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SEPARATION OF AROMATIC AMINO ACIDS ON β-CYCLODEXTRIN POLYURETHANE RESINS

YOSHIKAZU MIZOBUCHI*, MINORU TANAKA and TOSHIYUKI SHONO
Department of Applied Chemistry, Faculty of Engineering, Osaka University, Yamadakami, Suita, Osaka 565 (Japan)
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SUMMARY

The chromatographic behaviour of α- and β-cyclodextrin polyurethane resins cross-linked with diisocyanates have been studied using six aromatic amino acids as model compounds and aqueous phosphate buffer solutions of pH 5.5 or 8.2 as eluent. Phenylglycine, tyrosine, tryptophan and phenylalanine can be separated completely, although phenylalanine is very strongly retained. The amino acids are retained more strongly at pH 8.2 than at pH 5.5. The interactions between the cyclodextrin units in the resins and the amino acids are discussed briefly.

INTRODUCTION

Recently, free amino acids in water have been determined by high-performance liquid chromatography[1], and porous polymers, *e.g.*, Amberlite XAD copolymers[2,3], have been developed for the separation and collection of amino acids, organic acids, peptides, etc. The separation of amino acids and peptides has also been carried out by ion-exchange chromatography of their metal complexes[4] and by gel filtration chromatography using Sephadex gels[5].

We are interested in the specific interactions between adsorbents and adsorbates and have started a study of porous resins containing cyclodextrins. It is well known that cyclodextrins, which are soluble in water and some organic solvents, form inclusion complexes with various guest compounds. Cyclodextrins were cross-linked with diisocyanates and changed to an insoluble resin form. These cyclodextrin polyurethane resins are expected to exhibit specific adsorption based on inclusion complex formation, in contrast to the commercially available resins. The separation of amino acids on cyclodextrin polymers cross-linked with ethylene glycol di(epoxypropyl) ether was reported previously[6].

We have prepared cyclodextrin polyurethane resins having high cyclodextrin contents and investigated their specific adsorption behaviours in the gaseous phase[7]. In this paper we describe the separation and adsorption behaviours of aromatic amino acids on these resins.

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EXPERIMENTAL

Materials

DL-α-Phenylglycine, DL-tyrosine and L-kynurenine from Tokyo Kasei (Tokyo, Japan), DL-β-(3,4-dihydroxyphenyl)alanine from Wako (Osaka, Japan), DL-tryptophan from Kishida Kagaku (Osaka, Japan) and DL-phenylalanine from Tokyo Rigaku Yakuhin (Tokyo, Japan) were used without further purification. Water used for the eluting mixture was deionized. Inorganic salts were of analytical reagent grade. The preparation of the polyurethane resins for column packings has been described previously[7].

Procedures

The columns were glass tubings (35 × 0.52 cm I.D.) packed by a slurry packing technique. The bottom of each column was plugged with a silicone rubber plug and quartz-wool and the top with another plug of silicone rubber. The column was packed to a height of 29.5 cm with 149–177 μm particles of the polyurethane resin or 40–120 μm particles of Sephadex G-15.

An Atto Model UV monitor II equipped with 254- and 280-nm UV detectors, a 60-μl flow cell and Adzuma Model MF-1 syringe driving equipment with a 100-ml syringe was used. A 5 × 0.1 cm I.D. stainless-steel tube was inserted into the silicone rubber at each end of the column. The syringe containing the eluting solution, the column and the UV monitor were connected with PTFE tubing.

Sample solutions (0.68 m*M*) were prepared by dissolving the aromatic amino acids in the eluting solution of aqueous phosphate buffer (pH 5.5 or 8.2). Samples were stored in polyethylene bottles and kept in a refrigerator. A 2-ml syringe was used to inject 0.65 ml of a sample into the chromatographic system (at the top of the column). The flow-rate was 20 ml/h in all experiments. Aromatic amino acids were detected at 254 and/or 280 nm.

RESULTS AND DISCUSSION

Properties of cyclodextrin polyurethane resins

Table I shows the main physical properties of cyclodextrin polyurethane resins. The specific surface areas were determined by means of the B.E.T. method and the

TABLE I
PHYSICAL PROPERTIES OF CYCLODEXTRIN POLYURETHANE RESINS
Cyclodextrin: α = α-cyclodextrin; β = β-cyclodextrin. Solvent: P = pyridine. Diisocyanate: HDI = hexamethylene diisocyanate; H6XDI = 1,3-bis(isocyanatomethyl)cyclohexane; XDI = 1,3-bis(isocyanatomethyl)benzene. Precipitant: M = methanol; A = acetone. BDOL = 1,4-butanediol.

Resin	*Specific surface area (m^2/g)*	*Cyclodextrin content (%, w/w)*	*Gel bed volume (ml/g)*	*Dry bed volume (ml/g)*
β-HDI-P-5.5-A	260	63.9	2.3	2.3
β-H6XDI-P-6.0-M	250	67.7	3.0	2.1
β-XDI-P-5.8-A	170	65.0	2.7	2.5
α-HDI-P-4.9-A	180	78.1	1.9	1.5
BDOL-HDI-P-M	160	–	3.7	4.9
BDOL-XDI-P-A	40	–	4.3	5.6
Sephadex G-15	–	–	2.8	1.3

cyclodextrin contents were calculated from elemental analyses. The β-cyclodextrin polyurethane resins are porous and have high cyclodextrin contents (*ca.* 65 %, w/w). The polyurethane resins slightly swell in the aqueous phosphate buffer solutions used. The β-cyclodextrin polyurethane resins and the polyurethane resins containing no cyclodextrin units are very stable in organic solvents, except in strongly acidic solutions. However, the α-cyclodextrin polyurethane resin is unstable in organic solvents.

Retention behaviour of aromatic amino acids on cyclodextrin polyurethane resins

The cyclodextrin resins exhibit strong interactions with guest molecules such as benzene and pyridine containing π electrons and/or heteroatoms and may be used to distinguish between the configurations of the guest isomers[7]. It is of interest to investigate the retention behaviour of aromatic amino acids on the polyurethane resins.

Table II shows the retention times of various aromatic amino acids relative to phenylglycine at pH 5.5. Three kinds of β-cyclodextrin polyurethane resins, β-HDI-P-5.5-A, β-H6XDI-P-6.0-M and β-XDI-P-5.8-A, exhibit similar retention behaviour for the aromatic amino acids. The retention time of phenylalanine is much longer than that of phenylglycine, and may reasonably be interpreted as follows. Two interactions are important in retaining the aromatic amino acids: the hydrophobic interaction between cyclodextrin cavities and the amino acids; and the hydrogen-bonding interaction between the hydroxyl groups at the sides of the cyclodextrin torus and the amino acids. Phenylalanine readily fits into the cyclodextrin cavity and because the phenyl group is attached to the β-carbon atom both the hydrophobic and hydrogen-bonding interactions are strong. In the case of phenylglycine, however, the phenyl group is attached to the α-carbon atom. Consequently, the hydrophobic and/or hydrogen-bonding interaction is weakened, because the distance between the phenyl group and the hydrogen-bonding site is not as long as that of phenylalanine.

It is of interest to investigate the effect of substitution of the phenyl group of the aromatic amino acids by a hydrophilic hydroxyl group on their interaction with the β-cyclodextrin resins. The retention times were found to increase in the order: β-

TABLE II
RETENTION TIMES (RELATIVE TO PHENYLGLYCINE) OF AMINO ACIDS ON POLYURETHANE RESINS AND DEXTRAN GEL AT pH 5.5
Actual retention times (min) are given in parentheses.

Amino acid	*β-HDI-P-5.5-A*	*β-H6XDI-P-6.0-M*	*β-XDI-P-5.8-A*	*α-HDI-P-4.9-A*	*BDOL-HDI-P-M*	*BDOL-XDI-P-A*	*Sephadex G-15*
Phenylglycine	1.00 (23.1)	1.00 (28.5)	1.00 (37.4)	1.00 (17.7)	1.00 (21.0)	1.00 (20.2)	1.00 (19.9)
Tyrosine	2.17	2.23	2.96	1.07	1.02	1.01	1.25
Tryptophan	5.11	6.28	7.77	1.36	1.06	1.00	2.32
Kynurenine	5.22	7.84	10.52	1.00	1.04	1.02	1.09
Phenylalanine	7.40*	25.82*	20.24*	1.06	0.97	1.02	1.22
β-(3,4-Dihydroxyphenyl)alanine	1.03	1.15	1.12	0.84	1.00	1.02	1.39

* Initial retention time is shown because the peak is too broad.

(3,4-dihydroxyphenyl)alanine < tyrosine < phenylalanine. The introduction of one or two hydroxyl groups into the benzene ring of phenylalanine results in a large decrease in the retention time. It is suggested that the hydrophobic interaction is weakened by the presence of the hydrophilic hydroxyl group in the apolar benzene ring included in the cyclodextrin cavity.

Tryptophan and kynurenine are eluted faster than phenylalanine. This is presumably due to the steric effect of the substituent at the *ortho* position of the benzene ring. In this way, the benzene rings of tryptophan and kynurenine cannot enter so deeply into the cyclodextrin cavity as can phenylalanine.

The α-cyclodextrin polyurethane resin, α-HDI-P-4.9-A, and the polyurethane resins containing no cyclodextrin units, BDOL-HDI-P-M and BDOL-XDI-P-A, do not enable the separation of the amino acids studied in this work, the actual retention times of all the amino acids being nearly equal on these three resins. This indicates that the α-cyclodextrin units in α-HDI-P-4.9-A exhibit little interaction with the amino acids under these conditions. On the column packed with Sephadex G-15, tryptophan is the most strongly retained of the amino acids. As shown in Table II, the retention times of amino acids on the β-cyclodextrin polyurethane resins are dependent upon the cross-linking reagent and increase in the order β-HDI-P-5.5-A < β-H6XDI-P-6.0-M < β-XDI-P-5.8-A.

Fig. 1 shows chromatograms of mixtures of three amino acids on the β-cyclodextrin polyurethane resins. These amino acids are not separated completely on β-HDI-P-5.5-A, but are separated on both β-H6XDI-P-6.0-M and β-XDI-P-5.8-A at pH 5.5.

The retention behaviour of the amino acids on β-H6XDI-P-6.0-M and β-XDI-P-5.8-A was investigated in basic solution of pH 8.2. Chromatograms of a mixture of

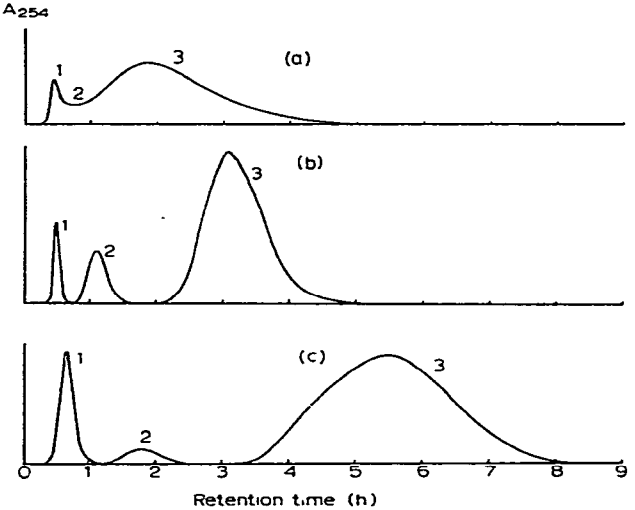

Fig. 1. Chromatograms of aromatic amino acids on β-cyclodextrin polyurethane resins at pH 5.5. Resins: a = β-HDI-P-5.5-A; b = β-H6XDI-P-6.0-M; c = β-XDI-P-5.8-A. Aromatic amino acids: 1 = phenylglycine; 2 = tyrosine; 3 = tryptophan.

phenylglycine, tyrosine and tryptophan are shown in Fig. 2. The retention time of each amino acid is longer on β-XDI-P-5.8-A than on β-H6XDI-P-6.0-M. However, the separation of phenylglycine and tyrosine on β-XDI-P-5.8-A is incomplete. These three amino acids can be separated completely on β-H6XDI-P-6.0-M.

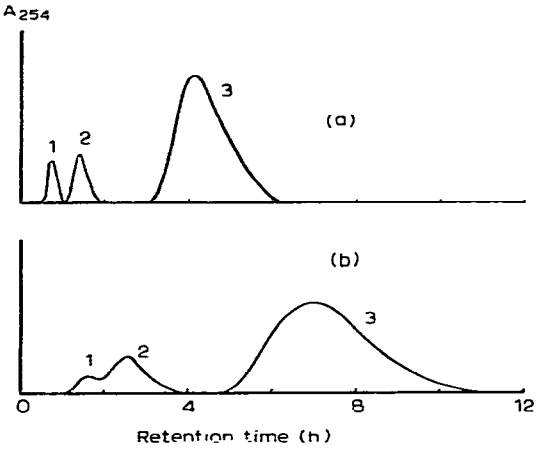

Fig. 2. Chromatograms of aromatic amino acids on β-cyclodextrin polyurethane resins at pH 5.5. Resins: a = β-H6XDI-P-6.0-M; b = β-XDI-P-5.8-A. Amino acids as in Fig. 1.

Table III summarizes the retention times of the amino acids on these two β-cyclodextrin resins at pH 8.2. The actual retention time is longer at pH 8.2 than at pH 5.5 except for phenylalanine. Since the carboxyl groups of the amino acids are dissociated to greater extents at pH 8.2 than at pH 5.5, and the carboxylate ions will form strong hydrogen bonds with the hydroxyl groups of the cyclodextrin, the amino acids are retained more strongly by the resins at pH 8.2 than at pH 5.5 because of the greater hydrogen-bonding interaction. Of the five aromatic amino acids in Table III, phenylalanine is very strongly retained by the β-cyclodextrin polyurethane resins and gives too broad a peak. The retention time of phenylalanine is not affected by the pH value. This may be due to the fact that the degree of dissociation of the carboxyl groups is the same at pH 5.5 as that at pH 8.2 because of the low isoelectric point of phenylalanine.

TABLE III
RETENTION TIMES (RELATIVE TO PHENYLGLYCINE) OF AMINO ACIDS ON β-CYCLODEXTRIN POLYURETHANE RESINS AT pH 8.2
Actual retention times (min) are given in parentheses.

Amino acid	*β-H6XDI-P-6.0-M*	*β-XDI-P-5.8-A*
Phenylglycine	1.00 (45.3)	1.00 (86.7)
Tyrosine	1.91	1.87
Tryptophan	5.43	5.08
Kynurenine	7.00	5.81
Phenylalanine	11.03*	8.94*

* Initial retention time is shown because the peak is too broad.

The effect of pH on the retention of tyrosine and tryptophan on β-XDI-P-5.8-A was investigated in the pH range 5.5–8.2. The retention times of both amino acids increase considerably at pH values above 7 as shown in Fig. 3. It is suggested that, in addition to the hydrophobic guest–host interaction between the phenyl groups of the amino acids and the β-cyclodextrin cavity, in a basic medium the hydrogen-bonding interaction between the carboxylate ions of the aromatic amino acids and the hydroxyl groups of the β-cyclodextrin also plays an important role in the retention.

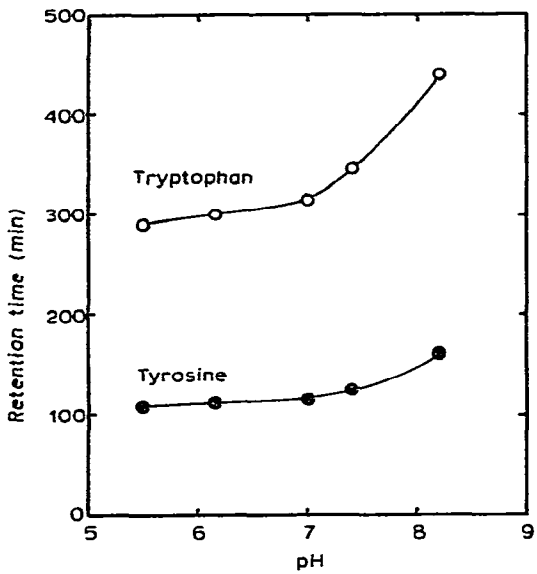

Fig. 3. Effect of pH on retention times of tyrosine and tryptophan on β-XDI-P-5.8-A.

It is found that the aromatic amino acids can be separated on the column packed with the β-cyclodextrin polyurethane resins. The results also indicate the possibility of using these β-cyclodextrin resins as adsorbents selectively to collect the aromatic amino acids in water.

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