Journal of Chromatography, 148 (1978) 536–538
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CHROM. 10,493

Note

Ion-exchange chromatography of amino acids and aromatic amino acid derivatives by a single-column method

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(First received January 31st, 1977; revised manuscript received July 5th, 1977)

Amino acids in proteins have been analyzed by use of an automatic analyzer or an accelerated method[1,2]. However, these methods are not always sufficient for the determination of some amino acids and their derivatives which are constituents of chemically modified proteins and peptides. Several studies have been made of the analysis of iodotyrosine derivatives by liquid column chromatography[3–6]. Sokolovsky *et al.*[7] used an amino acid analyzer for the analysis of nitrotyrosine derivatives. The analyses of these special amino acids have become increasingly important for the studies of protein and peptide chemistry.

In this work, 3-iodotyrosine (MIT), 3,5-diiodotyrosine (DIT), 3,5-dibromotyrosine (DBT), 3-nitrotyrosine (MNT), 3,5-dinitrotyrosine (DNT), 3-aminotyrosine (AT) and 4-nitrophenylalanine (pNP) were analyzed by a single-column system in the presence of all of the amino acids which are usually found in protein hydrolyzates. Moreover, the amino acids usually found can be quantitated simultaneously from the same chart. This method does not need any special devices or special column packing materials, but only an amino acid analyzer.

MATERIALS AND METHODS

The standard amino acids (acidic, neutral and basic amino acids) were purchased from Ajinomoto, Tokyo, Japan. MIT, DIT and DBT were obtained from Tokyo Kasei Chemical Industry, Tokyo, Japan. MNT, DNT and pNP were synthesized by the method of Greenstein and Winitz[8]. AT was prepared from MNT by the method of Sokolovsky and co-workers[10,11].

The analysis was performed in a Hitachi KLA-2 amino acid analyzer in which the separation column and the eluting buffers for separating the aromatic amino acid derivatives were modified. The column (650 × 9 mm) was packed with Amberlite CG-120 Type III (400 mesh). The particle size of the resin was homogenized by decantation to obtain a well resolved chromatogram. The column chromatography was carried out at 50°. The composition of eluting buffers is described in Table I.

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TABLE I

COMPOSITION OF BUFFERS

Buffer	*Composition*	*pH*
I	0.2 *N* Sodium citrate 0.5 % Thiodiglycol 0.2 % Brij-35 0.01 % Caprylic acid	3.25
II	0.2 *N* Sodium citrate 0.5 % Thiodiglycol 0.2 % Brij-35 0.01 % Caprylic acid	4.25
III	0.01 *M* Borax–HCl 0.8 *N* Sodium citrate 0.2 *M* NaCl	7.70

Buffers I and II have the same compositions as those used previously. The flow-rate of the buffers was 30 ml/h. Ninhydrin solution, prepared as described previously[1], was pumped at a flow-rate of 15 ml/h.

RESULTS AND DISCUSSION

A chromatogram of aromatic amino acid derivatives and ordinary amino acids obtained by this single-column method is shown in Fig. 1. The amount of each amino acid was 0.2 μmole, except for proline which was 0.4 μmole. The eluting buffer was changed immediately after the elution of valine from buffer I to buffer II, and after leucine from buffer II to buffer III. The time required for a complete analysis is about half of that by the original method[1], without serious losses in resolution of the amino acids. The tyrosine derivatives were well separated from the other amino acids, but pNP was not separated from lysine. Lysine and pNP could be separated and quantitated by changing slightly the pH or ionic strength of buffer III, whence, however, some tyrosine derivatives overlapped with the other amino acids. The condition for buffer III described in Table I was best for the resolution and quantification of the tyrosine derivatives.

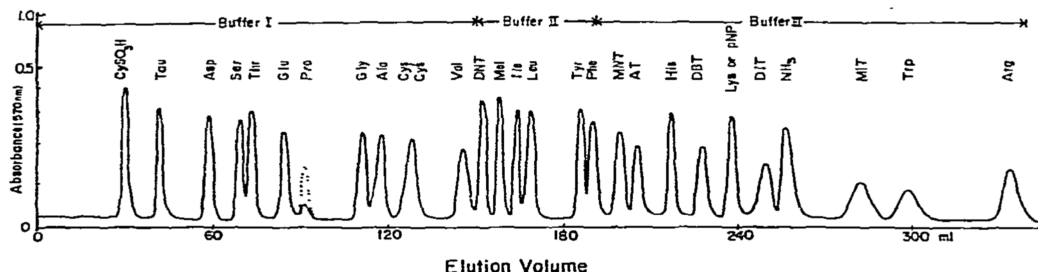

Fig. 1. Chromatogram of aromatic amino acid derivatives in the presence of ordinary amino acids. Column: Amberlite CG-120 (650 × 9 mm). Absorbance at 570 nm (———), and at 440 nm (- - -) for proline.

The amino acids were quantitated by the usual $H \times W$ method (net peak height $\times$ width of peak at half the net height). The $H \times W$ values shown in Table II are average values from six analyses of the standard amino acid mixture. The coefficient of variation for each $H \times W$ value is less than 5.1% as shown in Table II.

TABLE II

$H \times W$ VALUES OF AROMATIC AMINO ACID DERIVATIVES

Amino acid	$H \times W$*	*Coefficient of variation*
DNT	3.64	2.6
MNT	3.74	2.7
AT	2.60	4.7
DBT	3.16	2.9
DIT	3.17	5.1
MIT	3.13	4.7

* Per 0.2 μmole; each value is the average from six analyses of a standard mixture.

Hamilton[9] reported a single-column method for the analysis of some tyrosine derivatives on ion-exchange resin. However, this method needs *ca.* 21 h, and DNT and methionine are co-eluted, as are MIT and tryptophan. Sokolovsky and co-workers[10,11] analyzed AT on the short column of a two-column amino acid analyzer at 25° or 27°, but did not separate the other ordinary amino acids. Our method requires *ca.* 11 h for a complete analysis, and this analysis time may be reduced by using a higher flow-rate of the buffers, if the analyzer can withstand the pressure. Since this is a single-column system and the quantity of sample required is half of that for a two-column amino acid analyzer, this method will be useful for studies of proteins, peptides and amino acids where only small quantities of the sample are available.

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