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Short Communication

Optimization of naphthylethylurea multiple-bonded chiral stationary phases for optical resolution of enantiomeric amino acid derivatives

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ABSTRACT

The polyethyleneamine spacer length of naphthylethylurea multiple-bonded chiral stationary phases (CSPs) was optimized for optical resolution of *p*-bromophenylcarbamyl (Br-PC) amino acid enantiomers. Four multiple-bonded CSPs having different lengths of polyethyleneamine spacer, diethylenetriamine, triethylenetetramine, tetraethylenepentamine and pentaethylenehexamine, were prepared via an activated carbamate intermediate from aminopropylsilyl silica gel. The resolution data of Br-PC amino acids on these CSPs by elution with an aqueous mobile phase were compared. The CSP with the triethylenetetramine spacer showed the best resolution of the enantiomers in the four CSPs. The other conditions, pH, concentration and temperature, etc., were also investigated using this CSP. They influenced the retention time of Br-PC amino acids, but they hardly affected the enantio recognition of the CSP.

INTRODUCTION

Recently, optical resolution methods using high-performance liquid chromatography (HPLC) have been rapidly advanced. In particular, the chiral stationary phase (CSP) methods have become of interest, and numerous CSPs have been developed [1–3]. We have also reported a versatile method for the preparation of chemically bonded phase-type CSPs via activated carbamate type silica gel [4,5]. In addition, the optical resolution of *p*-bromophenylcarbamyl (Br-PC) derivatives of enantiomeric amino acids by the prepared CSP,the naphthylethylurea multiple-bonded CSP, in reversed-phase mode has been reported [4]. In this case, we have confirmed that when optically active 1-(α-naphthyl) ethylamine (NEA) as a chiral source is introduced into the packing, the use of pentaethylenehexamine as a spacer and a chiral centre-increasing agent gives better resolution of Br-PC amino acids than the naphthylethylurea monolayer CSP [5].

In this study, polyethyleneamine spacer length on

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the multiple-bonded CSP was optimized for the Br-PC derivatives of enantiomeric amino acids. Multiple-bonded CSPs having various lengths of polyethyleneamine spacer were prepared. The resolution data of the enantiomeric Br-PC amino acids on these CSPs were compared.

EXPERIMENTAL

Chemicals

Nucleosil 5-NH_2 (aminopropylsilyl silica gel) was obtained from Macherey–Nagel (Düren, Germany). Distilled water was purified by passage through a Milli-Q Labo system (Nihon Millipore, Tokyo, Japan). Optically active NEA and pentaethylenehexamine (n = 4) were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Tetraethylenepentamine (n = 3), HPLC-grade acetonitrile and methanol were purchased from Kanto Chemical (Tokyo, Japan). Disuccinimido carbonate (DSC) was purchased from Chemiscience (Tokyo, Japan). Diethylenetriamine (n = 1), triethylenetetramine (n = 2) and other reagents were obtained from Wako Pure Chemicals (Osaka, Japan). Succinimido p-bromophenylcarbamate (SIBr-PC) and optically active succinimido 1-(α-naphthyl)ethylcarbamate (SINEC) were prepared as described previously [6,7].

Chromatographic conditions

The HPLC system consisted of an LC-6A high-pressure pump (Shimadzu Seisakusyo, Kyoto, Japan), a Model 7125 loop injector (Rheodyne, Cotati, CA, USA) and a UVIDEC 100-III spectrophotometric detector (Jasco, Tokyo, Japan). The detector wavelength was set at 250 nm. Column temperature was thermostated by Thermo Minder Mini-80 and Coolpipe 150L (Taiyo Scientific, Tokyo, Japan). Mobile phases are shown in the table and figures.

Samples

Br-PC derivatives of amino acid enantiomers were prepared by the reaction of SIBr-PC with amino acids as described previously [6].

Preparation of CSPs

Multiple-bonded CSPs having various lengths of polyethyleneamine spacer were prepared by the successive reaction of Nucleosil 5-NH_2 prepacked into a stainless-steel column (150 × 4.6 mm I.D.) with

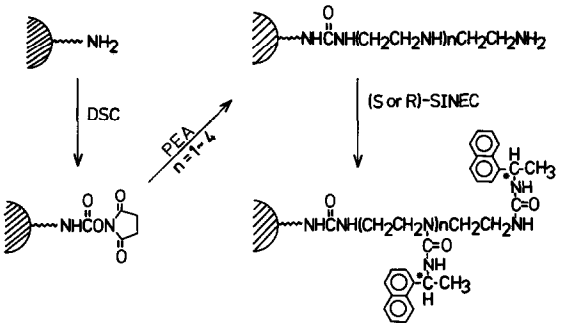

Fig. 1. Reaction scheme for the preparation of naphthylethylurea multiple-bonded CSPs having various lengths of polyethyleneamine spacer. DSC = Disuccinimido carbonate; PEA = polyethyleneamine; SINEC = succinimido 1-(α-naphthyl)ethylcarbamate.

DSC, polyethyleneamine and SINEC as described previously [4]. The reaction scheme is shown in Fig. 1. The CSPs prepared from each polyethyleneamine, n = 1–4, are called columns 1–4, respectively, in the following text.

Elemental analysis for the initial packing was 3.28% C, 0.85% H, 1.08% N; for column 1, 9.04% C, 1.38% H, 3.02% N; for column 2, 13.33% C, 1.73% H, 3.32% N; for column 3, 12.68% C,

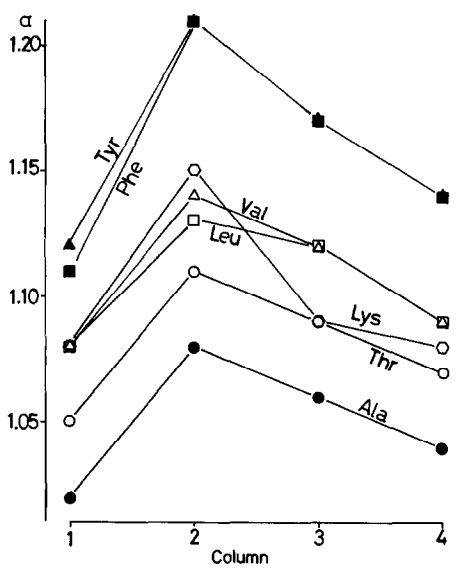

Fig. 2. Effect of the polyethyleneamine length of multiple-bonded CSPs on the separation factor of seven Br-PC amino acids enantiomers.

1.76% H, 3.74% N; for column 4, 15.91% C, 2.07% H, 4.08% N.

RESULTS AND DISCUSSION

Fig. 2 shows the relationship between polyethyleneamine length and the separation factor (α) of Br-PC amino acid enantiomers. The maximum α value was obtained with column 2. The separation data, capacity ratios (k'), α values and resolution values (R_s), are indicated in Table I. Columns 3 and 4 retained the enantiomers longer than column 2, however column 2 exhibited the maximum R_s value. In spite of this, since the R_s values of columns 1 and 2 are easily compared, the retention time of the enantiomers on column 1 was controlled by the mobile phase. Column 2 exhibited a better R_s value than column 1. These results evidently prove that column 2 gave the best resolution of Br-PC amino acids enantiomers on the four CSPs. Chromatograms of Br-PC valine and phenylalanine enantiomers are shown in Figs. 3 and 4, respectively.

This finding can be confirmed by C/N ratios calculated from elemental analysis. The C/N ratios suggest that the amount of naphthylethylurea residue introduced to column 2 was not less than that introduced into the other columns.

In order to optimize other conditions, column 2 was tested under various conditions. Component salts of buffer, acetate, phosphate, tartarate and citrate affected the retention time, but did not affect the separation factor of the enantiomers. Sodium acetate in the concentration range 0.05–0.15 *M* and mobile phase pH in the range 4.0–6.0 produced the same results. The effect of column temperature was investigated in the range 10–50°C in steps of 10°C. The theoretical plate number increased with a rise in temperature, but the separation factor was hardly affected.

TABLE I

SEPARATION OF ENANTIOMERIC Br-PC AMINO ACIDS ON FOUR MULTIPLE-BONDED CSPs

t_0 = 1.5 min; k', α and R_s refer to the capacity ratio, separation factor and resolution value for a pair of enantiomers, respectively. Mobile phase: 0.15 *M* sodium acetate (pH 5.0)–acetonitrile (30:70, v/v).

Sample	Column 4			Column 3			Column 2			Column 1		
	k'	α	R_s	k'	α	R_s	k'	α	R_s	k'	α	R_s
Thr L	9.59	1.07	1.18	7.00	1.09	0.98	7.30[a]	1.11	1.38	10.77[b]	1.05	0.82
D	10.27			7.65			7.80			11.29		
Ala L	10.32	1.04	0.69	8.19	1.06	0.70	9.91[a]	1.07	0.96	13.81[b]	1.02	–
D	10.77			8.68			10.63			14.12		
Val L	9.48	1.09	1.42	8.04	1.12	1.41	5.21	1.14	1.61	6.47[a]	1.08	1.09
D	10.33			8.99			5.97			6.97		
Leu L	9.96	1.09	1.60	8.69	1.12	1.47	5.59	1.13	1.61	7.41[a]	1.08	1.15
D	10.88			9.69			6.37			8.00		
Phe L	11.52	1.14	2.55	10.37	1.17	2.31	6.56	1.21	2.46	9.08[a]	1.11	1.60
D	13.19			12.16			7.91			10.09		
Tyr L	19.49	1.14	2.10	14.77	1.17	2.01	8.63	1.21	2.01	9.28[a]	1.12	1.85
D	22.19			17.35			10.47			10.45		
Lys L	18.23	1.08	1.55	16.01	1.09	1.45	9.83	1.15	1.87	14.11[a]	1.08	0.93
D	19.83			17.84			11.32			15.21		

[a] 0.15 *M* Sodium acetate (pH 5.0)–acetonitrile (50:50, v/v).
[b] 0.15 *M* Sodium acetate (pH 5.0)–acetonitrile (70:30, v/v).

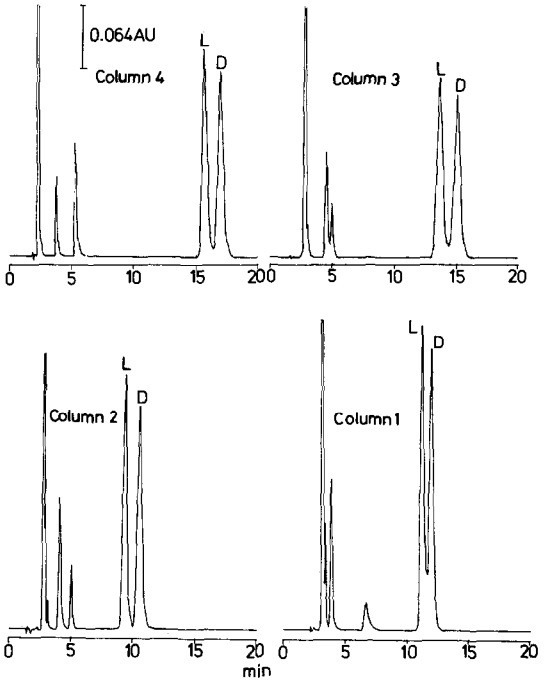

Fig. 3. Separation of enantiomeric Br-PC valine on four multiple-bonded CSPs. Mobile phase: 0.15 *M* sodium acetate (pH 5.0)–acetonitrile (30:70, v/v, for columns 2–4; 50:50, v/v, for column 1). Flow-rate: 1.0 ml/min. Column temperature: room temperature. Each peak corresponds to 500 ng of enantiomeric valine.

Thus, a multiple-bonded CSP with greater enantio recognition (column 2) for Br-PC amino acid enantiomers than the previous type (column 4) is presented. Column 2, in spite of showing high enantio recognition, exhibitis retention behaviour to Br-PC amino acids that is the same as column 4. Therefore column 2 will give superior simultaneous analysis of Br-PC amino acid enantiomers than that previously reported by us [4].

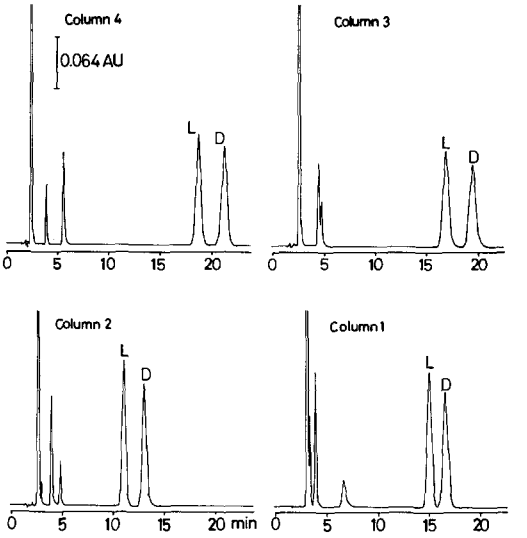

Fig. 4. Separation of enantiomeric Br-PC phenylalanine on four multiple-bonded CSPs. Conditions as in Fig. 3.

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