CHROM. 3549

The use of Sephadex G-25 in thin-layer electrophoresis and chromatoelectrophoresis of amino acids and low molecular weight peptides

Since the introduction of the thin-layer gel filtration technique in 1962[1,2] various modifications of this method have been described, but the papers dealing with this subject are almost exclusively connected with the separation of high molecular weight compounds. The possibility of using thin-layer electrophoresis for amino acids and peptides was briefly mentioned in the article by DOSE AND KRAUSE[3], but, neither results not details of the technique used were given.

In this paper we present the separation of amino acids and some low molecular weight peptides obtained by means of thin-layer electrophoresis and thin-layer chromatoelectrophoresis. It should be mentioned here that our attempts to apply thin-layer chromatography to mixtures of the above-mentioned compounds were not successful.

Material and methods

The separation medium used was the commercially available Sephadex G-25 fine (Pharmacia, Uppsala, Sweden). The separation of amino acids and peptides was carried out by means of thin-layer electrophoresis and chromatoelectrophoresis (a combination of paper chromatography and thin-layer electrophoresis).

(*a*) *Thin-layer electrophoresis*. Sephadex G-25 was equilibrated for 24–48 h in a solvent system composed of acetic acid–formic acid–water (3:1:135, v/v/v), pH 1.9. The degreased glass plates (4 × 36 cm) were coated with a 0.5 mm thick layer of gel and the samples of test solutions were applied 3 cm from one end of the plate. Having found that the application of sample directly on the gel resulted in very poor resolution, we applied the sample first to a Whatman 3MM paper strip (30 × 2 mm) and we then put this paper on the wet surface of the gel. Time of electrophoresis was 3 h; voltage, 600 V.

(*b*) *Chromatoelectrophoresis*. The mixture of amino acids and peptides was applied to a Whatman No. 4 paper strip and developed by descending chromatography in the solvent system pyridine–methanol–water (1:20:5, v/v/v). The paper chromatogram was then dried in air and put 3 cm from the short edge of the plate (24 × 36 cm), covered with a wet, 0.5 mm thick layer of Sephadex G-25 fine gel. The electrophoresis was carried out as before.

In both methods (a and b), after electrophoretic separation the plates were thoroughly dried in air at room temperature, and the spots were dyed with 2% acetone solution of ninhydrin.

Regeneration of the gel. Sephadex was regenerated by successively washing with an aqueous solution of ethyl alcohol (about 20%), a large volume of water, a 0.02 *N* solution of NaOH and once more with water until a neutral reaction was obtained. After equilibration of the gel with the appropriate solvent, it can be used for further separations. Results obtained are reproducible.

Results and discussion

Examples of the separation of some amino acids and low molecular weight peptides by means of thin-layer electrophoresis are given in Figs. 1 and 2. The results show that the method offers good possibilities for the resolution of amino acid groups which are very difficult to separate by paper chromatography. This method also permits good differentiation between low molecular weight peptides and their amino acid components.

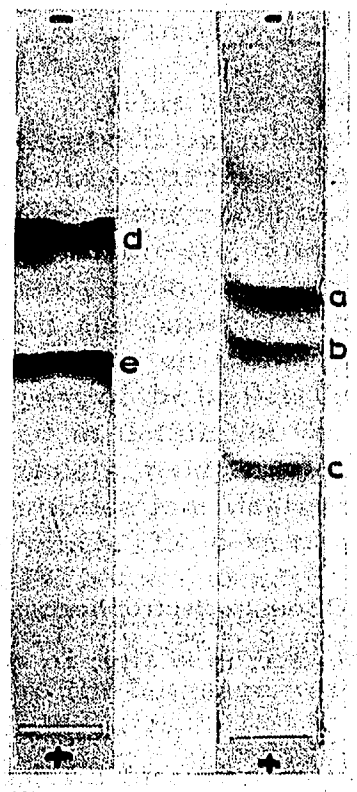

Fig. 1. Electrophoretic separation of amino acids. a = Val; b = Met; c = Trp; d = Gly; e = Ser.

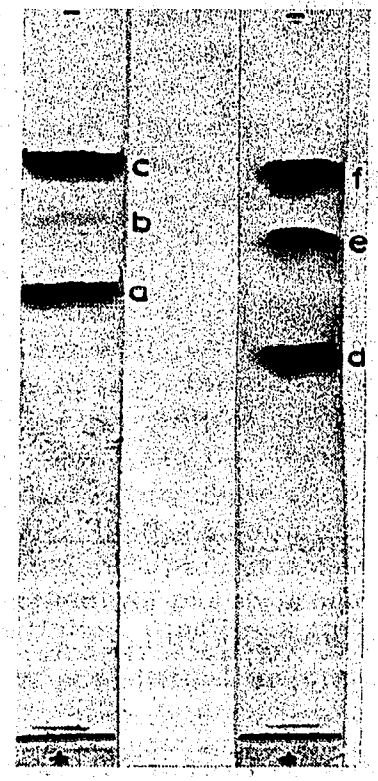

Fig. 2. Electrophoretic separation of low molecular weight peptides and their amino acid components. a = Leu; b = Gly-Leu; c = Gly; d = Glu; e = Gly-Glu; f = Gly.

Fig. 3 shows the complete separation of the mixture composed of 12 amino acids and 8 di- and tripeptides by means of chromatoelectrophoresis. It is necessary to emphasize that even in these spots, which on the photograph seem to contain a single substance, separate components can be distinguished by the various colours resulting from the use of ninhydrin. For instance, the yellow colour of the reaction product between diglycylglycine and ninhydrin allows one to differentiate the peptide from glycine. The same applies to glycylleucine, alanine and leucylglycine, where only the first substance yields the yellow-coloured product with ninhydrin.

We believe that thin-layer chromatoelectrophoresis on Sephadex will find an application in the analysis of minute amounts of amino acids and lower peptides. Attempts are now being made to apply this method to the analysis of protein hydrolysates and biological fluids. Further work in this field, with the use of Sephadex G-25 superfine and G-10 fine, is in progress.

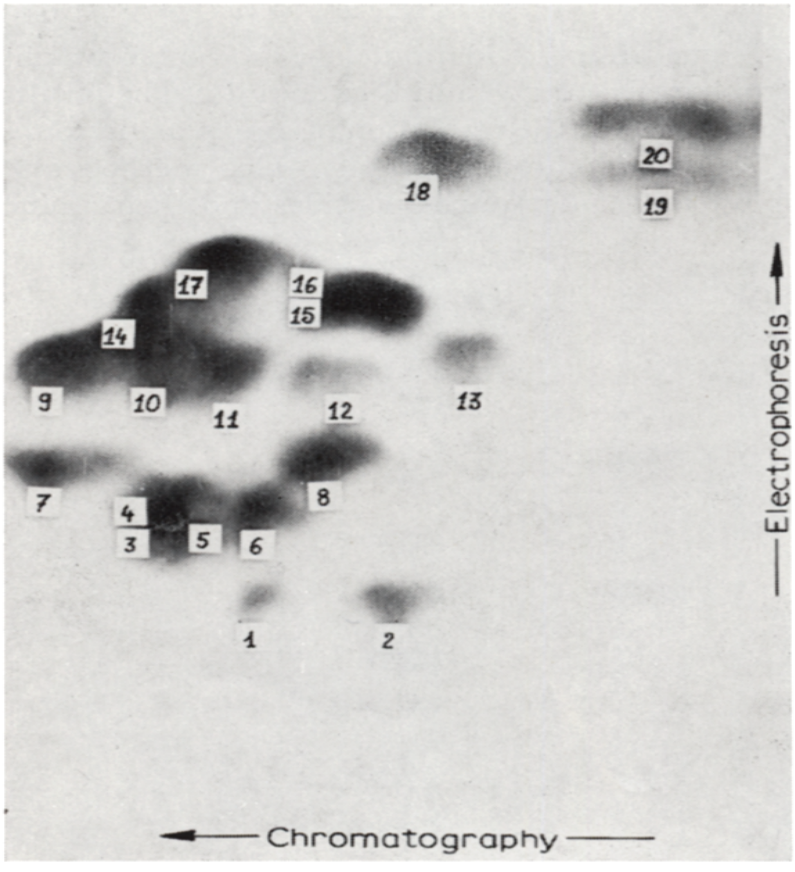

Fig. 3. Separation of amino acids and low molecular weight peptides by chromatoelectrophoresis. 1 = Gly-Cys-Glu; 2 = Trp; 3 = Phe; 4 = Met; 5 = Pro; 6 = Glu; 7 = Leu; 8 = Ser; 9 = Leu-Gly; 10 = Gly-Leu; 11 = Ala; 12 = Gly-Glu; 13 = Gly-Asn; 14 = Ala-Ala; 15 = Gly; 16 = Gly-Gly-Gly; 17 = Ala-Gly; 18 = His; 19 = Arg; 20 = Lys.

We are indebted to Dozent M. SARNECKA-KELLER Sc.D. for help and advice given throughout the course of this work.

Department of Biochemistry and Biophysics,
Jagellonian University, Kraków (Poland)

J. CHUDZIK
A. KLEIN

1 H. DETERMAN, *Experientia*, 18 (1962) 430.
2 G. B. JOHANSSON AND L. RYMO, *Acta Chem. Scand.*, 16 (1962) 2067.
3 K. DOSE AND G. KRAUSE, *Naturwiss.*, 49 (1962) 349.

Received April 10th, 1968