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Short communication

Chiral separation with novel (*S*)-biotin-bonded silica gel for liquid chromatography

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Abstract

Liquid chromatographic separation of enantiomers was accomplished using a chiral stationary phase (CSP) derived from (*S*)-biotin on silica gel. In both nonaqueous and aqueous media, this CSP (**1**) permitted separation of racemic amino acid derivatives based on hydrogen bonding with a urea moiety of the biotin moiety.

1. Introduction

Liquid chromatography (LC) using chiral stationary phases (CSPs) is a sophisticated method for separating enantiomeric compounds and determining their compositions [1,2]. For CSPs that form diastereomeric associations between enantiomers, hydrogen bonding is the most significant contributor to these associations [3]. A biotin known as a strong binder to avidine [4] contains a urea unit and an asymmetric center on a bent bicyclic backbone. The urea unit that can participate in hydrogen bonding is located far from the asymmetric carbon, but its bonding affinity is affected by the carbon configuration owing to a bent structure. Thus, silica gel modified with (*S*)-biotin (CSP **1**) was prepared to determine its ability to separate enantiomers such as amino acid derivatives.

2. Experimental

NMR spectra were obtained using a 300 MHz instrument (Varian Mercury-300). The internal standard was either chloroform (7.26 ppm) or tetramethylsilane for ^{1}H NMR and tetramethylsilane for ^{13}C NMR.

2.1. Preparation of (S)-biotin-bonded silica gel (CSP *1*)

2.1.1. (S)-Biotin 4-nitrophenyl ester (*1a*)

(*S*)-Biotin (3.68 g, 16.35 mmol) was suspended in dry pyridine (80 ml), to which was added 4-nitrophenyl trifluoroacetate (15.0 g, 63.75 mmol) and 4-nitrophenol (2.08 g, 15.00 mmol). After the mixture stirred at 55 °C for 2 h, the solvent was removed under reduced pressure. The residue was filtrated, washed with diethyl ether, and then recrystallized in ethanol to give 4.76 g of the desired material (yield, 80.0%): m.p. 155.5–157 °C; ^{1}H NMR ($CDCl_3$) δ 0.52–1.64 (brm, 2H), 1.70–1.89 (brm, 4H), 2.64 (t, 2H, J = 7.1 Hz), 2.75 (d, 1H, J = 13.4 Hz), 2.95(dd, 1H, J = 5.0, 12.8 Hz), 3.16–3.24 (m, 1H), 4.31–4.46 (m, 1H), 4.52–4.57 (m, 1H), 4.77 (brs, 1H), 5.14(brs, 1H), 7.29 (d, 2H, J = 9.2 Hz), 8.28 (d, 2H, J = 9.2 Hz); ^{13}C NMR ($CDCl_3$) δ 25.16, 28.92, 34.51, 41.14, 55.98, 60.67, 62.57, 112.10, 123.06, 125.79, 164.06, 171.74, 172.24; Anal. Calcd for $C_{16}H_{19}O_5N_3S$: C, 52.29; H, 5.24; N, 11.50. Found: C, 52.40; H, 5.22; N, 11.50.

2.1.2. (S)-Biotinyl 10-undecenylamine (*1b*)

Compound **1a** (2.93 g, 8.0 mmol) was dissolved in dimethylformamide (DMF, 40 ml). To this solution was added 10-undecenylamine (2.54 g, 15.0 mmol). After the mixture was stirred at room temperature for 24 h, the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography with 4% (v/v)

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methanol–chloroform mixture as the eluent to give 2.71 g of the desired material (yield, 85.8%): m.p. 180–182 °C; ^{1}H NMR ($CDCl_3$) δ 1.26–1.61 (brm, 16H), 1.64–1.74 (m, 4H), 1.99–2.06 (m, 2H), 2.18 (t, 2H, J = 7.5 Hz), 2.72 (d, 1H, J = 12.9Hz), 2.90 (dd, 1H, J = 5.1, 12.9 Hz), 3.11–2.23 (m, 3H), 4.27–4.31 (m, 1H), 4.47–4.51 (m, 1H), 4.89–5.01 (m, 1H), 5.36 (brs, 1H), 5.75–5.84 (m, 1H), 5.75–6.02 (brd, 2H); ^{13}C NMR ($CDCl_3$) δ 25.68, 26.94, 28.11, 28.45, 28.89, 29.07, 29.30, 29.40, 29.49, 33.75, 35.77, 39.44, 40.28, 55.60, 60.07, 61.83, 113.68, 138.70, 163.89, 173.79; Anal. Calcd for $C_{21}H_{37}O_2N_3S$: C, 63.76; H, 9.43; N, 10.62. Found: C, 63.33; H, 9.44; N, 10.48.

2.1.3. (S)-Biotinyl 11-(chlorodimethylsilyl) undecanylamine (**1c**)

Compound **1b** (2.37 g, 6.0 mmol) and a catalytic amount of hydroplatinic acid hexahydrate were dried at room temperature under reduced pressure for 1 h. The mixture was suspended in dry chloroform (45 ml) under argon atmosphere and the suspension heated at 60 °C with stirring. After dissolution of **1b**, trimethylchlorosilane (2 ml, 18 mmol) was added to the mixture, followed by further heating for 1 h. The solvent was removed under reduced pressure to give the desired material, confirmed by disappearance of olefinic proton signals from **1b** in NMR spectra. This material was used for the following step without further purification.

2.1.4. (S)-Biotin-bonded silica gel (**1d**)

Spherical silica gel (2.05 g; Nucleosil 100-5 (diameter, 5 μm), Macherey-Nagel, Düeren, Germany) was heated under reduced pressure at 170 °C for 16 h. Compound **1c** from the previous step was added to the dried silica gel after the silica gel had cooled to room temperature under argon atmosphere. A total of 20 ml of dry pyridine was used for this addition. The mixture was heated at 45 °C for 6 h. The modified silica gel was filtered, washed successively with chloroform, methanol, and acetone, and then dried under reduced pressure to yield 2.52 g of the desired material: Anal. Found: C, 15.91; H, 2.92; N, 2.31.

2.1.5. Trimethylsilylated (S)-biotin-bonded silica gel (CSP **1**)

After the modified silica gel **1d** (2.51 g) was dried under reduced pressure at 80 °C, it was suspended in dry chloroform (26 ml) under argon atmosphere and trimethylimidazole (6 ml) was added. The mixture was heated at 65 °C for 12 h. The modified silica gel was filtered, successively washed with chloroform, methanol, and acetone, and dried under reduced pressure to give 2.51 g of the final product: Anal. Found: C, 17.04; H, 3.16; N, 2.21.

2.2. Liquid chromatography

The modified silica gel was packed into a stainless-steel column (1.5 (i.d.) × 150 mm) in accordance with the procedure described previously [5].

Liquid chromatography was conducted with a system consisting of a Labo System Intelligent pump 301 (Tokyo), SPD-2AM UV detector (Shimadzu Co., Kyoto) equipped with a 0.5 μl flow cell, LC-300 column oven (Chromato Science Co., Osaka), and CR-3A data processor (Shimadzu Co., Kyoto). Solute elution was detected at 254 nm. Chromatographic conditions were: eluent, either 2% (v/v) 2-propanol in *n*-hexane or 50% (v/v) methanol in water; flow rate, 60 μl/min; column temperature, 25 °C.

3. Results and discussion

3.1. Synthesis of CSP **1**

(*S*)-Biotinyl 11-(chlorodimethylsilyl)undecanylamine (**1c**) was introduced at a ratio of 0.55 mmol/g silica gel, which was estimated from the nitrogen content (2.31%) in the elemental analysis of (*S*)-biotin-bonded silica gel (**1d**) without the exhaustive trimethylsilylation of remaining silanols. After end capping of **1d** with trimethylsilylimidazole, the carbon content increased from 15.91 to 17.04%. This capping is a prerequisite for selective hydrogen bond formation on the chiral recognition site, i.e., the cyclic urea unit of the biotin moiety, in nonaqueous media (Fig. 1).

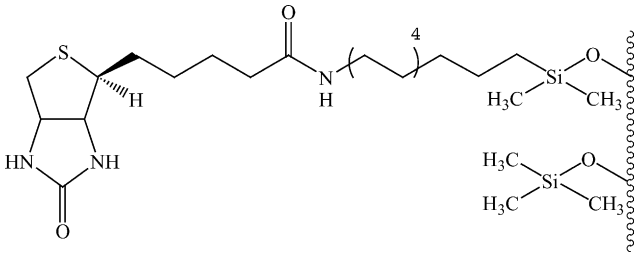

Fig. 1. Structure of CSP **1**.

3.2. Chromatography with CSP **1** in nonaqueous medium

Table 1 shows separation of enantiomeric amino acid derivatives on CSP **1** under nonaqueous phase operation

Table 1
Enantiomer separation of two types of racemic *N*-3,5-dinitrobenzoyl (DNB)-amino acid derivatives on CSP **1** using 2% (v/v) 2-propanol-*n*-hexane as a eluent[a]

Amino acid	*N*′,*N*′-Diethylamide			Isopropyl ester		
	k'_D[b]	α	R_s	k'_D[b]	α	R_s
Alanine	4.68	1.29	2.10	8.56	1.08	0.95
Valine	2.32	1.15	1.26	4.71	1.10	1.12
Leucine	2.66	1.13	1.16	5.99	1.08	0.85
Phenylalanine	4.56	1.14	1.33	9.22	1.11	1.20

[a] Other chromatographic conditions are described in Section 2.
[b] Retention factor (k') of the D-enantiomer eluted first.

Table 2
Enantiomer separation of two types of racemic *N*-3,5-dinitrobenzoyl (DNB)-amino acid derivatives on CSP **1** using 50% (v/v) methanol–water as a eluent[a]

Amino acid	*N*′,*N*′-Diethylamide			Isopropyl ester		
	k'_D[b]	α	R_s	k'_D[b]	α	R_s
Alanine	4.00	1.14	1.22	7.20	1.04	0.25
Valine	9.41	1.10	1.12	16.38	1.07	0.96
Leucine	15.93	1.18	2.14	29.16	1.08	1.09
Phenylalanine	22.62	1.14	1.87	38.15	1.07	1.04

[a] Other chromatographic conditions are described in Section 2.
[b] Retention factor (k') of the D-enantiomer eluted first.

using 2% (v/v) 2-propanol-*n*-hexane as an eluent. Derivatives used were either *N*-3,5-dinitrobenzoyl (DNB)-*N*′,*N*′-diethylamides or *N*-DNB-*O*-isopropyl esters. The former derivatives possessed higher separation factors than the latter and the L enantiomer was retained stronger than was the D enantiomer for all amino acid derivatives, indicating that the L enantiomer provided a more stable association to the (*S*)-biotin moiety.

Among the solutes resolved, the largest separation factor (1.29) was obtained by *N*-DNB-alanine *N*′,*N*′-diethylamide (**2**), compared to a value of 1.08 obtained with *N*-DNB-alanine isopropyl ester. This difference indicates that the carbonyl group of the *C*-terminal ternary amide unit of **2** functions as a hydrogen bond acceptor stronger than that of the ester derivative. Therefore, the cyclic urea unit of the (*S*)-biotin moiety should interact with both *N*-terminal amides and *C*-terminal carbonyl groups of the derivatives.

3.3. Chromatography with CSP *1* in aqueous medium

Aqueous phase operation of CSP **1** using 50% (v/v) methanol–water as an eluent produced enantiomer separation for all derivatives, as shown in Table 2. The *N*′,*N*′-diethylamide derivatives provided separation factors larger than those for the ester derivatives and as large as those found for nonaqueous phase operation. For elution order, the D enantiomer was followed by the L enantiomer. Retention was dictated by the hydrophobicity of the amino acid residues and the ester derivatives were more strongly retained than were the ternary amide derivatives when comparing two derivatives with the same amino acid residues. Fig. 2(A) illustrates the separation of racemic *N*-DNB-leucine *N*′,*N*′-diethylamide, which provided the largest separation factor on CSP **1**. Chromatogram of *N*-DNB-leucine isopropyl ester whose R_s value was the highest in the isopropyl ester derivatives was also depicted in Fig. 2(B).

We have observed that silica gel modified with (10-undecenoyl)-L-valine *tert*-butylamide via hydrosilylation (CSP **3**) is capable of separating enantiomers of amino acid derivatives [5,6] using aqueous phase operation. This separation was thought to occur when hydrogen bonds form in the hydrophobic environment shielded from the bulk aqueous phase. (Using acetyl-L-valine *tert*-butylamide as a model compound in the chiral selection area of CSP **3**, the hydrogen bonding between this region and 4-nitrobenzoyl (NB)-L-leucine isopropyl ester was measured using NMR [3]).

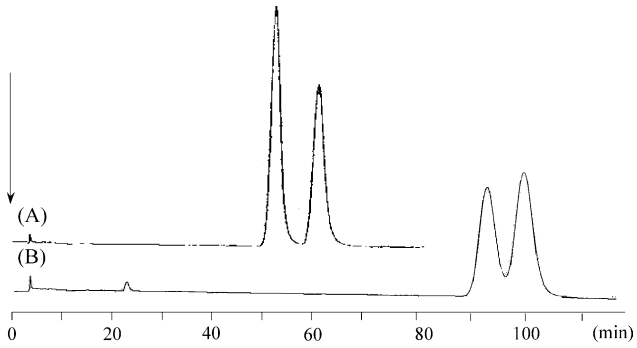

Fig. 2. Enantiomer separation of racemic *N*-3,5-dinitrobenzoyl(DNB)-leucine *N*′,*N*′-diethylamide and *N*-DNB-leucine isopropyl ester on CSP **1** with 50% (v/v) methanol–water as a eluent. (A) *N*-DNB-DL-leucine *N*′,*N*′-diethylamide; (B) *N*-DNB-DL-leucine isopropylester; other chromatographic conditions are as described in Section 2.

However, separation was observed in a high concentration of methanol. Under the conditions mentioned above, the hydrophobic phase would be broken by the distribution of methanol to the interfacial phase. It is difficult to describe whether or not hydrogen bonding affinity is capable of promoting diastereomer formation between the chiral biotin moiety and racemic amino acid derivatives even in 50% methanol–water. However, the biotin-bonded silica gel (CSP **1**) was effective during aqueous phase operation, thus hydrogen bonding probably occurs even in the situation of competition from highly polar solvents such as methanol and water. The leucine and phenylalanine isopropyl ester derivatives were weakly but definitely separated with the separation factor of 1.06 (k'_D, 2.45) and that of 1.07 (k'_D, 3.00), respectively, even when methanol concentration increased to 70%.

The (*S*)-biotin can function as a chiral resolving agent based on hydrogen-bonding interactions on the silica gel surface. Complexation of the biotin and *N*-3,5-dinitrobenzoyl (DNB)-amino acid *N*′,*N*′-diethylamide probably occurs via bidentate hydrogen bonding between the cyclic urea unit and the amino acid derivatives, at least in nonaqueous media.

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