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A modified two-column procedure for the analysis of the basic amino acids found in elastin, collagen and antibiotics

The analysis of most protein hydrolysates can be accomplished satisfactorily using the accelerated two-column system[1,2]. However, resolution of the amino acids from hydrolysates of elastin, collagen, and various antibiotics, with their unusual complement of basic amino acids, cannot be achieved by this procedure. A modified system[3] devised for the analysis of amino acids found in plant hydrolysates likewise will not resolve all of the basic amino acids found in elastin and collagen. This system also suffers from the disadvantage of requiring a column and buffer other than those used in routine analysis. It was our intent to devise a rapid and simple method that could be used when necessary to resolve these unusual amino acids, and at the same time would utilize the same column and buffers as the standard procedure.

Methods

A Beckman model 116 amino acid analyser equipped with an automatic buffer-change module for both the long (acidic and neutral) and the short (basic) column was used for these experiments. The short column, 0.9 × 23 cm, was packed with PA-35 resin to a column height of 11 cm. Flow rates were 50 ml/h for the buffer and 20 ml/h for the ninhydrin reagent. Water jacket temperature was maintained at 55° throughout the run.

All reagents and buffers were prepared as described in the Beckman manual. Distilled water used in the preparation of the buffers was redistilled over sulfuric acid to remove ammonia. The short column was equilibrated with 0.20 *M* sodium

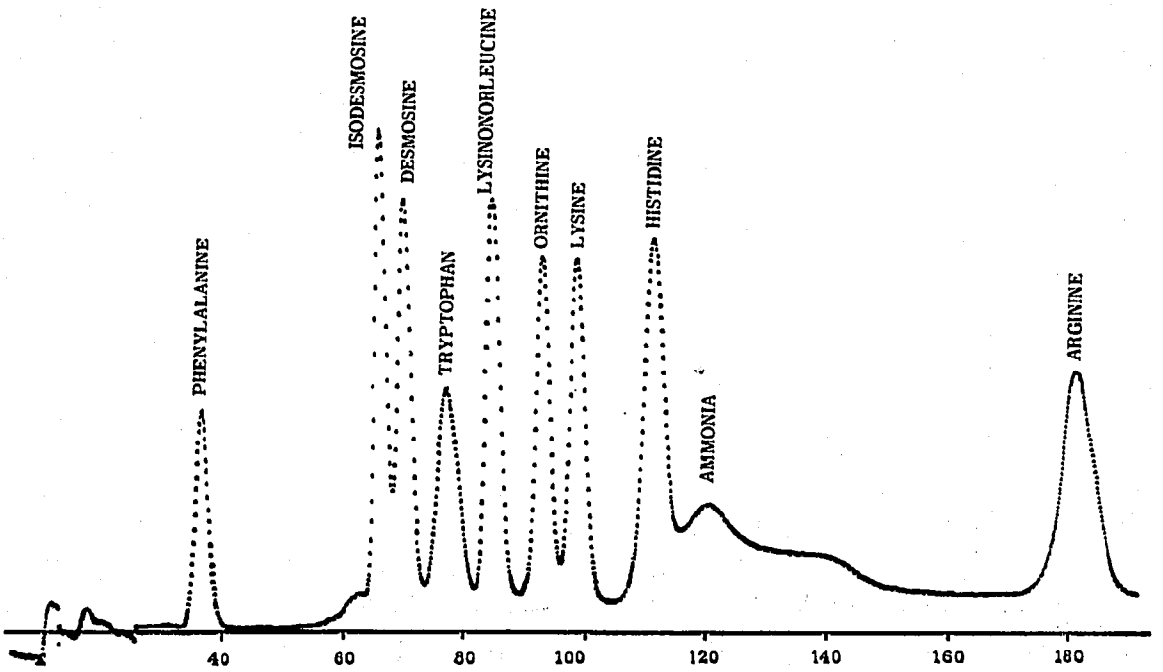

Fig. 1. Chromatogram of a synthetic mixture of basic amino acids using the procedure as described in the text.

citrate, pH 4.30, prior to the start of the run. Elution was started with this buffer and switched after 25 min to 0.35 *M* sodium citrate buffer, pH 5.25.

Results and discussion

Fig. 1 shows a chromatogram of a synthetic mixture of amino acids. Retention times for these and for other amino acids not shown on the chromatogram are listed in Table I. Although some of the amino acids listed in Table I have the same retention

TABLE I

PEAK ELUTION TIME OF BASIC AMINO ACIDS FROM A 0.9 × 11 CM PA-35 RESIN COLUMN

Amino acid	*Retention time (min)*
Tyrosine	33
Phenylalanine	37
Isodesmosine	66
γ-Amino butyric acid	66
Desmosine	70
Tryptophan	77
Merodesmosine	78
Hydroxylysine	83
Lysinonorleucine	84
Ornithine	93
Diamino butyric acid	98
Lysine	99
Diaminopropionic acid	107
Histidine	111
Ammonia	118
Arginine	182

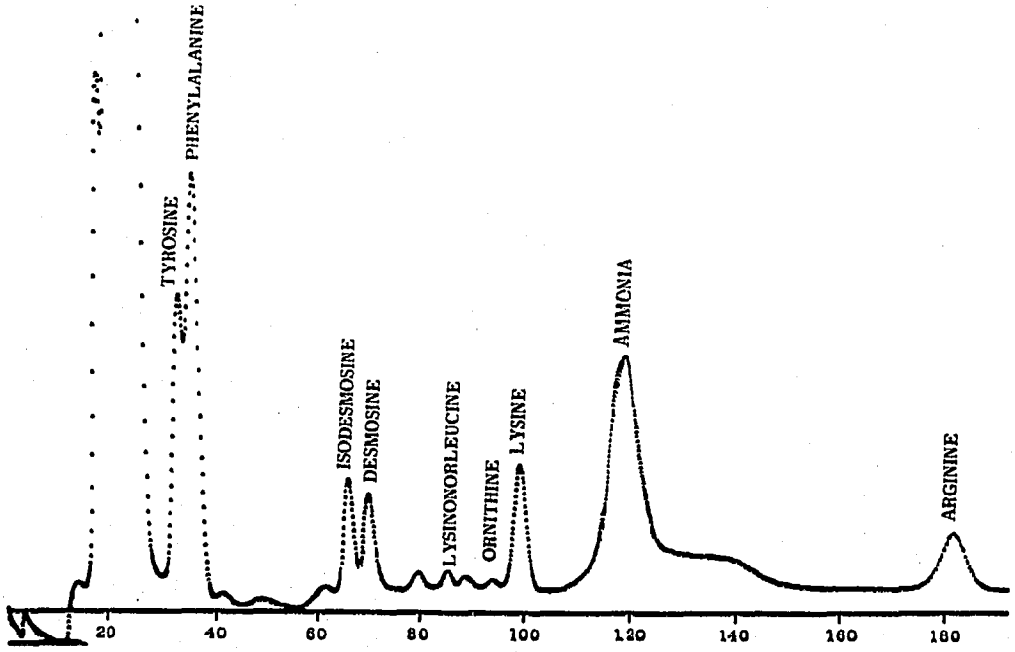

Fig. 2. Chromatogram of a 24-h hydrolysate of aortic elastin from 3 week old chicks. Procedure used is described in the text.

times, this has not created difficulties, since none of the proteins or antibiotics that we have encountered have contained two unresolvable amino acids.

Elastin has an unusual complement of basic amino acids, some of which are present in very small amounts. The only method of resolution of these amino acids has been a single-column gradient elution system[4]. Fig. 2 shows a chromatogram of the basic amino acids from a hydrolysate of purified chick aortic elastin, obtained with our modified two-column system. As can be seen in this chromatogram, the basic amino acids contained in the elastin hydrolysate have been well resolved. Further, because this system allows one to increase the size of the sample introduced onto the basic column without affecting the analysis of the acidics and neutrals, accurate measurement of the small basic peaks normally obtained becomes possible.

Increasing the length of the basic column to 11 cm increases the time required for an amino acid run using the standard procedure. Retention times in minutes for standard amino acids are: tryptophan, 31; lysine, 46; histidine, 55; ammonia, 68; and arginine, 104.

Difficulty may be encountered if the distilled water used in making the buffers is contaminated with ammonia. Such contamination will cause either a large-skewed ammonia peak or a dramatic increase in the baseline.

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1 D. H. SPACKMAN, W. H. STEIN AND S. MOORE, *Anal. Chem.*, 30 (1958) 1190.
2 R. W. HUBBARD, *Biochem. Biophys. Res. Commun.*, 19 (1965) 679.
3 R. D. DURBIN, G. F. PEGG AND M. STRMECKI, *J. Chromatog.*, 28 (1967) 429.
4 E. J. MILLER AND K. A. PIEZ, *Anal. Biochem.*, 16 (1966) 320.

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