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Note

Single column amino acid analysis using an ammonia filtration system and a new buffer

When a single column system is used for the amino acid analysis of protein hydrolysates[1], the recovery of the basic amino acids is limited by ammonia contamination of the buffers. Many procedures have been described to minimize ammonia artifacts[2–4]. When ammonia filters of high capacity are installed, histidine and lysine are eluted together with the third buffer (commonly used are 1.2 *N* Na^+, pH 6.45, Bio-Cal Laboratory Manual; 1.2 *N* Na^+, pH 6.28, Beckman Manual; and 1.6 *N* Na^+, pH 6.35, Durum Resin Report). To carry out amino acid analyses with an ammonia filtration system without loss of time, buffers of 2.0 *N* Na^+ are recommended[4]. These buffers with high ionic strength are a rough treatment for the ion-exchange resins usually used in amino acid analyzers.

This paper describes a small column of Dowex used to reduce the ammonia contamination of the first two buffers and a modification of the third buffer, thus resolving all basic amino acids.

Experimental

In these experiments a Bio-Cal BC 200 amino acid analyzer was used, equipped with a long column, 69 × 0.9 cm I.D., packed to column height of 54 cm with Aminex A6 resin (Bio-Rad, Richmond, Calif., U.S.A.). The temperature of the water jacket

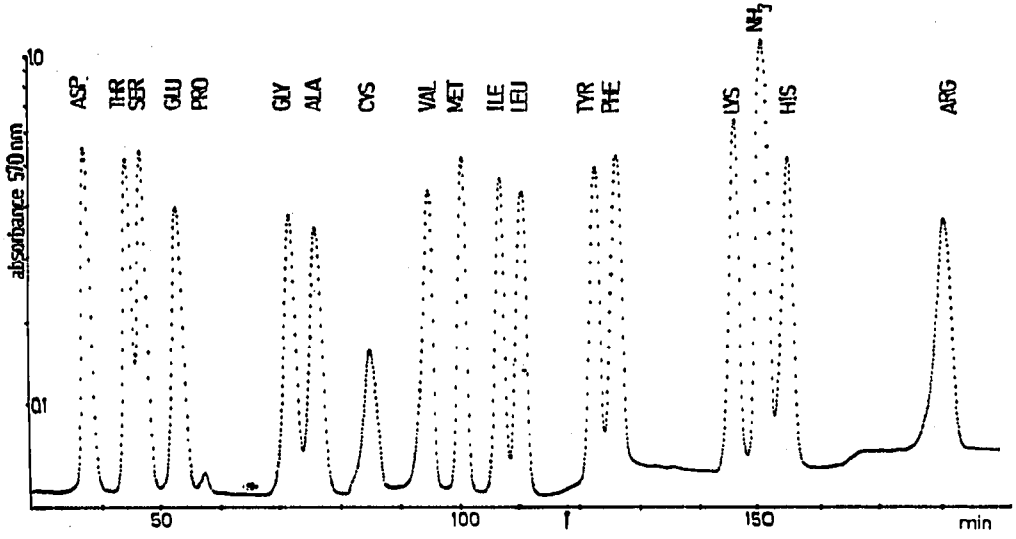

Fig. 1. Elution profile of 100 nmole of a standard mixture of amino acids, obtained with the buffers described in the text. The time scale commences 30 min after the application of the sample to the column. The arrow indicates the occurence of buffer 3.

was maintained at 52°. Buffer and ninhydrin reagent had flow-rates of 100 and 40 ml/h, respectively.

The amino acids were eluted by means of a single pump with 0.2 *N* sodium citrate buffers of pH 3.25 and 4.25. Buffer 3 consisted of 0.2 *N* sodium citrate and 1.0 *N* sodium chloride adjusted with HCl to pH 5.25. Before use, the buffer was subjected to a vacuum and 0.1 volume of methanol was added.

The ammonia filter was a column, 54 × 0.9 cm, packed to a height of 40 cm with Dowex 50W-X8, 20–50 mesh, introduced into the buffer line of buffers 1 and 2 before the pump. The resin was regenerated with sodium hydroxide (0.4 *N*, Bio-Cal Manual) and buffer 1 after each run. No further precautions were taken to remove ammonia from the salts and the water used to prepare the buffers.

Results and discussion

Tyrosine, phenylalanine and the basic amino acids were eluted with buffer 3 (pH 5.25). A typical elution profile is shown in Fig. 1. Good separation of all amino acids was achieved, and only a small increase of the base-line occurred. Methanol must be added to buffer 3 so as effect the separation of lysine and ammonia. If the buffer was adjusted to pH 5.5, a similar elution profile could still be obtained, thus indicating the high resolving power of buffer 3 in this pH range. A good separation of ammonia from histidine (10 nmole) could be obtained even in the presence of large amounts of ammonia (300 nmole). The valley between the two peaks was deep enough to determine the amount of histidine quantitatively.

A vacuum was applied to all buffers before use. By this procedure, degassing within the pump could be hindered while the degassing occurring in the ammonia filter could be neglected.

Buffer 3 was successfully applied to nearly 50 amino acid analyses of hydrolysates of proteins and peptides without any difficulties. As no gradient is needed for elution[3], this system seems to be advantageous. It is recommended for use in order to avoid expensive ammonia filter systems or special resins, or both.

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