The use of ion exchange cellulose columns with the Technicon AutoAnalyser technique for the fractionation of peptides

The fractionation of enzymic digests of proteins is an important step in techniques for determination of their amino acid sequence. One technique commonly used for this is chromatography using an ion exchange resin in conjunction with a fraction collector. The usual technique for the location of the peptides in the fractions is by estimation of the colour produced by ninhydrin with (a) suitable aliquots taken directly from each fraction and (b) similar aliquots which have been hydrolysed by alkali. CATRAVAS[1] has described a technique using the Technicon AutoAnalyser which permits the continuous monitoring of the column eluate. In this procedure the effluent stream from the column is split into three streams. Two of these streams are used for analysis giving recorder traces corresponding to "direct" and "hydrolysed" ninhydrin colours whilst the third stream goes to a fraction collector. However, the combined analytical streams require peptides from about 5 mg protein and take eluate from the column at a total rate of 24 ml/h and the *collection* of a total of 5 mg peptides thus requires a total flow rate of 50 ml/h through the column. Hence chromatography of 50–100 mg of peptides and the collection of 90 % of the material requires a pumping rate of 250 ml/h. This flow rate could lead to very high back-pressures with modern analytical ion exchange resins whilst the alternatives of very large columns or coarser resins would undoubtedly lead to loss of band resolution.

Our own problem is further complicated by the composition of the peptides under examination. We are interested in determining the sequence of a high-sulphur protein derived from wool, SCMK-B2[2,3]. In this protein approximately one residue in four is S-carboxymethylcysteine, and there are no lysine or histidine and few arginine residues. Peptides derived from the protein thus tend to be highly acidic and the special problems of separation of very similar acidic peptides have been little studied. It occurred to us that ion exchange celluloses might provide a solution to both these problems since they have a higher capacity for peptides and can be operated at much faster flow rates and lower back-pressures than conventional ion exchange resins[4].

Fig. 1(a) shows the AutoAnalyser record using CATRAVAS' procedure and illustrates the separation of peptides obtained from a chymotryptic digest of SCMK-B2. A total of 5 mg of peptides was used on a 0.9 × 50 cm column of DEAE-cellulose in the formate form. Development was at 25 ml/h and used a linear gradient from distilled water to 0.5 *N* formic acid. Essentially all the eluate was put through the analytical system. Fig. 1(b) shows a similar record except that 91 mg of peptides were loaded on to a 2.5 × 40 cm column operated at 230 ml/h, so that approximately 10 % was used for analysis and 90 % collected in a fraction collector. Comparison of the two traces shows that the separation is reproducible and that the scaling up for the preparative procedure has not involved any loss of resolving power. The separation obtained seems comparable with that given by conventional ion exchange resins[1], despite the fact that we are using an exchangeable buffer ion, and this is a condition which is not regarded as ideal[3].

Whilst the conditions reported are designed specifically for the separation of acidic peptides there would seem to be no reason why neutral and basic fractions could not also be fractionated by a suitable choice of conditions and ion exchange cellulose type. The especial feature to which we wish to draw attention is that the

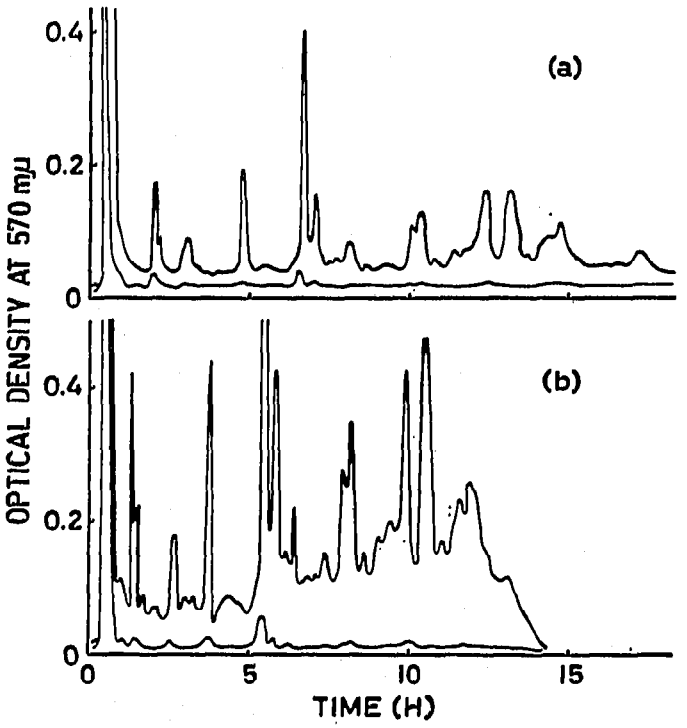

Fig. 1. Separation of peptides in a chymotryptic hydrolysate of SCMK-B2 on DEAE-cellulose in the formate form using a linear gradient from distilled water to 0.5 *N* formic acid. (a) 5 mg of peptides on a 0.9 × 50 cm column; flow rate 24 ml/h; (b) 91 mg of peptides on a 2.5 × 40 cm column; flow rate 230 ml/h. The lower curve in each trace represents the direct ninhydrin colour whilst the upper curves give the ninhydrin colour after alkaline hydrolysis.

technique overcomes one of the problems of the original CATRAVAS technique by allowing a considerable increase in the scale of the preparative procedure[5].

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Received July 20th, 1967

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