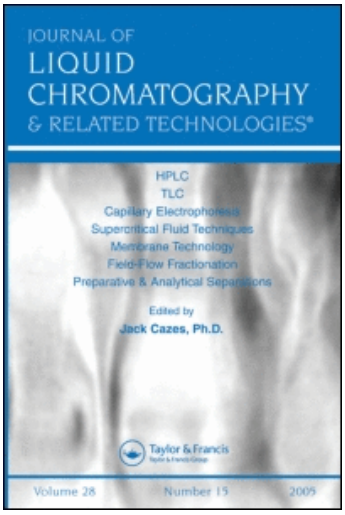

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Covalently Bonded and Coated Chiral Stationary Phases Derived from Polysaccharide Derivatives for Enantiomer Separation of *N*-Fluorenylmethoxycarbonyl α-Amino Acids with Fluorescence Detection

Jing Yu Jin[a]; Wonjae Lee[a]; Jung Hag Park[b]; Jae Jeong Ryoo[c]

[a] College of Pharmacy, Chosun University, Kwangju, S. Korea [b] Department of Chemistry, Yeungnam University, Kyoungsan, S. Korea [c] Department of Chemistry Education, Kyungpook National University, Daegu, S. Korea

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Covalently Bonded and Coated Chiral Stationary Phases Derived from Polysaccharide Derivatives for Enantiomer Separation of *N*-Fluorenylmethoxycarbonyl α-Amino Acids with Fluorescence Detection

Jing Yu Jin and Wonjae Lee
College of Pharmacy, Chosun University, Kwangju, S. Korea

Jung Hag Park
Department of Chemistry, Yeungnam University, Kyoungsan, S. Korea

Jae Jeong Ryoo
Department of Chemistry Education, Kyungpook National University, Daegu, S. Korea

Abstract: Liquid chromatographic comparisons for enantiomer resolution of *N*-fluorenylmethoxycarbonyl (FMOC) α-amino acids with fluorescence detection were made on covalently bonded type chiral stationary phases (CSPs) (Chiralpak IA and Chiralpak IB) and coated type CSPs (Chiralpak AD and Chiralcel OD) derived from polysaccharide derivatives of the same chiral selectors. This is the first study reported of enantiomer resolution with fluorescence detection on covalently bonded type CSPs, Chiralpak IA and Chiralpak IB. In general, covalently bonded type CSPs (Chiralpak IA and Chiralpak IB) showed lower enantioseparation than coated type CSPs (Chiralpak AD and Chiralcel OD) for enantiomer resolution of these analytes, respectively. Owing to higher sensitivity and broader solvent compatibility in fluorescence detection on Chiralpak IA and Chiralpak IB than in UV detection, however, this analytical method is expected to enlarge their application

Address correspondence to Prof. Wonjae Lee , College of Pharmacy, Chosun University, 375 Seoseok-Dong, Dong-Ku, Kwangju, 501-759, Republic of Korea. E-mail: wlee@chosun.ac.kr

of enantiomer resolution, such as an online HPLC monitoring of asymmetric synthesis.

Keywords: Enantiomer separation, Chiralpak IA, Chiralpak IB, Fluorescence detection, Chiral stationary phase

INTRODUCTION

Chiral stationary phases (CSPs) derived from polysaccharide derivatives have been extensively used for enantiomer separation of a number of racemic compounds.[1,2] Since chiral selectors of polysaccharide derivatives are coated on a silica matrix, these types of CSPs have intrinsic drawbacks of column stability and a limitation of mobile phases. Therefore, the solvents such as halogenated solvents, tetrahydrofuran, ethyl acetate, and acetone, which partially or totally dissolve the chiral selectors of the polysaccharide derivatives must be excluded for mobile phases and analytes solvents.[1,2] These disadvantages are directly related to limitation of their applicability including preparative separation due to solubility of analytes. Many studies to solve these problems by covalently bonding the chiral selectors of polysaccharide derivatives to a silica matrix have been reported.[3–9] Recently, covalently bonded CSPs on silica matrix, Chiralpak IA[10–15] and Chiralpak IB,[16] which have the same chiral selectors, amylose and cellulose tris(3,5-dimethylphenylcarbamate) of coated type Chiralpak AD and Chiralcel OD, respectively, have been developed. Therefore, it is considered that Chiralpak IA and Chiralpak IB are the covalently immobilized CSP of Chiralpak AD and Chiralcel OD, respectively. In this study, we present the comparative liquid chromatographic enantiomer resolution of *N*-fluorenylmethoxycarbonyl (FMOC) protected α-amino acids on polysaccharide derived CSPs, covalently bonded type Chiralpak IA and Chiralpak IB, and coated type Chiralpak AD and Chiralcel OD with fluorescence detection.[17]

EXPERIMENTAL

Chromatographic analysis was carried out using an HPLC consisting of a Waters model 510 pump, a Rheodyne model 7125 injector with a 20 μL loop, a spectrofluorometric detector (Jasco FP-920), and an HP 3396 series II recorder. The excitation and emission wavelengths were 280 and 310 nm, respectively. Chiralpak IA, Chiralpak IB, Chiralpak AD, and Chiralcel OD columns (250 mm L × 4.6 mm I.D.) were purchased from Daicel Chemical Company (Tokyo, Japan). HPLC grade hexane (Hxn), 2-propanol, tetrahydrofuran (THF), ethyl acetate, and dichloromethane were obtained from J. T. Baker (Phillipsburg, NJ). Trifluoroacetic acid (TFA) was obtained from

Aldrich (Milwaukee, WI). The racemic and L-*N*-FMOC α-amino acids were prepared according to a reported procedure.[18]

RESULTS AND DISCUSSION

Tables 1 and 2 show the effect of mobile phase on the enantiomer separation of four *N*-FMOC α-amino acids on Chiralpak IA and Chiralpak IB with fluorescence detection. Due to their covalently bonded nature, these two CSPs are compatible with a large range of organic miscible solvents, including the halogenated solvent of dichloromethane or chloroform.[10,16] The separation factors and retention times on Chiralpak IA and Chiralpak IB are considerably influenced by the nature of mobile phase.[10,12,15] As shown in Tables 1 and 2, the highest enantioselectivities for all analytes were obtained using 5% 2-propanol in hexane with 0.1% TFA as a mobile phase, except for *N*-FMOC valine using 25% dichloromethane in hexane with 0.1% TFA. Interestingly, the elution orders of these analytes on Chiralpak IA are variable depending upon the used mobile phases in Table 1, while their elution orders on Chiralpak IB are unchanged regardless of the mobile phases in Table 2.

Especially, this fluorescence detection method for enantiomer separation on Chiralpak IA and Chiralpak IB has great advantages over a UV detection method. It provides higher sensitivity and much wider solvent versatility irrelevant to the cutoffs of the used mobile phases, compared to a UV detection method.[17] This is the first report for enantiomer resolution with fluorescence detection on covalently bonded type CSPs, Chiralpak IA and Chiralpak IB.

Tables 3 and 4 show the comparative results of enantiomer separation of *N*-FMOC α-amino acids on covalently bonded type CSPs (Chiralpak IA and Chiralpak IB) and coated type CSPs (Chiralpak AD and Chiralcel OD) using 2-propanol in hexane with 0.1% TFA as a mobile phase with fluorescence detection. Chiralpak IB and Chiralcel OD derived from cellulose tris(3,5-dimethylphenylcarbamate) showed, in general, higher enantioselectivity than Chiralpak IA and Chiralpak AD derived from amylose tris(3,5-dimethylphenylcarbamate), respectively. Most of *N*-FMOC α-amino acids enantiomers were well separated on Chiralcel OD. Also, in general, Chiralpak IA and Chiralpak IB of covalently bonded type CSPs showed lower enantioseparation than Chiralpak AD and Chiralcel OD of coated type CSPs, respectively. It was reported that the reduction in enantioselectivity on covalently bonded CSPs is due to the lack of the ordered arrangement of polysaccharide derived chiral selectors bonded to the silica matrix.[4,6] It is interesting that the elution orders of the resolved *N*-FMOC α-amino acids on the covalently bonded type Chiralpak IB derived from cellulose tris(3,5-dimethylphenylcarbamate) are observed to be identical with those on coated type Chiralcel OD of the same chiral selector using 2-propanol in hexane with 0.1% TFA as a

Table 1. Effect of mobile phase on the enantiomer separation of some *N*-FMOC α-amino acids on Chiralpak IA

Mobile[a] phase / Analyte	5% 2-Propanol/Hxn with 0.1% TFA			15% THF/Hxn with 0.1% TFA			20% Ethyl acetate/Hxn with 0.1% TFA			25%Dichloromethane/Hxn with 0.1% TFA		
	α[b]	k_1'[c]	Conf.[d]	α[b]	k_1'[c]	Conf.[d]	α[b]	k_1'[c]	Conf.[d]	α[b]	k_1'[c]	Conf.[d]
Ala	1.09	5.28	D	1.07	6.15	D	1.00	5.76	—	1.00	4.37	—
Leu	1.26	4.82	L	1.00	5.21	—	1.07	4.08	L	1.16	3.04	L
PG	1.31	5.02[e]	D	1.09	9.92	D	1.11	6.35	L	1.18	8.40	L
Val	1.08	5.92	L	1.08	4.02	D	1.00	3.59	—	1.21	2.81	L

[a]Mobile phase; Hexane (Hxn), Tetrahydrofuran (THF); Flow rate = 1 mL/min.
[b]Separation factor.
[c]Capacity factor for the first eluted enantiomer.
[d]Indicates the absolute configuration of the second retained enantiomer.
[e]10% 2-Propanol/Hxn (V/V) with 0.1% TFA.

Table 2. Effect of mobile phase on the enantiomer separation of some *N*-FMOC α-amino acids on Chiralpak IB

Mobile[a] phase / Analyte	5% 2-Propanol/Hxn with 0.1% TFA			15% THF/Hxn with 0.1% TFA			20% Ethyl acetate/Hxn with 0.1% TFA			25% Dichloromethane/Hxn with 0.1% TFA		
	α[b]	k_1'[c]	Conf.[d]	α[b]	k_1'[c]	Conf.[d]	α[b]	k_1'[c]	Conf.[d]	α[b]	k_1'[c]	Conf.[d]
Ala	1.40	7.15	L	1.07	6.01	L	1.09	10.25	L	1.00	11.93	—
Leu	1.42	4.65	D	1.13	3.72	D	1.18	5.53	D	1.24	7.33	D
PG	1.34	3.65[e]	D	1.18	6.65	D	1.19	9.61	D	1.00	13.45	D
Val	1.21	4.61	D	1.10	3.12	D	1.20	5.04	D	1.17	6.21	D

[a]Mobile phase; Hexane (Hxn), Tetrahydrofuran (THF); Flow rate = 1 mL/min.
[b]Separation factor.
[c]Capacity factor for the first eluted enantiomer.
[d]Indicates the absolute configuration of the second retained enantiomer.
[e]10% 2-Propanol/Hxn (V/V) with 0.1% TFA.

Table 3. Enantiomer separation of *N*-FMOC α-amino acids on Chiralpak IA and Chiralpak AD

		Chiralpak IA				Chiralpak AD			
Entry	Analyte	α^a	$k_1'^b$	Rs[c]	Conf.[d]	α^a	$k_1'^b$	Rs[c]	Conf.[d]
1	ABA[e]	1.07	6.90	0.96	D	1.13	3.46	1.40	D
2	ACA[f]	1.06	5.98	0.85	—	1.13	2.95	1.36	—
3	Ala	1.09	5.28	1.25	D	1.12	3.05	1.46	D
4	Asn	1.28	8.19[g]	1.96	L	1.20	6.97	2.03	L
5	Asp	1.07	3.70[g]	0.72	D	1.17	4.90	0.94	D
6	Gln	1.10	6.67[g]	0.54	L	1.42	5.64	3.79	L
7	Glu	1.11	4.01[g]	1.14	L	1.27	5.30	2.69	L
8	Ileu	1.08	7.26	1.07	L	1.17	3.99	1.86	D
9	Leu	1.26	4.82	3.34	L	1.19	3.22	1.85	L
10	Met	1.05	12.11	0.71	L	1.00	5.57	—	—
11	Norleu	1.04	6.75	0.45	L	1.07	9.69[h]	0.92	D
12	Norval	1.00	7.12	—	—	1.12	10.63[h]	1.63	D
13	PG	1.31	5.02[g]	3.86	D	1.40	7.62	4.56	D
14	Phe	1.08	3.31[g]	0.91	L	1.13	4.89	1.50	L
15	Ser	1.07	3.65[g]	0.60	L	1.09	4.78	1.05	L
16	Thr	1.09	3.76[g]	0.92	D	1.14	5.05	1.55	D
17	Tyr	1.25	15.35[g]	2.76	L	1.31	26.55	3.79	L
18	Val	1.08	5.92	1.15	L	1.14	4.24	1.43	D

Mobile phase; 5% and 10% 2-propanol/hexane (V/V) containing 0.1% TFA on Chiralpak IA and Chiralpak IB, respectively; Flow rate = 1 mL/min. [a]Separation factor. [b]Capacity factor for the first eluted enantiomer. [c]Resolution factor. [d]Indicates the absolute configuration of the second eluted enantiomer. [e]2-Aminobutyric acid. [f]2-Aminocaprylic acid. [g,h]10% and 5% 2-propanol/hexane(V/V) containing 0.1% TFA, respectively.

mobile phase in Table 4, as the similar results using different mobile phases are shown in Table 2. However, Chiralpak IA and Chiralpak AD derived from amylose tris(3,5-dimethylphenylcarbamate) showed three exceptions of the elution orders (entries 8, 11, and 18) in Table 3.

In conclusion, an HPLC fluorescence analysis of enantiomer separation of *N*-FMOC α-amino acids on covalently bonded type CSPs (Chiralpak IA and Chiralpak IB) and coated type CSPs (Chiralpak AD and Chiralcel OD) was performed. In general, cellulose tris(3,5-dimethylphenylcarbamate) derived CSPs, Chiralpak IB and Chiralcel OD showed higher enantioselectivity than amylose tris(3,5-dimethylphenylcarbamate) derived CSPs, Chiralpak IA and Chiralpak AD, respectively. Also, Chiralpak IA and Chiralpak IB showed, in general, lower enantioselectivity than Chiralpak AD and Chiralcel OD, respectively. However, this analytical method using fluorescence detection affords high sensitivity and compatibility with a much wider range of solvents on covalently bonded type CSPs

Table 4. Enantiomer separation of *N*-FMOC α-amino acids on Chiralpak IB and Chiralcel OD

		Chiralpak IB				Chiralpak OD			
Entry	Analyte	α^a	$k_1'^b$	Rs[c]	Conf.[d]	α^a	$k_1'^b$	Rs[c]	Conf.[d]
1	ABA[e]	1.23	6.28	2.02	L	1.38	5.23	2.89	L
2	ACA[f]	1.12	4.78	1.55	—	1.41	4.48	2.78	—
3	Ala	1.40	7.15	3.95	L	1.80	5.86	5.25	L
4	Asn	1.10	4.37[g]	0.48	L	1.60	5.05[g]	1.81	L
5	Asp	1.22	5.02[h]	1.50	L	1.58	6.77[g]	2.80	L
6	Gln	1.00	4.81[g]	—		1.17	14.01	0.86	D
7	Glu	1.16	6.00[h]	1.15	L	1.33	9.69	1.80	L
8	Ileu	1.51	4.59	5.42	D	1.51	4.30	3.62	D
9	Leu	1.42	4.65	3.97	D	1.26	5.19	2.01	D
10	Met	1.07	4.35[h]	0.76	L	1.14	7.80	1.11	L
11	Norleu	1.07	5.84	0.80	L	1.15	4.69	1.19	L
12	Norval	1.05	6.06	0.68	L	1.07	4.98	0.61	L
13	PG	1.34	3.65[h]	3.14	D	1.71	7.88	3.79	D
14	Phe	1.08	3.69[h]	0.70	L	1.10	8.49	0.70	L
15	Ser	1.65	6.25[h]	3.73	L	2.56	2.70[g]	5.01	L
16	Thr	1.11	4.66[h]	0.74	L	1.48	7.29	2.98	L
17	Tyr	1.08	7.76[g]	0.56	L	1.10	20.50[g]	0.55	L
18	Val	1.21	4.61	2.25	D	1.13	4.55	1.10	D

Mobile phase; 5% and 10% 2-propanol/hexane (V/V) containing 0.1% TFA on Chiralpak IB and Chiralpak OD, respectively; Flow rate = 1 mL/min. [a]Separation factor. [b]Capacity factor for the first eluted enantiomer. [c]Resolution factor. [d]Indicates the absolute configuration of the second eluted enantiomer. [e]2-Aminobutyric acid. [f]2-Aminocaprylic acid. [g,h]20% and 10% 2-propanol/hexane (V/V) containing 0.1% TFA, respectively.

(Chiralpak IA and Chiralpak IB). It permits the creation of new applications of enantiomer separation for fluorescent analytes, including a direct online HPLC monitoring for determination of enantiomeric purity during asymmetric synthesis procedures on these covalently bonded CSPs.[13]

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