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Ligand-Exchange Chromatography of Some Amino Acids on Co(II)-Loaded CMDAE-Sporopollenin Resin

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TECHNICAL NOTE

Ligand-Exchange Chromatography of Some Amino Acids on Co(II)-Loaded CMDAE-Sporopollenin Resin

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ABSTRACT

Sporopollenin obtained from *Lycopodium clavatum* has been modified as an ligand-exchange material. In this study, the possibility of using sporopollenin as a ligand exchanger in the chromatographic separation of amino acids is examined. Since sporopollenin has important advantages (it is stable to chemicals and has a constant mesh size), it can be used as a column packing material. By first treating sporopollenin with 1,2-diaminoethane and then with bromoacetic acid, carboxylated diaminoethylsporopollenin (CMDAE-sporopollenin) was obtained. This new resin was loaded with Co(II) transition element and used as a ligand-exchange material in the separation of amino acids. Using the ligand-exchange technique on Co(II)-loaded carboxylated diaminoethylsporopollenin is a suitable chromatographic method for the separation of most common amino acids.

INTRODUCTION

Ligand-exchange chromatography has been studied by Helfferich from the fundamental work of Walton and Stokes (1, 2). It has been employed during the past decades with great success in separation processes. This principle has been utilized to separate amino acids from peptides and to concentrate and remove amino acids from salt solutions such as seawater (3).

The principle upon which ligand-exchange chromatography is based has a transition metal fixed on a solid support, and this solid sorbent can be used for the exchange of bound ligands of the metal. An exchange of the

ligands takes place between the external solution and the coordination shells of the metal ions in the resin. By using ligand exchange, ligands such as amines, purines, and primidine bases and amino acids can be removed from their media by the formation of complexes with the metal attached to the solid support (4, 5). An application of ligand-exchange chromatography is the resolution of optical isomers on chiral-bonded phases loaded with a transition metal (6).

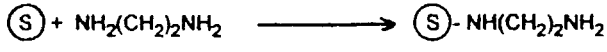
Sporopollenin, which is highly resistant to chemicals, occurs naturally as a component of spore walls. *Lycopodium clavatum* has a stable, cross-linked structure with an aromatic character that contains carbon, hydrogen, and oxygen with a stoichometry of $C_{90}H_{144}O_{27}$. Sporopollenin is produced by oxidative polymerization of carotenoids, and it leads to the proposed monomer structures of macromolecular stability. Therefore, very high column pressures can be applied without difficulty, and there are no problems because of swelling or collapse of pollen grains (7–9). Sporopollenin obtained from *L. clavatum* can be a suitable skeleton for the ligand-exchange resin structure.

This study was about the modification of *L. clavatum* as a ligand-exchanger. Ligand-exchange chromatography on cobalt-loaded functionalized sporopollenin can be a useful alternative method for the separation of amino acids.

EXPERIMENTAL

Materials. The resin was *L. clavatum*, 20 μm mesh, from BDH Chemicals. The amino acids were purchased from Merck Chemical Corp. The columns (4 mm i.d. and 50 cm long), the fittings, and the peristaltic pump were supplied by Pharmacia Fine Chemicals. A Shimadzu UV-visible spectrophotometer (UV-160 A) was used as a UV monitor in the separation process. The pH was monitored with an Orion model SA-720 pH meter using a combined electrode.

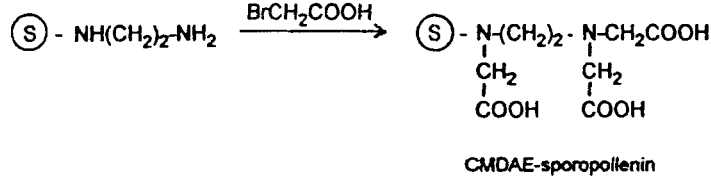
Preparation of DAE-sporopollenin. A suspension of sporopollenin from *L. clavatum* in dry toluene containing 1,2-diaminoethane was aminated by refluxing for 18 hours (10). The reaction is as follows;



where Ⓢ indicates sporopollenin.

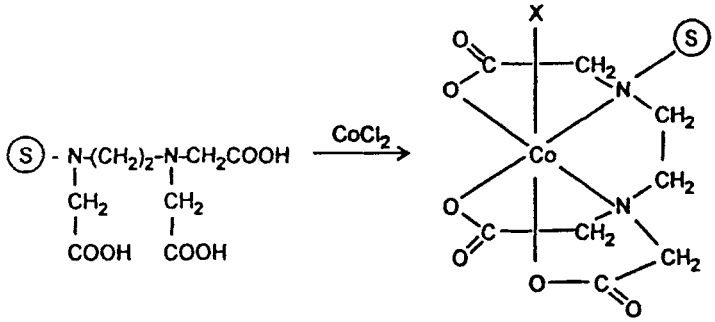
Preparation of CMDAE-sporopollenin. A suspension of DAE-sporopollenin was placed in a reaction vessel, and bromoacetic acid neutral-

ized by 2 M NaOH and 1 M $NaHCO_3$ was added to the resin. By stirring the suspension for 24-hours and washing it with water and dilute acetic acid, CMDAE-sporopollenin was obtained (10). The reaction is as follows:



CMDAE-sporopollenin

Preparation of Co(II)-Loaded CMDAE-sporopollenin. A suspension of CMDAE-sporopollenin was placed in a reaction vessel, and a 1 M $CoCl_2$ solution was added to the resin. The solution pH was adjusted to about 3.5. By adding 1 M $CoCl_2$ solution to the CMDAE-sporopollenin, cobalt cations are fixed to the resin matrix. The structure of the metal–ligand complex is as follows:



RESULTS AND DISCUSSION

Ligand-exchange chromatography has been a preferred method for analyzing amino acids, nucleosides, nucleic-acid bases, and related substances (10–12). The ligand-exchange packing exhibits good efficiency and permits rapid separation at moderate column operations.

In this study, new "CMDAE-sporopollenin" was prepared from *L. clavatum*, diaminoethane, and bromoacetic acid. Ethylenediamine groups have a very stable structure with little possibility of dissociating. They are very suitable functional groups for a ligand exchanger matrix. These functional groups hold cations strongly.

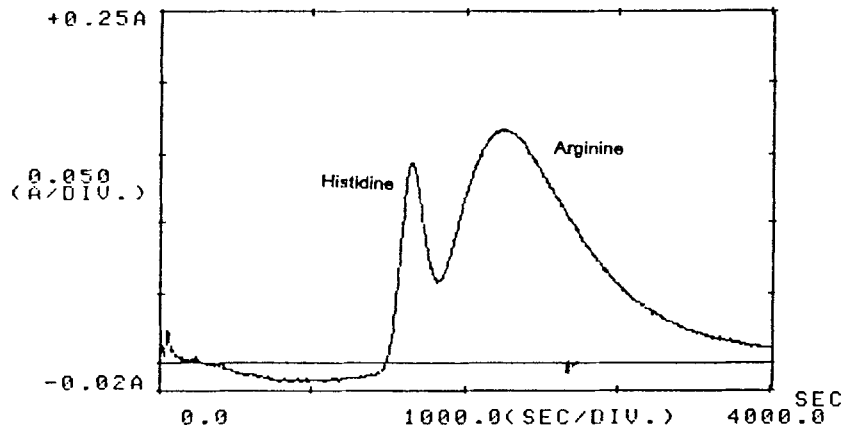


FIG. 1 Elution of amino acids with 1 M NH_4OH in acetonitrile–water mixture (30:70). Column = 0.4 × 50 cm, V = 15 mL, flow rate = 0.22 mL·min^{-1}, λ = 225 nm.

For chromatographic separations, various amounts of amino acid solutions were placed on the top of the column which was filled with cobalt-loaded CMDAE-sporopollenin. Mixtures of amino acids were separated with CMDAE-sporopollenin in about 50–60 minutes as indicated in Figs. 1 and 2. The amino acids can be easily detected without using their derivative forms.

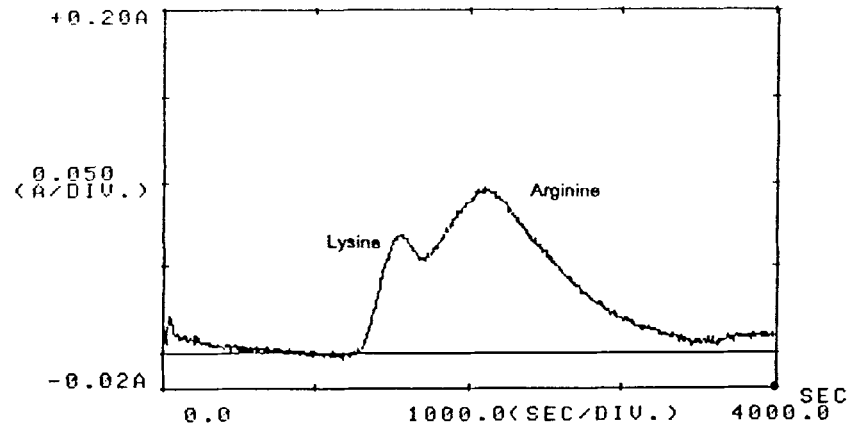


FIG. 2 Elution of amino acids with 1 M NH_4OH in acetonitrile–water mixture (30:70). Column = 0.4 × 50 cm, V = 17 mL, flow rate = 0.25 mL·min^{-1}, λ = 221 nm.

TABLE 1
Elution of Amino Acids

Amino acids	Peak	
	t (s)	V (mL)
Histidine (1×10^{-3} M, 50 μL)	1650	6.1
Arginine (6×10^{-2} M, 150 μL)	2250	8.4
Lysine (1×10^{-2} M, 50 μL)	1553	6.7
Arginine (4×10^{-2} M, 150 μL)	2097	9.0

These separations were carried out with a 0.4 × 30 cm column by linear elution chromatography using a carrier of 1 M NH_4OH with a acetonitrile–water mixture (30:70). A single eluant was employed, and no regeneration of the column was required following the analysis. Consequently, the column was ready for another separation. These ligand-exchange packings undergo no detectable change when the pH of the solution and the concentration of the eluant are changed. (See Table 1.)

The Chelex-100 ligand-exchange technique has some disadvantages. The elution of histidine and arginine from cobalt-Chelex-100 was not quantitative. Complete displacement of the amino acids from cobalt-Chelex-100 was accomplished with 6 M ammonium hydroxide, which causes a slight leakage of the metal, but metal leakage has also been observed during elution with 3 M ammonium hydroxide (4). On the other hand, the elution of such basic amino acids as histidine and arginine from Co(II)-CMDAE-sporopollenin was quantitative, and metal leakage from the column was not observed. To test for metal leakage, a 6 M ammonia solution was passed through the column and the ammonia-eluted material was then tested with 1-nitroso-2-naphthol.

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