Dual-mode of Reversed and Normal-phase Liquid Chromatographic Enantiomer Separation on Coated Cellulose Derived Chiral Stationary Phases

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For their broad applicability of enantiomer separation of a number of chiral compounds, many chiral stationary phases (CSPs) based on polysaccharide derivatives have been developed and extensively used.[1,2] Among these CSPs, cellulose tris(3,5-dimethylphenylcarbamate) derived CSP (Chiralcel OD or Chiralcel OD-H) and cellulose tris(4-methylbenzoate) derived CSP (Chiralcel OJ or Chiralcel OJ-H) have proven to be highly useful CSPs.[1-3] These type CSPs are usually prepared with coating or adsorbing the polysaccharide derivatives of the chiral selectors on a silica matrix. It has been used with a limited range of solvents and, therefore, it has been mainly employed with normal phase eluents, such as hexane/2-propanol mixtures.[4] Compared to normal-phase enantiomer separation, however, reversed-phase enantiomer separation using aqueous mobile phases may often provide advantages.[5] Especially, it is important for samples that have problems for analysis under normal phase conditions because of their solubility and intrinsic aqueous biological samples or synthetic analytes with aqueous solvents. Since these coated cellulose derived CSPs like Chiralcel OD and Chiralcel OJ have been applied with normal phase eluents, only a few of results for enantiomer separation using aqueous mobile phases on these CSPs have been reported.[6-10] The first attempt of reversed-phase enantiomer separation on the polysaccharide derived CSP has been reported by Ikeda *et al.* Four chiral drug analytes have been enantioseparated with good separation factors (α = 1.10-1.61) on Chiralcel OD using aqueous buffer-acetonitrile solutions.[6] The second report on enantiomer separation of one hydantoin biological analyte of a major metabolite of phenytoin on Chiralcel OJ using aqueous ethanolic solution (Rs = 2.2) has been published by Eto *et al.*[7] Also, the enantiomer resolution of four drug analytes on Chiralcel OJ using aqueous acetonitrile solution has been performed by Ishikawa *et al.*[8] A series of quaternary tropane alkaloids have been resolved with good separation factors on Chiralcel OD using aqueous ion pair mobile phases and normal phases by Hempe *et al.*, respectively.[9] Weinz and his co-workers have reported on enantiomer resolution of only two biological samples of glutethimide and its analog analytes on Chiralcel OD using aqueous acetonitrile solution (α = 1.21, 1.14).[10] In this study, we present enantiomer resolution of *N*-fluorenylmethoxycarbonyl (FMOC) α-amino acids on Chiralcel OJ-H and Chiralcel OD-H under the dual-mode of reversed as well as normal phase conditions. This is the first report on both reversed and normal phase liquid chromatographic enantiomer separation using Chiralcel OJ-H and Chiralcel OD-H, coated cellulose based CSPs.

Table 1 and 2 show liquid chromatographic results for the separation of the enantiomers of *N*-FMOC α-amino acids on Chiralcel OJ-H and Chiralcel OD-H under reversed and normal phase conditions. In general, the enantioselectivities on these two CSPs for the resolution of *N*-FMOC α-amino acids under reversed phase condition of 40% acetonitrile in 50 mM phosphate buffer are lower than those under normal

Table 1. Enantiomer Separation of *N*-FMOC α-Amino Acids on Chiralcel OJ-H under Reverse Phase and Normal Phase

Analyte		Reverse mode				Normal mode			
		α^a	$k'_1{}^b$	Rs[c]	Conf.[d]	α^a	$k'_1{}^b$	Rs[c]	Conf.[d]
1	Ala	1.00	2.02	–	–	1.47	3.67	1.32	D
2	ABA[e]	1.12	2.42	1.12	D	2.38	3.01	2.78	D
3	Asn	1.00	0.45	–	–	1.16	9.37	0.76	D
4	Asp	1.00	0.75	–	–	1.21	7.09	0.86	D
5	Gln	1.00	0.81	–	–	1.32	13.29	1.03	L
6	Glu	1.00	0.75	–	–	1.48	10.13	1.21	L
7	Ileu	1.35	5.51	2.77	D	3.53	1.37	1.82	D
8	Leu	1.63	4.89	4.73	D	1.41	1.33	2.20	D
9	Met	1.06	4.43	0.82	D	1.74	6.34	2.05	D
10	Norleu	1.18	5.75	2.10	D	2.69	1.52	1.50	D
11	Norval	1.25	3.81	1.55	D	3.64	1.94	2.39	D
12	PG	1.10	8.99	1.06	L	1.00	12.75	–	–
13	Phe	1.00	7.37	–	–	1.11	5.20	0.69	D
14	Ser	1.00	0.74	–	–	1.10	7.27	0.73	D
15	Thr	1.20	0.92	0.10	D	4.68	4.48	2.75	D
16	Tyr	1.05	2.82	0.75	D	1.75	41.27	3.36	D
17	Val	1.17	3.90	1.49	D	2.89	1.74	2.90	D

Mobile phase; 40% acetonitrile in 50 mM phosphate buffer (pH 2) for reverse mode and 10% 2-propanol/hexane(V/V) containing 0.1% TFA for normal mode on Chiralcel OJ-H, respectively; Flow rate = 0.5 mL/min (reverse mode), 1 mL/min (normal mode); Detector UV 254 nm. [a]Separation factor. [b]Capacity factor for the first eluted enantiomer. [c]Resolution factor. [d]indicates the absolute configuration of the second retained enantiomer. [e]2-Aminobutyric acid.

Table 2. Enantiomer Separation of *N*-FMOC α-Amino Acids on Chiralcel OD-H under Reverse Phase and Normal Phase

	Analyte	Reverse mode				Normal mode			
		α^a	$k'_1{}^b$	Rs[c]	Conf.[d]	α^a	$k'_1{}^b$	Rs[c]	Conf.[d]
1	Ala	1.88	10.10	6.82	L	1.94	5.73	3.38	L
2	ABA[e]	1.59	13.40	4.20	L	1.44	4.97	2.18	L
3	Asn	1.46	1.68	2.23	L	2.53	27.29	1.97	L
4	Asp	1.37	2.75	1.97	L	1.93	8.12	2.75	L
5	Glu	1.33	2.91	1.41	L	1.47	8.86	1.92	D
6	Gln	1.45	1.59	1.58	D	1.09	13.70	0.97	D
7	Ileu	1.21	29.31	4.71	–	1.20	5.64	1.10	D
8	Leu		ne[f]			1.26	4.84	1.63	D
9	Met		ne			1.27	9.00	1.68	D
10	Norleu	1.17	48.62	2.92	L	1.32	5.11	1.98	L
11	Norval		ne			1.22	4.92	1.46	L
12	PG	1.15	49.43	2.02	L	1.82	7.90	2.09	L
13	Phe	1.19	74.30	2.34	L	1.32	8.47	1.54	L
14	Ser	1.98	4.02	4.13	L	2.70	6.98	4.15	D
15	Thr	1.56	4.75	3.41	L	1.41	5.11	1.57	L
16	Tyr	1.11	18.61	1.34	L	1.13	15.90[g]	0.76	L
17	Val	1.45	30.52	3.55	L	1.00	4.40	–	–

Mobile phase; 40% acetonitrile in 50 mM phosphate buffer (pH 2) for reverse mode and 10% 2-propanol/hexane(V/V) containing 0.1% TFA for normal mode on Chiralcel OD-H, respectively; Flow rate = 0.5 mL/min (reverse mode), 1 mL/min (normal mode); Detector 254 nm. [a]Separation factor. [b]Capacity factor for the first eluted enantiomer. [c]Resolution factor. [d]indicates the absolute configuration of the second retained enantiomer. [e]2-Aminobutyric acid. [f]No elution until 500 min retention time. [g]30% 2-propanol/hexane(V/V) containing 0.1% TFA.

phase condition of 2-propanol/hexane(V/V) containing 0.1% TFA (trifluoroacetic acid), respectively. It is observed that the enantioselectivities using Chiralcel OJ-H on normal phase mode are greater or lower than those using Chiralcel OD-H. However, the separation factors on Chiralcel OD-H under reverse phase conditions are greater than those on Chiralcel OJ-H and, therefore, all investigated *N*-FMOC α-amino acids enantiomers on Chiralcel OD-H showed fairly good enantioselectivities (α = 1.11-1.98) except three analytes (entries 8,9 and 11) in Table 2. Typical chromatograms of resolution of *N*-FMOC valine enantiomers on Chiralcel OJ-H and Chiralcel OD-H under reversed phase conditions are presented in Figure 1, respectively. Especially, all resolved analytes in reverse mode were base-line separated on Chiralcel OD-H (Rs = 1.34-6.82) and most of the examined analytes showed base-line resolution in normal mode on the same CSP (Rs = 0.76-3.38). Therefore, Chiralcel OD-H is effective under not only normal-phase conditions but also reversed-phase conditions for enantiomer resolution of *N*-FMOC α-amino acids. It is interesting that the elution orders of the resolved *N*-FMOC α-amino acids on Chiralcel OD-H in reverse mode are not always identical with those in normal mode. Since the chiral recognition mechanisms related to enantiomer separations may be affected by the used mobile phases, reversals of the elution orders on the polysaccharide derived CSP are often observed by the change of mobile phase.[11,12]

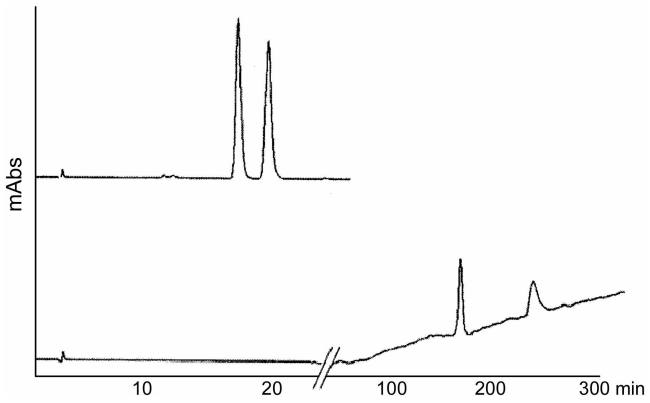

Figure 1. Chromatograms of enantiomer separation of *N*-FMOC valine on Chiralcel OJ-H (the top) and Chiralcel OD-H (the bottom) under reversed phase conditions. The chromatographic conditions are given in Tables 1 and 2.

In summary, we demonstrated enantiomer separation of *N*-FMOC α-amino acids on coated cellulose derived CSPs, Chiralcel OJ-H and Chiralcel OD-H under both normal and reversed-phase conditions. Although the separation factors for resolution of *N*-FMOC α-amino acids in reverse mode are generally lower than those in normal mode, these CSPs can be usefully applied in dual mode, not only normal-phase conditions but also reversed-phase conditions for enantiomer resolution of *N*-FMOC α-amino acids. In particular, this reversed-phase analytical method using Chiralcel OD-H is expected to be quite useful for determination of the enantiomeric purity of analytical samples in biological matrices of serum or plasma and in asymmetric synthesis using aqueous solvents.

Experimental Section

Chromatographic analysis was performed at room temperature using an HPLC consisting of an SCL-10A system controller, LC-10AD pump and SPD-10AVP diode array detector (Shimadzu, Kyoto, Japan). Chiralcel OJ-H and Chiralcel OD-H (250 mm L × 4.6 mm I.D., 5 μm) were purchased from Daicel Chemical Company (Tokyo, Japan). HPLC-grade acetonitrile, hexane and 2-propanol were obtained from J. T. Baker (Phillipsburg, NJ). Trifluoroacetic acid (TFA) was obtained from Aldrich (Milwaukee, WI). The racemic and enantiomerically pure *N*-FMOC α-amino acids were prepared according to the conventional methods.[13] The reversed mobile phase was consisted of acetonitrile and 50 mM NaH_2PO_4 buffer. Mobile phases were adjusted to pH 2 with 10% phosphoric acid. The pH of the buffers was adjusted before mixing with acetonitrile. The mobile phases for HPLC were filtered through a Millipore membrane filter (0.45 μm) and degassed before use.

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