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Note

Rapid and highly sensitive amino acid analysis using a precolumn packed with cation-exchange resin

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Ion-exchange chromatography has long been used as a standard method of amino acid analysis. Many investigators have made efforts to improve the hard ware to attain rapid and highly sensitive analyses of amino acids. Thus, improvements of pump, ion-exchange resin, narrow-bore column, detector with small-volume flow cell and information processing computer have made it possible to perform amino acid analysis in the subnanomole range within 1 h.

For highly sensitive assay of amino acids, a fluorescence detection system with *o*-phthalaldehyde (OPA) is most suitable[1,2]. This method is employed in some commercial instruments. It allows us to perform amino acid analysis at the level of a few picomoles, but impurities having relatively high fluorescence disturb the assay of amino acids such as lysine, tryptophan and arginine[1,2]. These impurities are called the "ammonia plateau", and are not easily removed completely by distillation and by passing through a column of ion-exchange resin as they are contained in water. Oda *et al.*[3] reported that water distilled twice in a dust-free room enabled a clear separation of amino acids; however, such a room is not always available.

This communication deals with a high speed separation of amino acids within 30–35 min using a precolumn packed with cation-exchange resin situated between the main pump and sample injector.

EXPERIMENTAL

Chemicals

The amino acid mixture, tryptophan and OPA were purchased from Wako (Osaka, Japan). Sodium citrate, sodium hydroxide, perchloric acid, Brij 35, *n*-propanol, ethanol and boric acid were of special reagent grade for amino acid analysis (Nakarai, Kyoto, Japan). Cation-exchange resin for amino acid analysis, MCI GEL CK10F (7 μm), was purchased from Mitsubishi (Tokyo, Japan). AG 50W-X2 (200–400 mesh) and AG 50W-X16 (200–400 mesh) were obtained from Bio-Rad Labs. (Richmond, CA, U.S.A.) and DC-3 from Durrum (Palo Alto, CA, U.S.A.). Once-deionized and distilled water was used; its electric conductivity was less than 10 μS/m.

Equipment

The apparatus for amino acid analysis was obtained from Shimadzu (Kyoto,

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Japan). The liquid chromatograph was a Model LC-3A with step gradienter (SCR-1A). A column for amino acid analysis (150 × 4.0 mm I.D.) was packed by the slurry packing method with MCI GEL CK 10F in the first buffer containing 30% glycerine. The pressure was set at 21.6 MPa (220 kg/cm^2).

Procedure

The elution buffer and the elution program are shown in Table I. After elution with the fifth buffer, the column was recycled by use of the sixth buffer for 3 min and the first buffer for 15 min. The flow-rate was 0.6 ml/min and the pressure was 10.8–11.8 MPa (110–120 kg/cm^2) at 55°C.

TABLE I

BUFFER SYSTEM AND TIME PROGRAM FOR AMINO ACID ANALYSIS

Step	*pH*	*Buffer*	*Time (min)*
1st	3.25	0.067 *M* sodium citrate–perchloric acid in 7% 1-propanol	0
2nd	4.25	0.067 *M* sodium citrate–perchloric acid	6
3rd	8.0	0.2 *M* sodium citrate	4
4th	11.0	0.2 *M* sodium citrate–sodium hydroxide	18
5th	14	0.2 *M* sodium hydroxide	3
6th	2.80	0.067 *M* sodium citrate–perchloric acid in 7% 1-propanol	3
Recycle		1st buffer	15

OPA reagent was prepared by mixing 400 mg of OPA in 7 ml of ethanol with 500 ml of 0.5 *M* boric acid–NaOH buffer (pH 10.5), 1 ml of 2-mercaptoethanol and 5 ml of 10% Brij 35. The reagent was pumped into the reaction coil (2 m, CRB-1B) at 0.6 ml/min with a minipump (PRR-1A) and reacted with amino acids at 55°C.

The eluates were monitored by fluorescence at 450 nm with excitation at 350 nm using a fluorescence photometer RF-530. The results were displayed on a Model C-RIA recording integrator.

RESULTS AND DISCUSSION

When amino acid analysis was performed without a precolumn, a large amount of impurities was eluted with the fourth buffer, and amino acids eluted with the same buffer were not easily analyzed below 100 pmoles (Fig. 1).

For elimination of these impurities, we tried a small precolumn. A stainless-steel column (250 × 4.0 mm I.D.) was packed with cation-exchange resin by the slurry packing method, and set between the main pump and the sample injector. Fig. 2 shows an example of a chromatogram of standard amino acids obtained with a precolumn packed with AG 50W-X16. As can be seen, the ammonia plateau can be shifted to behind the arginine peak and the baseline becomes flat. Accordingly, the peaks of lysine, tryptophan, ammonia and arginine were clearly separated and their amounts were assayed precisely.

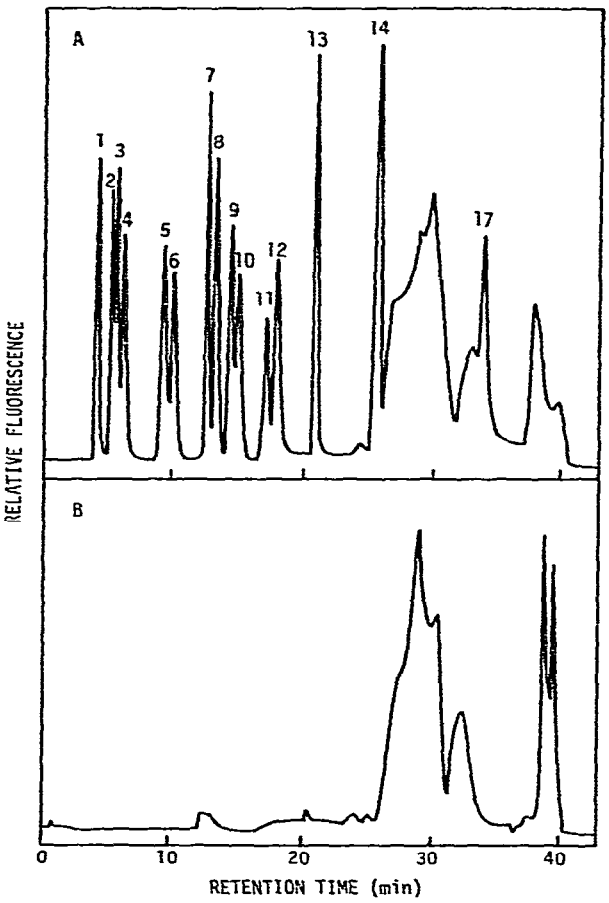

Fig. 1. Chromatogram of a mixture of standard amino acids and baseline blank without the precolumn. Amino acid analysis was performed as described in the text. A, A mixture of standard amino acids at a concentration of 250 pmoles; B, baseline blank. Peaks: 1 = Asp; 2 = Thr; 3 = Ser; 4 = Glu; 5 = Gly; 6 = Ala; 7 = Val; 8 = Met; 9 = Ile; 10 = Leu; 11 = Tyr; 12 = Phe; 13 = His; 14 = Lys; 17 = Arg.

Since the column pressure of this precolumn was 1.96–2.94 MPa (20–30 kg/cm^2) at 9.9 ml/min, pressure drop was negligible at 0.6 ml/min. Under these conditions, the analysis was completed within about 35 min and the total running time for one cycle was 50 min.

Since a precolumn of cation-exchange resin placed between the main pump and sample injector was effective in removing the ammonia plateau, we checked the efficiency of some cation-exchange resins, namely, two Dowex resins, AG 50W-X2 (200–400 mesh) and AG 50W-X16 (200–400 mesh), and Durrum ammonia-trapping gel, DC-3. Each resin was packed as described above. These precolumns were compared with each other according to the shape of their baselines, which are a clear reflection of the elimination of impurities. Among the resins examined, AG 50W-X16 (Fig. 2A) was the best, followed by AG 50W-X2 and then DC-3. These results can be explained in terms of exchange capacity and resin particle size; the exchange capacity of AG 50W-X16 (2.6 mequiv./ml) is larger than that of AG 50W-X2 (0.7 mequiv./ml)

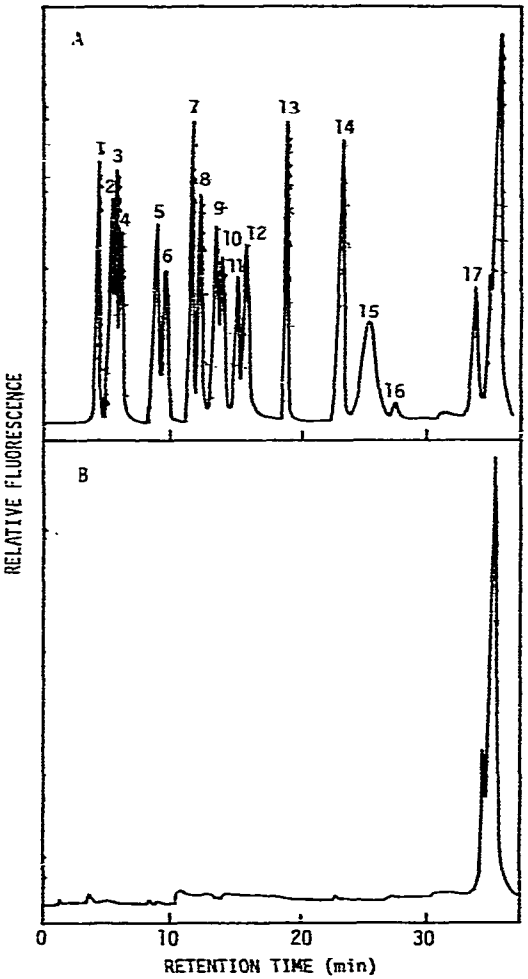

Fig. 2. Chromatogram of a mixture of standard amino acids and baseline blank with a precolumn of Dowex AG 50W-X16. Details as in Fig. 1. Peaks: 15 = Trp; 16 = NH_3.

and the particle size of Dowex resin (about 50 μm) is smaller than that of DC-3 (about 1 mm). Accordingly, the resins of smaller particle size and with high capacity packed in a small column are recommended for high speed amino acid analysis, although any cation-exchange resin commercially available might be used for the precolumn.

The described procedure should enable a high speed and highly sensitive amino acid analysis by the OPA method.

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