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A simplified buffer system for automated column chromatography of amino acids including methionine sulphone

A simplified buffer system for gradient elution in amino acid analysis was proposed recently by ELLIS AND PRESCOTT[1]. This system, for use on a 130-cm column of Technicon "Chromobeads, Type B", eliminated one of the original buffers, reduced the number of volumetric additions to the Autograd from 14 to 2, and involved the manipulation of only two Autograd valves. The gradient has been tested in these laboratories and the simplicity combined with resolution of the amino acids confirmed. However, in the amino acid analysis of proteins, cystine and methionine are usually determined as cysteic acid and methionine sulphone after acid hydrolysis of performic acid-oxidised protein. On adding these two amino acids to the standard mixture, and using the simplified buffer system, cysteic acid was frontally eluted as expected but methionine sulphone was not adequately resolved from aspartic acid. The effect of lowering the pH value of the initial eluting buffer has been studied.

Experimental

A Technicon amino acid analyser (Technicon Instruments Co. Ltd., Chertsey, Surrey) was used. The 140-cm column was packed to a height of 126 cm with Technicon "Chromobeads, Type A" and was maintained at 60°. Buffers of pH 2.50 and 5.00 were prepared as directed by Technicon Instruments Co. Ltd. With only the first and sixth valves of the nine-chambered Autograd closed, a mixture of 280 ml of pH 2.50 buffer and 20 ml of methanol were placed in the section comprising chambers 1–5, and 240 ml of pH 5.00 buffer in section 6–9 such that each chamber contained 60 ml solution after reaching hydrostatic equilibrium. After applying the sample, dissolved in 0.1 *N* hydrochloric acid, to the column which was equilibrated with the pH 2.50 buffer without methanol, valves 1 and 6 were opened and pumping was commenced at a flow rate of 0.50 ml/min. After the emergence of histidine the eluting buffer was changed from the Autograd to pH 5.00 in order to elute arginine.

Results

Altering the pH of buffer No. 1 of the simplified system from pH 2.88 to 2.50, together with the inclusion of 6.67% by volume of methanol instead of 10% by volume of ethylene glycol, resulted in the separation of methionine sulphone from aspartic acid. The other common amino acids including the standard norleucine were still resolved, the only major differences being that cystine was brought closer to methionine and ammonia emerged sooner after phenylalanine. This system may therefore be used as a general one for hydrolysates of proteins with or without prior performic acid oxidation and retains the simplicity of the buffer system of ELLIS AND PRESCOTT[1].

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