CHROM. 5113

Detection of several non-protein amino acids in the presence of protein amino acids

Since there are indications in the literature of possible confusion of some non-protein amino acids with protein amino acids, we wish to present evidence that the following non-protein amino acids are detectable and simultaneously distinguishable from protein amino acids utilizing a Beckman amino acid analyzer: α-amino butyric acid, β-alanine, taurine, norvaline, sarcosine, norleucine, homocystine, betaine, hydroxyproline, and L,L-α,ε-diaminopimelic acid. Urea was also run. We wish to present new data for *cis*-Δ^4-dehydrolysine, and 3,5-diaminohexanoic acid which appear close to lysine in the amino acid analysis chromatogram.

Experimental

a. *Materials.* The Beckman Model 120 amino acid analyzer was used in these studies. Basic column buffer pH 5.25 and column length 20 cm. Neutral-acidic column length 50 cm, pH 3.25, followed by pH 4.30. The temperature was 55.5°. Flow rate: 1 ml/min. The following is a list of compounds and the companies from which they were purchased: D,L-norvaline from K & K Laboratories, D,L-methionine from Mann Research Laboratories, D,L-homocystine, L-lysine, valine, threonine, L-histidine·HCl, β-alanine, urea, leucine, α-amino butyric acid, glutamic acid, taurine, betaine·HCl, sarcosine·HCl, hydroxyproline, and phenylalanine from Nutritional Biochemicals Corporation.

It is a pleasure to thank Dr. LIN TSAI at Dr. Thressa Stadtman's Laboratory at the National Institutes of Health for the 3,5-diamino hexanoic acid·2 HCl which was used as a standard in these studies.

cis-Δ^4-Dehydrolysine was prepared according to the literature[1]. L,L-α,ε-Diaminopimelic acid was prepared according to the literature[2]. The N-succinyl-L,L-α,ε-diaminopimelic acid from which the L,L-α,ε-diaminopimelic acid was prepared was a gift from CHARLES GILVARG. It was hydroloyzed and the resulting L,L-α,ε-diaminopimelic acid was isolated on a Dowex-50 Column, similar to work described previously[2].

b. *Chromatography.* Solutions of the standard compounds were made so that 1–60 mμ moles of each amino acid were applied to the appropriate "basic" or "neutral-acidic" columns after adjusting the pH of the amino acid solutions to pH 2. Chromatograms of the compounds were run singly as well as in mixtures.

Chromatograms of these compounds were also run at the Worthington Biochemical Corporation under the direction of Dr. A. L. BAKER and Mr. V. WORTHINGTON.

Results

The color constants for the non-protein amino acids as well as protein amino acid controls are indicated in Table I. Fig. 1 shows the elution profiles of the non-protein amino acids as well as the protein amino acids which are closest to them.

Thus, we especially note the separation between norvaline, L,L-α,ε-diaminopimelic acid, isoleucine, methionine, betaine, and leucine; homocystine and β-alanine; 3,5-diamino hexanoic acid, *cis*-Δ^4-dehydrolysine, and lysine.

TABLE I

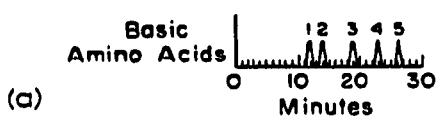
AMINO ACID ANALYZER COLOR CONSTANTS FOR AMINO ACIDS

The color constant is calculated with the use of the equation $Hw = CD$, where H is the height of the peak measured with the use of the Beckman expanded absorbance scale (4–5 mV over 10 in. span,) w is the number of dots in the peak above the halfheight, D is the number of μmoles of amino acid applied to the column, and C is the color constant. Phenylalanine or lysine may be considered standards for comparison to the literature. The 5700 Å data was used except for hydroxyproline and proline for which 4400 Å data was used .The conditions for the chromatographic runs are described in the text and are typical for the model of amino acid analyzer used.

Amino acid	*Color constant*
Homocystine	69
β-Alanine	11
Methionine	48
Urea	4
Taurine	25
Betaine	15
Sarcosine	5
Hydroxyproline	7
Phenylalanine	62
α-Amino-*n*-butyric acid	72
L,L-α,ε-Diaminopimelic acid	42
Isoleucine	21
Lysine	65
cis-Δ^4-Dehydrolysine	49
3,5-Diaminohexanoic acid	15
Norvaline	57
Norleucine	45

Discussion

Data showing the positions of 147 compounds in amino acid chromatograms have appeared in the literature[3] including non-protein amino acids such as *meso*-α,ε-diaminopimelic acid, norleucine, homocystine, hydroxyproline, taurine, urea, sarcosine, and β-alanine. However, not all of these compounds have been run concur-



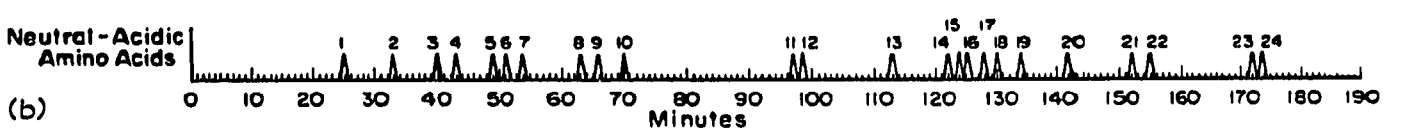


Fig. 1. Amino acid analyzer chromatograms of nor-protein and nearest protein amino acids. The distances between peaks are measured between apexes. Conditions are described in the text. (a) 1 = Tryptophan, 2 = 3,5 diaminohexanoic acid, 3 = lysine, 4 = *cis*-Δ^4-dehydrolysine, 5 = histidine. (b) 1 = Cysteic acid, 2 = urea, 3 = taurine, 4 = aspartic acid, 5 = methionine sulfone, 6 = hydroxyproline, 7 = threonine, 8 = glutamic acid, 9 = sarcosine, 10 = proline, 11 = α-amino-*n*-butyric acid, 12 = cystine, 13 = valine, 14 = betaine, 15 = methionine, 16 = L,L-α,ε-diamino pimelic acid, 17 = norvaline, 18 = isoleucine, 19 = leucine, 20 = norleucine, 21 = tyrosine, 22 = phenylalanine, 23 = homocystine, 24 = β-alanine.

rently. Often, compounds in the presence of different sets of compounds have been found to have non-reproducible chromatographic patterns. However, we have found that it is possible to reproducibly separate fifteen non-protein amino acids in the presence of the fourteen chromatographically nearest protein amino acids. There was reproducibility of time of appearance of the apexes of the amino acid analyzer peaks for several different runs of different sets of all these compounds as well as for when they were all run concurrently. We also determined the positions of several new amino acids whose positions have not previously been determined.

Leucine and norleucine have been shown to be separable[4,5]. However, difficulties were found in the separation of methionine and norvaline, and L,L- and *meso*-mixtures of α,ε-diaminopimelic acid, and also isoleucine and leucine[6,7].

Homocystine has been chromatographed[6-8] using several conditions. Different extents of separation were obtained depending on whether 50 cm or 150 cm columns were used. In one instance[6] homocystine was not readily separable from phenylalanine and tyrosine and in another instance[8] homocystine was not separable from β-alanine. However, the figure indicates that chromatographic separations are possible amongst all these compounds.

The diamino acids 3,5-diaminohexanoic acid[9] and *cis*-Δ^4-dehydrolysine[1] resemble lysine in structure. Whereas difficulty may have been expected in their chromatographic separation, in fact, they are distinguishable as shown.

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