

Synthesis of Copper(II) Complexes of Asymmetric Resins Prepared by Attachment of α -Amino Acids to Crosslinked Polystyrene

M. A. PETIT and J. JOZEFONVICZ, *Laboratoire de Recherches sur les Macromolécules, Université Paris-Nord, 93430 Villetaneuse, France*

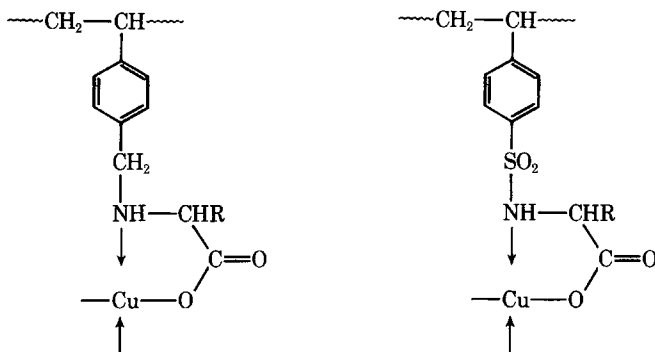
Synopsis

In order to use ligand exchange resins for chromatographic resolution of racemic compounds, two series of asymmetric sorbents were prepared by attachment of various optically active α -amino acids onto crosslinked polystyrene. Chloromethylated and chlorosulfonated styrene-divinylbenzene copolymers were prepared and then treated with α -amino acids. Results show that reaction of α -amino acids with polystyrene could be carried to a substitution of 15% to 40% of the aromatic rings depending on many parameters. Stable copper(II) complexes of the asymmetric resins were prepared. Potentiometric methods were adapted to the case of insoluble materials such as resins. These methods indicate that bis-chelate complexes are formed.

INTRODUCTION

Attempts to resolve racemic compounds by ligand exchange chromatography have been carried out in a few cases with various sorbents. Davankov et al. have prepared metal complexes of asymmetric sorbents obtained by reacting chloromethylated polystyrene with α -amino acids¹ or α -amino acid methyl esters.² It can be concluded from their results that the most satisfactory chromatographic resolutions of α -amino acid racemates can be made using copper complexes of L-proline or L-hydroxyproline stationary ligands.³⁻⁵ Other asymmetric sorbents obtained by reacting α -amino acids with chlorosulfonated polystyrene have been prepared by Vesa et al.⁶ The authors obtained partial resolution of numerous α -amino acid racemates using the asymmetric sorbent poly(styrylsulfonyl-L-phenylalanine) charged with copper ions.⁷ Snyder et al. reported the preparation of styrene-divinylbenzene copolymers containing the complex N-carboxymethyl-L-valine-copper(II). Their results show that partial resolution of several amino acids is possible on such a sorbent.⁸

In this paper, we report the preparation and the chemical properties of asymmetric sorbents similar to those prepared by Davankov and Vesa. The aim of this work was to elucidate the effect of certain parameters on the exchange capacities of the two series of asymmetric sorbents obtained from copper ions complexation of α -amino acids linked to chloromethylated or chlorosulfonated polystyrene:



We also report the use of potentiometric methods which allow precise determinations of the sorbents composition. Copper ions were chosen as complexing metal ions because of the high stability and the well-known structure of their α -amino acid complexes. The influence of methylene or sulfone bridges on the ability of fixed α -amino acid ligands to interact with copper ions and other free ligands will be compared in a future paper. The following describes the three successive experimental steps leading in each series to the final asymmetric, complexed sorbent: (1) preparation of chloromethylated or chlorosulfonated polystyrene; (2) attachment of α -amino acids onto the polymer, and (3) complexation of the asymmetric polymer by copper ions.

RESULTS AND DISCUSSION

Chloromethylation of Polystyrene

Crosslinked polystyrene was chloromethylated by pure or dilute chloromethyl methyl ether (CME) at various temperatures in the presence of different amounts of stannic chloride as catalyst. Results are shown in Figure 1, where the frac-

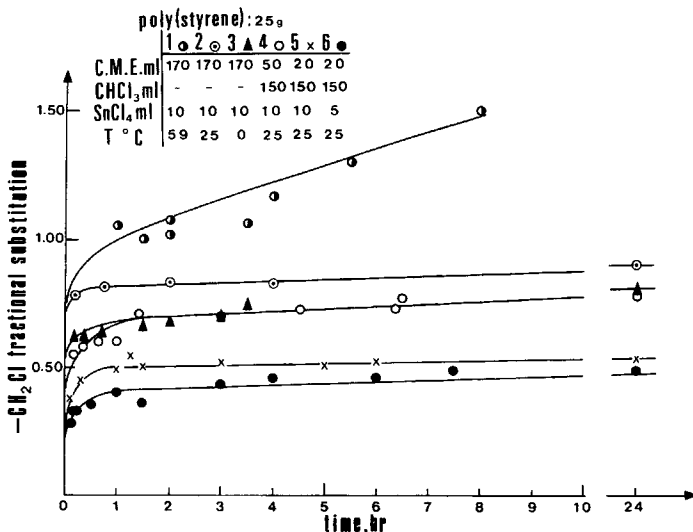


Fig. 1. Rate of chloromethylation of polystyrene: effect of temperature, dilution of chloromethyl methyl ether (CME), and catalyst amount.

TABLE I
Properties of Chloromethylated Resins

Fixed α -AA	—CH ₂ Cl content, % ^a	α -AA/ PSS ⁺ ^b	Reaction time, hr	α -AA content, %	α -AA capacity, meq/g ^c	Copper capacity, meq/g	
						Calcd. ^d	Found ^c
DL-Ala-1	116.1	2	52	17.2	1.10	—	—
DL-Ala-2	150.0	2.8	72	24.3	1.35	—	—
L-Ala-3	116.1	2	192	21.2	1.34	—	—
L-Ala-5	106.5	2	303	19.1	1.24	0.61	0.60
L-Phe-6	106.5	1.3	303	17.4	1.05	0.52	0.52
L-Tyr-10	98.0	2	63	16.7	1.04	0.51	0.78
L-Thr-9 ^e	101.2	1.7	48	3.1	0.22	—	—
L-Thr-12	93.0	2	96	34.1	1.96	0.96	1.02
L-Try-13	93.5	2	96	11.4	0.73	0.36	0.59
L-OH Pro 17	31.0	2	22	12.9	0.99	0.49	0.53
L-OH Pro 19	49.2	1.3	19	12.6	0.93	0.46	0.48
L-OH Pro 15	52.8	2	18	16.0	1.13	0.56	0.64
L-OH Pro 7	98.2	2	720	37.4	2.03	1.01	0.98
L-Pro 16	31.0	2	22	13.0	1.01	0.50	0.49
L-Pro 18	49.2	2	19	16.5	1.19	0.59	0.60
L-Pro 14	52.8	2	46	17.9	1.23	0.59	0.60
L-Pro 8	98.2	2	95	36.4	2.06	1.02	0.99
L-Pro 11	129.3	2	63	28.4	1.61	0.79	0.82

^a Percent —CH₂Cl content = 100 × fractional substitution of the polymer before attachment of α -amino acid (α -AA).

^b Molar ratio of α -amino acid to sulfonium groups (PSS⁺).

^c As determined in experimental section.

^d Calculated on the basis of one copper ion bound by two fixed α -amino acids.

^e The chloromethylated resin was iodomethylated to an 80% extent before reaction with L-threonine.

tional substitution (number of chloromethyl groups per benzene ring) is plotted versus the reaction time. The chloromethyl content was determined according to a procedure described in the experimental part.

In the pure ether, no limit value of the substitution could be reached when refluxing at 59°C (curve 1). Furthermore, no striking difference was found between the runs at 25° and 0°C after 1 hr (curves 2 and 3).

When diluting the ether in chloroform (curves 4 and 5) at 25°C, the limit of substitution was lowered to 0.53.

When decreasing the amount of catalyst, the limit of substitution was slightly lowered (curves 5 and 6).

Attachment of α -Amino Acid to Chloromethylated Polystyrene

Significant attachment of α -amino acids to chloromethylated polystyrene implied the activation of the polymer. Three procedures have been used as follows:

The polymer was iodomethylated according to known conditions.^{8,9} The exchange reaction of Cl by I proceeded to an extent of 80%. The resulting resin was not found to exhibit a sufficient reactivity toward α -amino acids (Table I, resin 9). Therefore, according to another procedure described elsewhere,¹ attempts were made to attach α -amino acids to chloromethylated polystyrene in

the presence of sodium iodide and a tertiary amine. Low attachment yields were found again.

Good yields could be obtained using the two following steps. In the first step, chloromethylated polystyrene was treated with dimethyl sulfide in a water-isopropanol mixture following a preparation of sulfonium derivatives outlined by Snyder et al.⁸ The conversion rate is shown in Figure 2. It is apparent that more than 80% of chloromethyl groups have reacted after 24 hr. Changing the reaction temperature did not affect significantly the reaction yield. The amount of displaced chlorine was determined using a method described in the experimental part. The reaction is reversible; therefore, the sulfonium polymer was not isolated. In the second step, the α -amino acid (sodium salt) dissolved in a water-isopropanol mixture was added to the sulfonium polymer suspension. A sodium hydroxide amount corresponding to the number of sulfonium groups was simultaneously added in order to neutralize the hydrochloric acid amount displaced by the attachment reaction. After refluxing for one to four days, the final polymer was filtered, washed, and titrated in order to determine the extent of α -amino acid attachment.

Results are summarized in Table I. Comparison of samples 14, 18 and 1, 3, and 5 shows that the reaction tends to level off within approximately 40 hr. Results observed on samples 15, 19 lead to the conclusion that there is no large influence of the molar ratio (α -amino acid/sulfonium groups) on the achieved α -amino acid content.

It may be pointed out that the determining parameter is the average number of chloromethyl groups per aromatic ring. When changing this factor from 0.31 to 0.98, the percentage of aromatic rings having fixed a L-proline or L-hydroxyproline group increases from 13% to 37%.

The particle size range of final resins is strongly affected by the extent of α -amino acid attachment. No significant change in the bead size of the starting polystyrene is observed when the percentage of substituted aromatic rings is less than 15%, which corresponds to a capacity of about 1 milliequivalent α -amino acid per gram resin (1 meq/g). Resins with larger substitution percentages could be obtained; but, unfortunately, the beads generally break down to fine powders unsuitable in the chromatographic investigations.

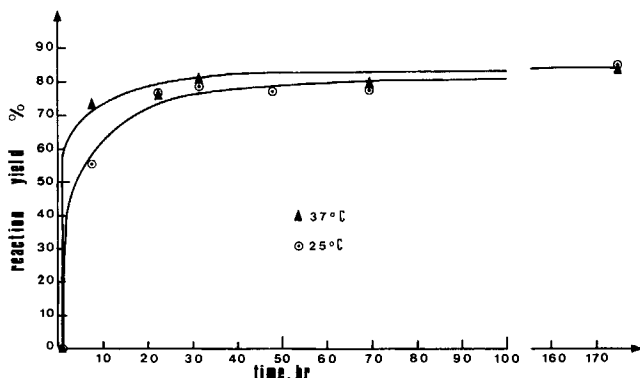


Fig. 2. Rate of reaction of chloromethylated polystyrene with dimethyl sulfide: effect of temperature.

TABLE II
Properties of Chlorosulfonated Resins

Fixed α -AA	—SO ₂ Cl content, % ^a	α -AA/—SO ₂ Cl ^b	α -AA content, %	α -AA capacity, meq/g ^c	Copper capacity, meq/g	
					Calcd. ^d	Found ^c
L-Phe-1	59.7	2	16.5	0.88	1.51	1.28
L-Pro-2	56.0	2	28.9	1.56	1.45	1.40
L-Phe-3	81.4	1.6	21.6	0.99	1.88	1.81
L-Thr-4	73.1	1.1	18.3	0.93	1.80	1.46
L-Ala-5	73.1	1.9	26.4	1.34	1.79	1.78
L-Pro-6	82.0	1	35.3	1.59	1.79	1.37
L-Glu-7	82.0	2	28.3	1.19	2.22	2.00
L-OH Pro-11	72.4	1.1	46.5	2.02	1.53	1.46

^a Percent —SO₂Cl content = 100 × fractional substitution of the polymer before attachment of α -amino acid (α -AA).

^b Molar ratio of α -amino acid to chlorosulfonated groups.

^c As determined in experimental section.

^d Calculated on the basis of one copper ion bound by two fixed α -amino acids or two sulfonate groups.

Chlorosulfonation of Polystyrene

Crosslinked polystyrene was chlorosulfonated according to a procedure previously described.¹⁰ The chlorosulfonated materials were found to have in all cases a chlorosulfonyl content between 4 and 4.5 meq/g (see experimental section). Analysis indicated a slight excess of sulfur with respect to chlorine. This suggests that chlorosulfonation of polystyrene involves partial crosslinking due to sulfone formation since no sulfonate groups could be found. However, the percentage of bridged aromatic ring calculated from analysis of sulfur and chlorine is less than 10%. Because chlorosulfonated resins are sensitive to hydrolysis, reactions with α -amino acids were immediately carried out.

Attachment of α -Amino Acids to Chlorosulfonated Polystyrene

Chlorosulfonated polystyrene was treated in a water-dioxane mixture containing an α -amino acid in the presence of alkali according to a method described by Vesa et al.⁶ The α -amino acid contents of the final resins were determined by acid-base titration (see experimental section). Results listed in Table II are expressed as the percentage of aromatic rings substituted by one α -amino acid group. The resin capacities are also reported in meq/g. Percentages have been calculated without considering crosslinkage by sulfone bridges since only few aromatic rings are bridged. The results show that the resins contain about 1 meq/g of α -amino acid. It can be calculated that approximately 20% to 30% of the aromatic rings of the polymer are substituted. It shall be pointed out that this substitution carried onto a chlorosulfonated resin is faster and more convenient than the one involving a chloromethylated resin.

Copper(II) Complexes of Polymer Derivatives

Two procedures were used to complex the α -amino acid groups linked to polystyrene. In the first procedure, a weighed portion of an asymmetric resin

dioxane and then progressively to pure methanol. Finally, it was dried under vacuum at 60°C.

The chloromethyl content was determined from the analytical value of Cl and through the following potentiometric procedure: a weighed portion (about 200 mg) of the resin was quaternized in 5 ml pure boiling *n*-butyl amine for 6 hr. The mixture was then poured into 50 ml water. After acidification of the heterogeneous medium with nitric acid, the Cl⁻ content was determined by potentiometric titration using a silver indicating electrode and a 0.1M silver nitrate solution as titrant. Good agreement was found between the values obtained from the two methods.

Sulfonium Polymer

A typical run was carried out according to the following procedure: a 20.0-g portion of dry chloromethyl polystyrene (130 mmoles Cl) was suspended in a mixture of 48 ml dimethyl sulfide (650 mmoles), 180 ml isopropanol, and 150 ml water. The solution was stirred at 25° or 37°C for several hours (Fig. 2). The reaction yield was determined using a potentiometric titration of the Cl⁻ ions contained in weighed aliquots of the suspension.

Attachment of α -Amino Acid to Sulfonium Polymer

To the preceding sulfonium polymer suspension containing about 100 meq —CH₂S⁺(CH₃)₂Cl⁻ groups, a mixture of 100 meq NaOH and an amount between 100 and 200 mmoles α -amino acid (sodium salt) dissolved in about 100 ml water was added. After refluxing for variable times (Table I), the resin was filtered and refluxed for 4 hr in 4M NH₄OH to remove remaining dimethyl sulfide. The polymer was filtered and carefully washed with water, 1M HCl solution, water, and 1M NaOH solution. Then, it was several times suspended in water and filtered until the filtrate was found neutral. Finally, the polymer was dried under vacuum.

Chlorosulfonated Polystyrene

Crosslinked polystyrene beads (200–400 mesh), 30 g, were swelled for 1 hr at 25°C in 250 ml methylene chloride. Then, a mixture of 200 ml nitromethane and 180 ml chlorosulfonic acid was added. The solution was stirred at 40°C for 6 hr. The crude polymer was filtered and washed carefully with nitromethane and acetone. Then, the resin was washed again for a short time with water and methanol. Finally, it was dried under vacuum. The number of chlorosulfonyl groups —SO₂Cl was determined according to the following method: a weighed sample (200 mg) of resin was hydrolyzed at reflux for 24 hr in 50 ml aqueous 0.1M sodium hydroxide solution. After acidification, the Cl⁻ ions were titrated by a 0.1M silver nitrate solution using a silver indicating electrode.

Attachment of α -Amino Acid to Chlorosulfonated Polymer

Between 100 and 200 mmoles α -amino acid was dissolved in 400 ml of a 5:3 water–dioxane mixture by adding the minimum amount of 4M NaOH. The pH was measured and about 25 g of the preceding chlorosulfonated resin (100 meq

—SO₂Cl) was added to the mixture. The pH was maintained at its original value by 4M NaOH additions. The reaction was stopped when no further NaOH was required. The polymer was filtered and carefully washed with water-dioxane mixtures, water, and 1M NaOH solution. Then, it was suspended several times in water and filtrated until the filtrate was found neutral. Finally, the polymer was dried under vacuum.

Determination of α -Amino Acid Contents

The following procedure was used for both chlorosulfonated and chloromethylated polystyrene resins containing α -amino acid groups. Polymers were suspended for 24 hr in an aqueous 0.1M sodium hydroxide solution, filtered, washed with aqueous 0.01M sodium hydroxide solution, and then dried. Under these conditons, all the α -amino acid groups were converted into their sodium salts. A weighed portion (1 g) of the sodium salt of the polymer was suspended in 30 ml water. An acidic titration was carried out very slowly by small additions of aqueous 0.1M hydrochloric acid. The observed equivalent point occurs for the amino group neutralization.

Determination of Copper Contents

A weighed portion (50 mg) of a copper complex of a resin was decomposed for a few hours in 50 ml of aqueous 1M nitric acid. After filtration, 1 ml of the solution was poured into the following mixture: 20 ml water, 10 ml 10% tartaric acid solution, and 10 ml 10% hydroxylamine hydrochloride solution. The pH was fixed between 5 or 6 by dropwise additions of concentrated ammonia. Then, 10 ml of 10⁻³M bisquinolin solution in isoamyl alcohol was added. The water phase was discarded and the concentration of the copper(II)-bisquinolin complex was determined by spectroscopic measurements of the optical density at 547 nm (extinction coefficient $\epsilon_m = 7620$ l./mole).

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