CHROM. 4321

A quick separation of the ε-N-methyl-lysines using the amino acid analyzer*

In view of the demonstration of the presence of ε-N-methyl-lysine in flagellar proteins[1], histones[2-6] and plasma[7], ε-N-dimethyl-lysine** in histones[3-5] and ε-N-trimethyl-lysine** in histones[4] and cytochrome *c*[8], it is desirable that a simple and quick method for the identification and estimation of these compounds be available. The standard amino acid analyzer systems do not separate the methyl-lysines, therefore various modifications of these have been used[2-8]. We have found that a short column of Aminex A-5 resin (Bio-Rad Laboratories, Richmond, Calif.) eluted with a pH 6.48 buffer at 25° is ideal for the separation of the methyl-lysines since it separates them from each other and from all of the protein amino acids.

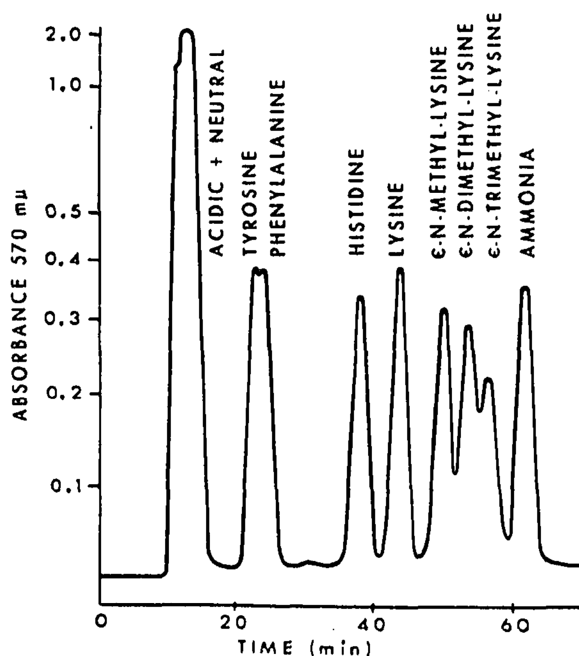

Fig. 1. Chromatography of the ε-N-methyl-lysines in the presence of a calibration mixture on a 15 × 0.9 cm column of Aminex A-5 resin eluted with 0.35 *N* sodium citrate, pH 6.48, at 25°. Buffer flow rate, 68 ml/h. The mixture contained, except for ammonia, 0.25 μmoles of each component.

The separations achieved with a Beckman model 120B amino acid analyzer are illustrated in Figs. 1 and 2. Fig. 1 is the curve obtained when the eluent was pumped through at the normal operation flow rate of 68 ml/h[9], while Fig. 2 is the curve obtained at a flow rate of 34 ml/h. It is seen that the slower system (120 min) gives a complete separation of all the basic components of the mixture, but that an adequate separation is also obtained with the faster (60 min) system. Both curves are readily reproducible since the positions of emergence of the peaks are not significantly altered

* Supported by a grant from the Medical Research Council of Canada.
** Though these terms are in common usage, the correct names are: ε-N,ε-N-dimethyl-lysine and ε-N,ε-N,ε-N-trimethyl-lysine.

by minor variations of the pH of the eluting buffer. If a pH 5.28 eluent is used, ε-N-dimethyl-lysine and ε-N-trimethyl-lysine emerge together, but all peaks are still completely separated from each other, with histidine appearing after ammonia. It is worth noting that the 440/570 mμ absorbance ratios of lysine and ε-N-methyl-lysine are similar, as are those of ε-N-dimethyl-lysine and ε-N-trimethyl-lysine, but that the ratios for the two pairs are very different.

A complete summary of the color constants and elution times obtained for the Aminex A-5 resin system as well as other systems appears in Table I. It should be

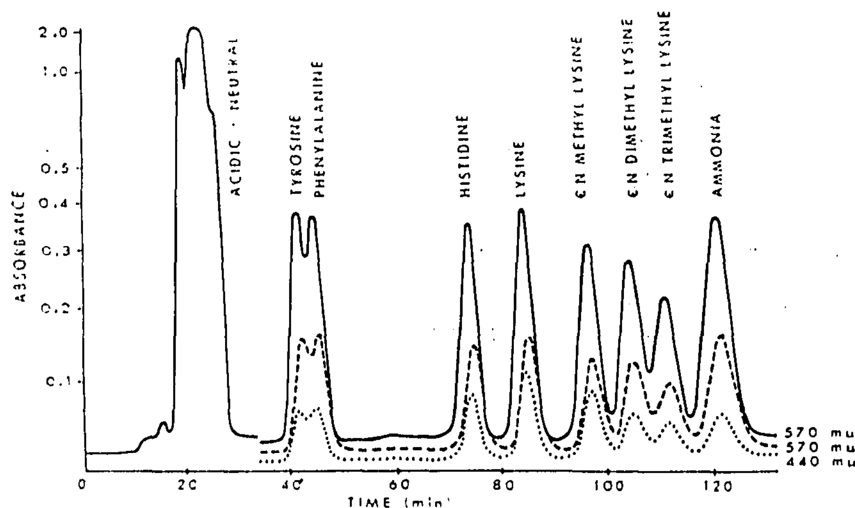

Fig. 2. Same as in Fig. 1, except a buffer flow rate of 34 ml/h.

TABLE I

DATA FROM THE BECKMAN AMINO ACID ANALYZER

Compound	*Eluting buffer, 0.35 N sodium citrate; Ninhydrin flow rate, 34 ml/h*					
	Eluent pH 5.28, 68 ml/h; 57°				*Eluent pH 6.48[a], 34 ml/h; 25°*	
	0.9 × 50 cm, AA-15 resin		*0.9 × 7 cm, PA-35 resin*		*0.9 × 15 cm, Aminex A-5 resin*	
	Time (min)	*Constant[b]*	*Time (min)*	*Constant[b]*	*Time (min)*	*Constant*
L-Lysine	164	22.06	25	23.80	84	43.5
ε-N-Methyl-L-lysine[10]	178	20.15	25	21.02	97.5	39.7
ε-N-Dimethyl-L-lysine[c]	178	19.48			105	39.2
ε-N-Trimethyl-L-lysine[d]	159	18.48			111.5	35.5
DL-Homolysine[13]	228	22.77	33	24.77		

[a] Prepared by adding NaOH to pH 5.28 buffer.
[b] Mean of five determinations for different amounts. Reproducibility was within ± 3 %.
[c] Solution prepared by refluxing α-N-acetyl-ε-N,ε-N-dimethyl-L-lysine·$2H_2O$[11] in 2 *N* HCl for 1.5 h.
[d] Solution prepared by heating α-N-benzoyl-ε-N,ε-N,ε-N-trimethyl-L-lysine gold salt[12] in 6 *N* HCl in a sealed tube at 110° for 72 h.

noted that the variation of the constants obtained with different systems is significant enough not to be ignored in calculations.

Department of Biochemistry, University of Ottawa, Ottawa 2, Ont. (Canada)

JOHN H. SEELY
SISTER R. EDATTEL
N. LEO BENOITON*

1 R. P. AMBLER AND M. W. REES, *Nature*, 184 (1959) 56.
2 K. MURRAY, *Biochemistry*, 3 (1964) 10.
3 W. K. PAIK AND S. KIM, *Biochem. Biophys. Res. Commun.*, 27 (1967) 479.
4 K. HEMPEL, H. W. LANGE AND L. BIRKOFER, *Naturwiss.*, 55 (1968) 36; *Z. Physiol. Chem.*, 349 (1968) 603.
5 W. C. STARBUCK, C. M. MAURITZEN, C. W. TAYLOR, I. S. SAROJA AND H. BUSCH, *J. Biol. Chem.*, 243 (1968) 2038.
6 R. J. DELANGE, D. M. FAMBROUGH, E. L. SMITH AND J. BONNER, *J. Biol. Chem.*, 244 (1969) 319.
7 T. L. PERRY, S. DIAMOND AND S. HANSEN, *Nature*, 222 (1969) 668.
8 R. J. DELANGE, A. N. GLAZER AND E. L. SMITH, *J. Biol. Chem.*, 244 (1969) 1385.
9 D. H. SPACKMAN, W. H. STEIN AND S. MOORE, *Anal. Chem.*, 30 (1958) 1190.
10 L. BENOITON AND L. BERLINGUET, *Biochem. Prep.*, 11 (1966) 80.
11 L. BENOITON, *Can. J. Chem.*, 42 (1964) 2043.
12 J. H. SEELY AND N. L. BENOITON, *Can. J. Biochem.*, to be published.
13 J. H. SEELY AND L. BENOITON, *Can. J. Biochem.*, 46 (1968) 387.

Received August 11th, 1969

* Associate of the Medical Research Council of Canada.

CHROM. 4324

A new accelerated fully automated system for amino acid analysis by ion-exchange chromatography

In a recent paper we reported on the development of a chromatographic system which separates the amino acids usually present in acidic protein hydrolysates within 63 min[1]. This was mainly achieved by improved column technologies and reduction of extra column contributions to band spreading in fittings and analytical system.

Since then, an improved resin has been developed for this system and precision data have been generated. Also, a final manifold for the peristaltic valve and pump has been assembled and evaluated.

Improved resin

The original resin used in the system tended to pack down during the first three runs and the top fittings of the columns had to be adjusted in order to avoid any dead space detrimental to resolution. The back pressure in the column for the separation of the acidic and neutral amino acids reached 600 p.s.i.