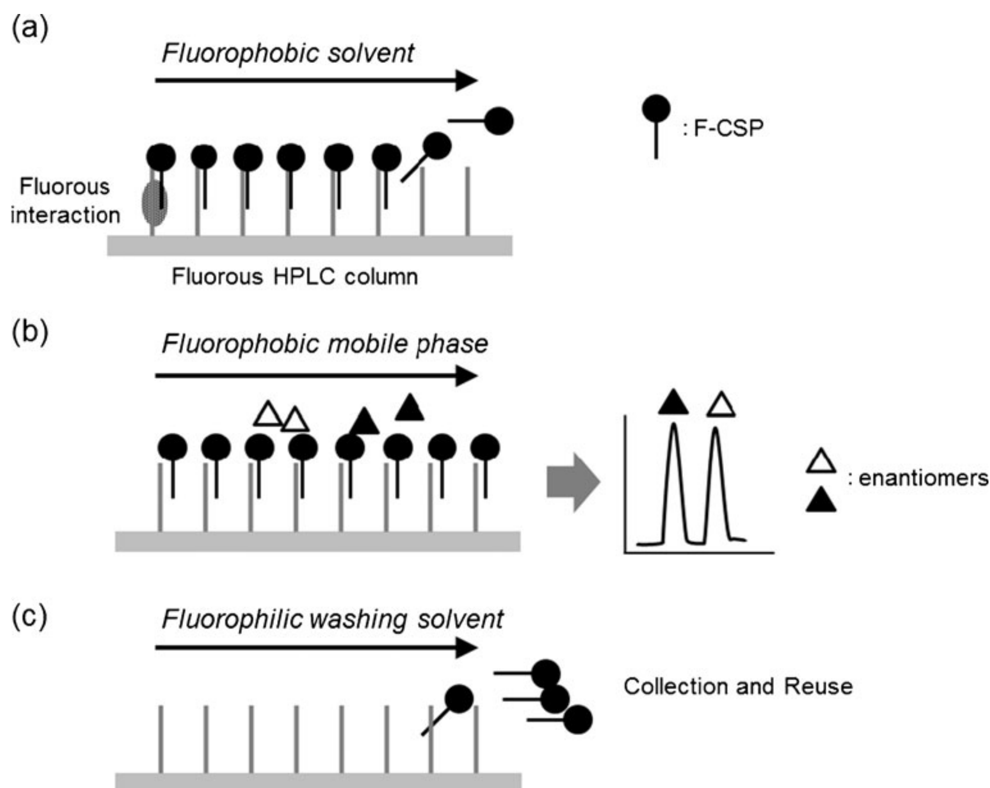

Fig. 1 Operating principle of a dress-up chiral column. **a** Dressing of the F-CSPs under fluorophobic elution conditions, **b** chiral separation of analytes under fluorophobic elution conditions, and **c** undressing of the F-CSPs under fluorophilic washing conditions

removal of immobilized ligands from such columns are affected by hydrophobicity of them. In contrast, in our proposed dress-up chiral columns, in which we employed perfluoroalkylated amino acid derivatives as stationary chiral ligands, we obtained selective adsorption of the ligands on the stationary phases without requiring them to be hydrophilic; in addition, complete removal of the ligands from the column became easier.

In this study, we employed new perfluoroalkylated D- and L-proline derivatives—Rf-D-Pro and Rf-L-Pro—as our F-CSPs. When we coated the dress-up chiral column with these F-CSPs, we could perform the enantioseparation of optically active amino acids. In addition, we also evaluated this system's ability to perform chiral separation as well as its durability and robustness.

Experimental section

Reagents, solutions, and apparatus Deionized and distilled water, purified using an Aquerius pwu-200 automatic water distillation apparatus (Advantec, Tokyo, Japan), was used to prepare all aqueous solutions. ^{1}H NMR (500 MHz) spectra were recorded using a JEOL (Tokyo, Japan) JNM-ECA-500 spectrometer and CD_3OD as the solvent. High-resolution mass spectrometry (HRMS) was performed using a Bruker Daltonics (Bruker, Germany) micrOTOF (ESI) mass spectrometer. *N-tert*-Butoxycarbonyl-L-proline, *N-tert*-butoxycarbonyl-D-proline, (*R*)-(−)-DBD-Py-NCS, and 1 M HCl in ethyl ether were purchased from Tokyo Chemical Industry (Tokyo, Japan). 4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-Heptadecafluoroundecylamine was purchased from Sigma–Aldrich (St. Louis, MO, USA). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and the D and L forms of 19 optically active natural amino acids were purchased from Wako Pure Chemical (Osaka, Japan). 4-Dimethylaminopyridine (DMAP) was purchased from Dojindo (Kumamoto, Japan). HPLC-grade MeCN and MeOH, and anhydrous CH_2Cl_2 and acetone were purchased from Kanto Chemicals (Tokyo, Japan). Copper(II) sulfate pentahydrate was purchased from Nakalai tesque (Kyoto, Japan). A stock solution of each D- and L-amino acid (1 mM) was prepared in water. All stock solutions were stored at 4 °C and then diluted further with water to the desired concentration prior to use. All other chemicals were of the highest purity available and were used as received.

HPLC system and conditions The HPLC system consisted of an SCL-10A VP system controller (Shimadzu, Kyoto, Japan), a SIL-20 AC VP auto sampler (Shimadzu), a CTO-20 AC column oven (Shimadzu), an SPD-M20A diode array detector (Shimadzu), and a 12-μL flow cell. The data were collected using LC solution (v. 1.21, Shimadzu); for quantification, peak areas and heights were estimated using the baseline-to-baseline method. The fluorous HPLC column was a Fluofix-II 120E column (5 μm, 150×2 mm I.D., Wako Pure Chemical). An L-column 2 ODS (5 μm, 150×2.1 mm I.D. CERI, Tokyo, Japan) was used to compare the elution profiles of the F-CSPs on fluorous and RP columns. The flow rate of the mobile phase and the column temperature were set at 0.2 mL/min and 40 °C, respectively; the sample injection volume and detection wavelength were 1 μL and 230 nm, respectively.

Synthesis of F-CSPs EDC (115 mg, 0.6 mmol), DMAP (73 mg, 0.6 mmol), and 4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecylamine (149 μL, 0.5 mmol) were added to a solution of *N-tert*-Butoxycarbonyl-D-proline or *N-tert*-butoxycarbonyl-L-proline (108 mg, 0.5 mmol) in CH_2Cl_2 (20 mL), and then the mixture was left at room temperature overnight. After washing with 1 M HCl, 1 M NaOH, and brine (each 3×20 mL), the organic phase was dried ($MgSO_4$) and concentrated. The residue was purified through column chromatography [SiO_2; CH_2Cl_2/acetone, 25:1 (*v*/*v*)] to afford a white powder, which was dissolved in CH_2Cl_2 (3 mL) and treated with trifluoroacetic acid (500 μL). After stirring ambient for 6 h, the solution was evaporated to dryness and the residue added to a solution of 1 M HCl in ethyl ether (1.5 mL). Evaporation of the solvent afforded the hydrochloride salt of (*R*)-2-[(4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecyl)carbamoyl]pyrrolidine-1-carboxylic acid (Rf-D-Pro, 153 mg, 0.25 mmol, 50 %) or (*S*)-2-[(4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecyl)carbamoyl]pyrrolidine-1-carboxylic acid (Rf-L-Pro, 177 mg, 0.29 mmol, 58 %) as a white powder. Each F-CSP was dissolved in MeOH to form a 50-mM solution and then further diluted to 3 mM with MeOH/water (1:2, *v*/*v*).

^{1}H NMR (500 MHz, CD_3OD): δ 1.84 (t, *J*=8.0 Hz, 2H), 2.02 (m, 2H), 2.06 (m, 2H), 2.23 (t, *J*=5.2 Hz, 2H), 2.42 (t, *J*=4.3 Hz, 2H), 2.80 (br, 2H), 4.21 (t, *J*=7.7 Hz, 1H) for Rf-D-Pro; 1.84 (t, *J*=8.0 Hz, 2H), 2.00 (m, 2H), 2.06 (m, 2H), 2.23 (t, *J*=4.6 Hz, 2H), 2.42 (t, *J*=4.3 Hz, H,), 2.82 (br, 2H), 4.21 (t, *J*=7.7 Hz, 1H) for Rf-L-Pro. HRMS (ESI) calcd for $C_{16}H_{15}F_{17}N_2O$ $[M]^+$: *m*/*z* 575.0991. Found: *m*/*z* 575.0990 (Rf-D-Pro) and 575.0990 (Rf-L-Pro). Optical purities of the F-CSPs were calculated from peak areas obtained by fluorescence chiral derivatization method to form fluorescent diastereomer with (*R*)-(−)-DBD-Py-NCS using RP-HPLC [32]. An RP column and mobile phase used were an L-column 2 ODS (5 μm, 150×2.1 mm I.D. CERI) and 60 % aqueous acetonitrile. The flow rate of the mobile phase and the column temperature were set at 0.2 mL/min and 40 °C; excitation and emission wavelengths were 460 and 550 nm; the sample injection volume and detection wavelength were 20 μL and 230 nm, respectively.

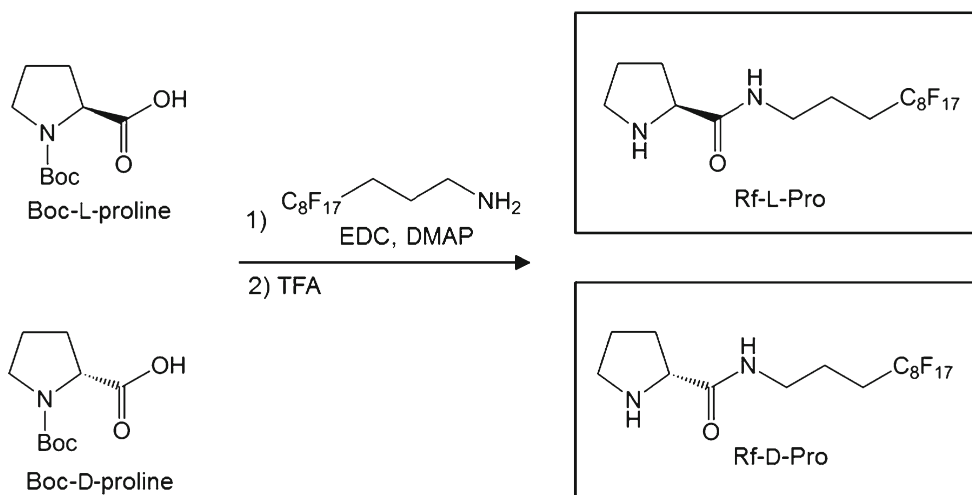

Fig. 2 Synthesis of Rf-L-Pro and Rf-D-Pro

Coating the F-CSPs on the fluorous stationary phase A 3-mM solution of each F-CSP (20 mL) was delivered into the fluorous HPLC column at a flow rate of 0.2 mL/min. The UV absorbance at 230 nm was recorded continuously until the baseline of the chromatograms increased (ca. 60 min).

Enantioseparation of amino acids Enantioseparation of amino acids was performed using 0.1 mM aqueous copper(II) sulfate solution/MeOH (98:2, *v*/*v*) as the mobile phase (flow rate, 0.2 mL/min) and a column temperature of 40 °C. The sample injection volume and detection wavelength were 1 μL and 230 nm, respectively.

Uncoating the F-CSPs from the dress-up chiral column and collection of F-CSP for reuse Uncoating of the F-CSPs from the dress-up chiral column was performed by the passage of MeOH containing 0.1 % formic acid as a removal solvent for 60 min after washing with MeOH/water (98:2, *v*/*v*) for 120 min. The eluted MeOH solution was collected and evaporated to dryness under reduced pressure. The residue was re-dissolved in MeOH/water (1:2, *v*/*v*) to a concentration of 3 mM for reuse as an F-CSP.

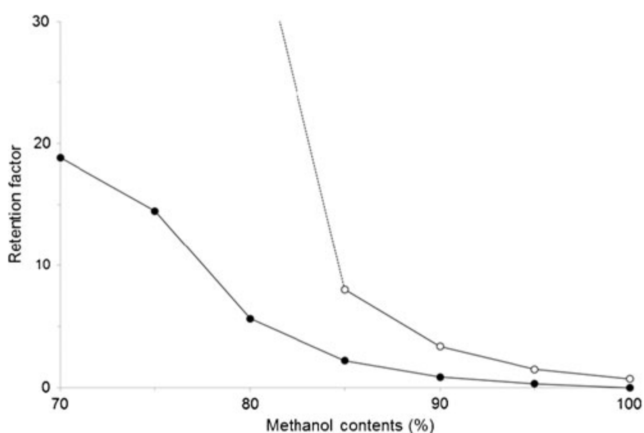

Fig. 3 Elution profiles of Rf-L-Pro separated through a fluorous HPLC column (*open circle*) and an ODS column (*closed circle*). HPLC conditions are described in the "Experimental section"

Results and discussion

Synthesis of F-CSPs

Figure 2 outlines the synthesis of Rf-L-Pro and the Rf-D-Pro. These F-CSPs were synthesized from corresponding

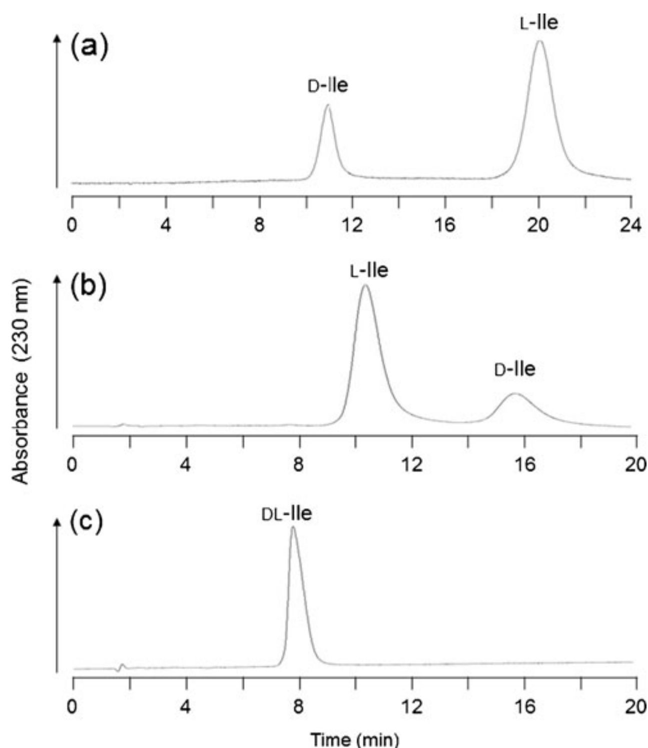

Fig. 4 Chromatograms of a mixture of 0.5 mM D-Ile and 1.5 mM L-Ile separated on chiral columns coated with **a** Rf-L-Pro and **b** Rf-D-Pro and **c** after removal **a** through washing with MeOH containing 0.1 % formic acid. HPLC conditions are described in the "Experimental section"

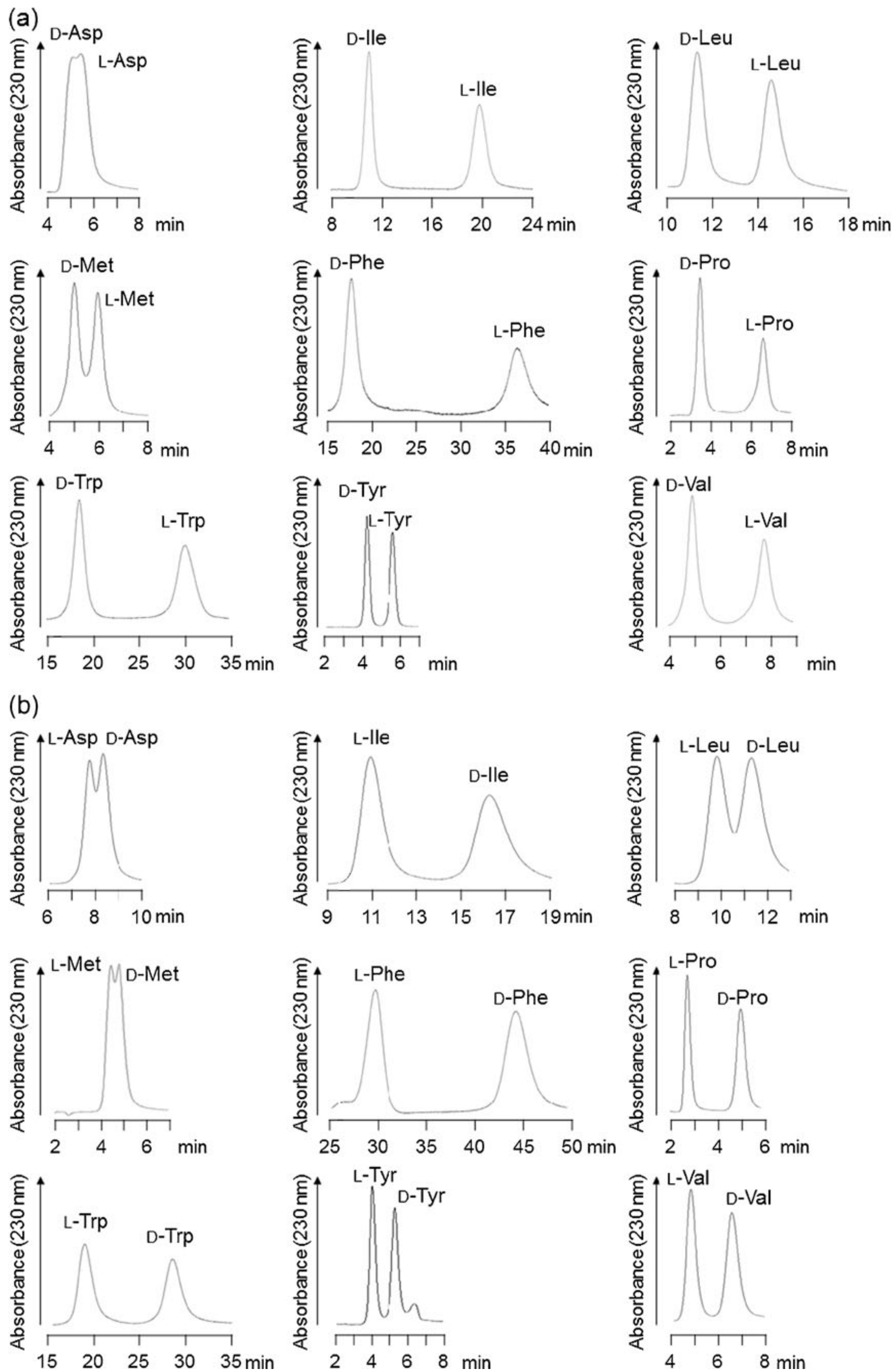

Fig. 5 Chromatograms of nine racemic amino acids (2 mM) separated through dress-up chiral columns coated with **a** Rf-L-Pro and **b** Rf-D-Pro

Table 1 Retention times (t_R), separation factors (α), and resolutions (R_s) of 19 kinds of racemic amino acids (2 mM) separated through dress-up chiral columns coated with Rf-L-Pro and Rf-D-Pro

Compound	Rf-L-Pro				Rf-D-Pro			
	t_{R1} (min)	t_{R2} (min)	α	R_s	t_{R1} (min)	t_{R2} (min)	α	R_s
Ala	2.43		–	–	1.99		–	–
Arg	1.80		–	–	1.61		–	–
Asn	2.13		–	–	1.82		–	–
Asp	5.17	5.52	1.22	0.05	7.73	8.33	1.11	0.48
Cys	3.56	4.37	2.32	0.77	3.22	4.25	2.26	0.28
Gln	2.26		–	–	1.88		–	–
Glu	4.93	5.17	1.17	0.03	7.19	7.76	1.11	0.47
His	3.18		–	–	2.54		–	–
Ile	10.95	19.80	2.08	5.69	10.93	16.24	1.59	2.52
Leu	11.71	15.83	1.51	2.51	9.82	11.36	1.21	1.11
Lys	1.76		–	–	1.58		–	–
Met	5.01	5.95	1.44	1.17	4.44	4.82	0.26	1.15
Phe	17.43	36.43	2.29	5.85	25.48	43.42	4.03	1.76
Pro	3.48	6.65	6.37	4.46	2.71	5.08	4.15	3.76
Ser	2.15		–	–	1.83		–	–
Thr	2.32		–	–	1.93		–	–
Trp	18.51	30.26	1.73	4.03	18.88	30.26	1.67	2.78
Tyr	4.18	5.63	1.81	2.70	4.04	5.20	1.56	2.13
Val	4.98	7.85	2.40	3.42	4.76	6.61	1.79	2.05

Mobile phase, 0.1 mM copper(II) sulfate in water/MeOH (98:2)

Boc-protected amino acids and perfluorooctylundecylamine in the presence of EDC as the condensation agent and DMAP as the catalyst. Fluorescent diastereomer-forming derivatization-HPLC analysis revealed that the optical purities of Rf-L-Pro and Rf-D-Pro were greater than 99.1 %. These compounds were stable for at least 3 months at 4 °C under a N_2 atmosphere.

Fluorophilicity of synthesized F-CSPs

To confirm the fluorophilicity of the synthesized F-CSPs, we compared their elution profiles from a fluorous HPLC column and an RP ODS column. Figure 3 reveals the effect of the MeOH content in the mobile phase on the retention factors of the Rf-L-Pro species. When using the RP ODS column, the retention times were delayed gradually according to the hydrophobicity of the mobile phase. When using a fluorous HPLC column, the retention factor of Rf-L-Pro increased dramatically when the MeOH content in the mobile phase was less than 85 %; Rf-L-Pro did not elute within 90 min when the aqueous MeOH content was less than 80 %. From these results, we deduced that the elution of the F-CPSs from the fluorous HPLC column could be controlled not by their hydrophobicity but by their fluorophilicity, and that the F-CSPs would hardly be eluted under fluorophobic elution conditions.

Dressing and undressing the F-CSPs on the fluorous HPLC columns

We confirmed the success of the dressing and undressing processes by checking the baseline level in the chromatograms

Table 2 Retention times, numbers of theoretical plates, and resolutions of D,L-Tyr and D,L-Trp, and their reproducibilities, when separated through a dress-up chiral column coated with Rf-L-Pro in 20 repeated analyses

Compound	Retention time (min)	RSD (%) (n=20)	N	RSD (%) (n=20)	R_s	RSD (%) (n=20)
D-Tyr	4.22±0.05	1.22	1,261.3±102.6	8.14	2.72±0.08	2.83
L-Tyr	5.87±0.34	5.76	1,810.1±48.9	2.70		
D-Trp	18.30±0.32	1.74	983.4±85.7	8.71	4.14±0.16	3.91
L-Trp	30.66±0.65	2.11	1,216.5±116.0	9.53		

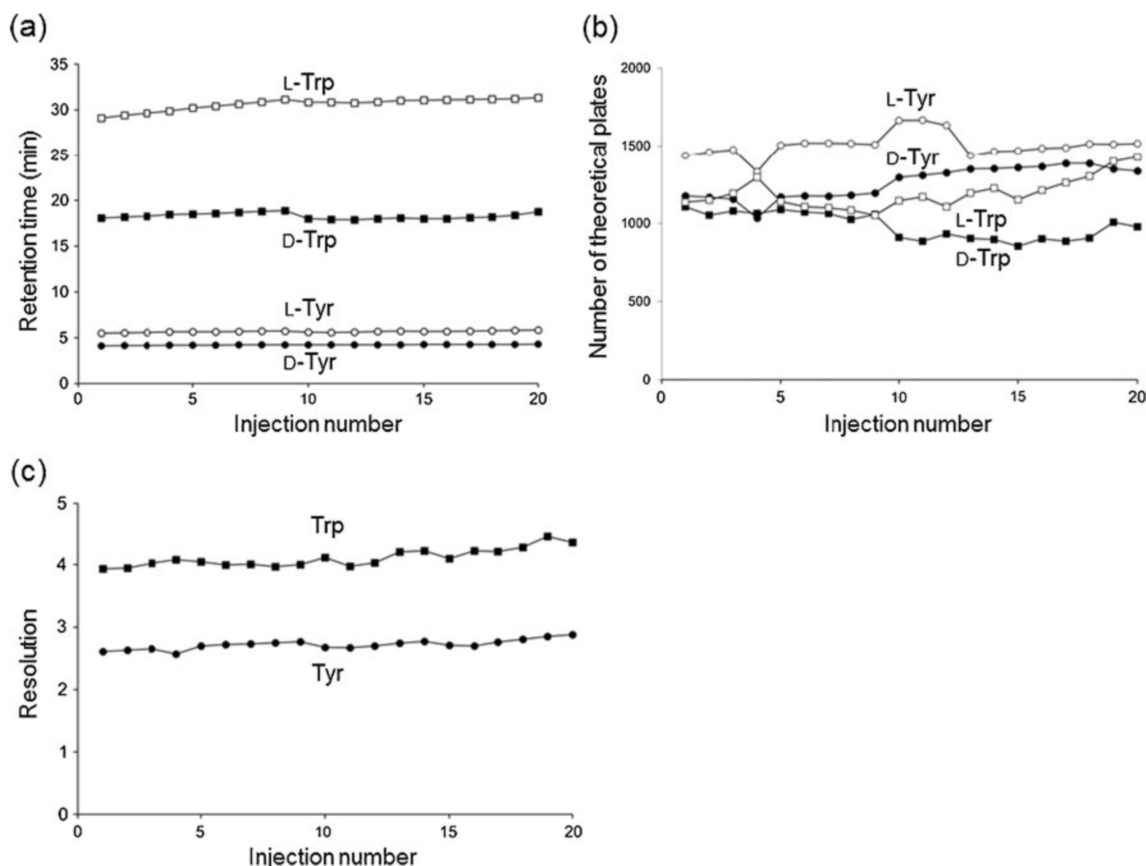

Fig. 6 Injection number-dependent change of **a** retention times, **b** numbers of theoretical plates, and **c** resolutions of D,L-Tyr and D,L-Trp, when separated through a dress-up chiral column coated with Rf-L-Pro in 20 repeated analyses

at a UV absorbance of 230 nm. We performed the dressing of the F-CSPs on the fluorous stationary phase through pumping of an F-CSP solution into the column; 90 min after the onset of pumping, the baseline levels in the chromatograms increased greatly as a result of saturation of the F-CSPs from the column (data not shown). Thus, we set 90 min as the column dressing time. We performed undressing of the coated F-CSPs from the column by passing MeOH containing 0.1 % formic acid, a fluorophilic solvent, into the column after washing with MeOH/water (98:2, *v*/*v*) for 120 min each. Baseline monitoring of the chromatograms revealed that undressing of the F-CSPs was complete within 60 min (data not shown).

Enantioseparation of amino acids through the dress-up chiral columns

Figure 4 displays chromatograms a mixture of 0.5 mM D-Ile and 1.5 mML-Ile separated on chiral columns coated with Rf-L-Pro and Rf-D-Pro and after removal of Rf-L-Pro through washing with the fluorophilic solvent THF. After coating with the F-CSPs, ligand exchange occurred among the analytes, the F-CSP, and the Cu(II) ions in the mobile phase; accordingly, racemic Ile was successfully enantioseparated with high resolution (2.98). When using the Rf-L-Pro-coated chiral column, D-Ile was initially eluted, followed by L-Ile

Table 3 Retention times, numbers of theoretical plates, and resolutions of D,L-Tyr and D,L-Trp, and their reproducibilities, when separated through a dress-up chiral column coated with Rf-L-Pro after four repeated preparations

Compound	Retention time (min)	RSD (%) (n=5)	N	RSD (%) (n=5)	R_s	RSD (%) (n=5)
D-Tyr	4.65±0.52	11.1	1,072.8±200.7	18.7	2.60±0.17	6.61
L-Tyr	6.28±0.80	12.7	1,342.3±244.4	18.2		
D-Trp	20.75±3.08	14.8	933.3±158.0	16.9	3.86±0.51	13.3
L-Trp	32.75±3.14	9.60	1,255.0±180.8	14.4		

(Fig. 4a). In contrast, when using the Rf-D-Pro-coated chiral column, the elution order of the analytes was inverted (Fig. 4-b). Furthermore, after washing the dress-up chiral column with the fluorophilic solvent THF, the adsorbed Rf-L-Pro desorbed from the column, thereby completely destroying its enantioseparation ability (Fig. 4c).

Figure 5 presents chromatograms of nine racemic amino acids (2 mM each) separated through the Rf-L-Pro- and Rf-D-Pro-coated chiral columns; Table 1 summarizes the retention times, separation factors, and resolutions of these examined compounds. When using the Rf-L-Pro- and Rf-D-Pro-coated chiral columns, six of the racemic amino acids were well separated within 50 min with the resolution of greater than 1.5. On the Rf-L-Pro-coated column, the retention of the L isomers of the amino acids was always stronger than that of the D isomers; on the Rf-D-Pro-coated column, the retention properties were all inverted. Among the examined amino acids, Ile, Phe, Pro, Tyr, Trp, and Val exhibited especially large separation factors and resolutions in both columns. In contrast, we could not enantioseparate the racemic hydrophilic amino acids; we suspect that the retention of hydrophilic amino acids was very weak because of the low hydrophobicity of the short-length perfluoroalkyl chains of the packing material.

Robustness of the dress-up chiral columns

We examined the robustness of the prepared dress-up Rf-L-Pro-coated chiral column through 20 repeated analyses of D,L-Tyr and D,L-Trp. Table 2 and Fig. 6 summarize the retention times, numbers of theoretical plates, and resolutions for these analytes and their reproducibilities. We obtained similar chromatograms for both the first and twentieth analyses. The retention times of D-Tyr, L-Tyr, D-Trp, and L-Trp each peak were 4.22±0.05, 5.87±0.34, 18.30±0.32, and 30.66±0.65 min, respectively; the numbers of theoretical plates were 1,261.3±102.6, 1,810.1±48.9, 983.4±85.7, and 1,216.5±116.0, respectively; the resolutions of the two peaks for Tyr and Trp were 2.72±0.08 and 4.14±0.16, respectively. Because the relative standard deviations of the retention times, numbers of theoretical plates, and resolutions all remained less than 9.53 %, we conclude that the Rf-L-Pro coating was not stripped from the column during the analyses performed under the tested HPLC conditions.

Reproducibility of the preparation of the dress-up chiral columns

Table 3 lists the reproducibility of the column performance as examined from four repeated cycles of dressing and undressing. The relative standard deviations of the retention times, the numbers of theoretical plates, and the resolutions for D,L-Tyr and D,L-Trp on the Rf-L-Pro-coated column for each of the four preparations were less than or equal to 14.8, 18.7, and 6.61 %, respectively.

Conclusion

We have developed a new type of chiral column—a so-called dress-up chiral column—that functions through a fluorous separation technique. By using this chiral column, we performed the successful enantioseparation of seven racemic amino acids. The robustness of the performance of the column and the reproducibility of its preparation were both relatively good. Unlike other existing dynamically coated chiral columns, the adsorption and removal criteria for the CSPs on the column packing material were not determined by its hydrophobicity, but rather by its fluorophilicity. Therefore, it was possible to ensure the non-elution of the CSPs during analyses as well as their complete removal from the column at the completion of analyses. This concept should be applicable not only for microanalysis but also for large-scale organic syntheses or industrial production. In summary, we believe that dress-up chiral columns could become powerful tools for the enantioseparation of chiral amino acids; using this concept, it might become possible to provide breakthrough chiral columns to suit a wide variety of user demands. To improve the response to a variety of analytes and to ensure better separation, our future goal is to synthesize different types of F-CSPs that will function in chiral separation modes other than ligand exchange chromatography.

Acknowledgments This study was supported in part by a Grant-in-Aid for Young Scientists (B) (23790061) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References

1. Welch CJ (1994) J Chromatogr A 666(1–2):3–26
2. Davankov VA (2003) J Chromatogr A 1000(1–2):891–915
3. Davankov VA (1994) J Chromatogr A 666(1–2):55–76
4. Okamoto Y, Kaida Y (1994) J Chromatogr A 666(1–2):403–419
5. Yashima E, Okamoto Y (1995) Bull Chem Soc Jpn 68(12):3289–3307
6. Lammerhofer M, Lindner W (1996) J Chromatogr A 741(1):33–48
7. Maier NM, Nicoletti L, Lammerhofer M, Lindner W (1999) Chirality 11(7):522–528
8. Zhang W (2003) Tetrahedron 59(25):4475–4489
9. Zhang W (2009) Chem Rev 109(2):749–795
10. Curran DP (1999) Med Res Rev 19(5):432–438
11. Zhang W (2009) Green Chem 11(7):911–920
12. Todoroki K, Yoshida H, Hayama T, Itoyama M, Nohta H, Yamaguchi M (2011) J Chromatogr B 879(17–18):1325–1337
13. Sakaguchi Y, Yoshida H, Todoroki K, Nohta H, Yamaguchi M (2009) Anal Chem 81(12):5039–5045

14. Sakaguchi Y, Yoshida H, Hayama T, Yoshitake M, Itoyama M, Todoroki K, Yamaguchi M, Nohta H (2011) J Pharm Biomed Anal 55(1):176–180
15. Sakaguchi Y, Yoshida H, Hayama T, Itoyama M, Todoroki K, Yamaguchi M, Nohta H (2011) J Chromatogr A 1218(33):5581–5586
16. Hayama T, Sakaguchi Y, Yoshida H, Itoyama M, Todoroki K, Yamaguchi M, Nohta H (2012) Anal Chem 84(19):8407–8414
17. Hayama T, Sakaguchi Y, Yoshida H, Itoyama M, Todoroki K, Yamaguchi M, Nohta H (2010) Rapid Commun Mass Sp 24 (19):2868–2874
18. Todoroki K, Etoh H, Yoshida H, Nohta H, Yamaguchi M (2009) Anal Bioanal Chem 394(1):321–327
19. Todoroki K, Hashimoto H, Mikawa T, Itoyama M, Hayama T, Kojima E, Yoshida H, Nohta H, Yamaguchi M (2010) Anal Bioanal Chem 397(6):2409–2416
20. Todoroki K, Hashimoto H, Machida K, Itoyama M, Hayama T, Yoshida H, Nohta H, Nakashima M, Yamaguchi M (2013) J Sep Sci 36(2):232–238
21. Umehara T, Kamiya S, Itoh A, Chiba K, Haraguchi H (1997) Anal Chim Acta 349:231–238
22. Twohill E, Paull B (2002) J Chromatogr A 973:103–113
23. Chambers SD, Glenn KM, Lucy CA (2007) J Sep Sci 30:1628–1645
24. Umemura T, Kitaguchi R, Inagaki K, Haraguchi H (1998) Analyst 123:1767–1770
25. Umemura T, Tsunoda K, Koide A, Oshima T, Watanabe N, Chiba K, Haraguchi H (2000) Anal Chim Acta 419:87–92
26. Schmid MG, Schreiner K, Reisinger D, Gubitz G (2006) J Sep Sci 29(10):1470–1475
27. Natalini B, Sardella R, Macchiarulo A, Pellicciari R (2008) J Sep Sci 31(4):696–704
28. Oi N, Kitahara H, Kira R (1992) J Chromatogr 592(1–2):291–296
29. Pawlowska M (1991) Chirality 3:136–138
30. Bo T, Wiedmer SK, Riekkola ML (2004) Electrophoresis 25:1784–1791
31. Pittler E, Grawatsch N, Paul D, Gubitz G, Schmid MG (2009) Electrophoresis 30(16):2897–2904
32. Toyo'oka T, Liu Y-M (1995) J Chromatogr A 689(1):23–30