Electrochromatography of Amino Acid Mixtures in Fermentation Liquors

M. K. EL-MARSASY, 1) Z. ŘEHÁČEK and ABDEL-GAWAD ZENAB 2)

Department of Antibiotics and Experimental Mycology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague 6

Received March 6, 1963

ABSTRACT

The substitution of the phenolic chromatography dimension, by electrophoresis at 500 to 1000 volts, in a veronal buffer, pH 8.6, µ 0.02 followed by triple rechromatography in a completely miscible butanol, acetic acid, water solvent system, leads to clear, easily reproducible electrochromatography patterns. A reliable economical chromatography cabinet is described. The technique allows the analysis of many samples conveniently, and some important characteristics of unidentifiable components can be readily predicted.

An interpretable pattern resolving mixtures of amino acids, can be achieved by the combination of the same technique, under different conditions, or the combination of two different techniques, perpendicularly on a sheet of filter paper. The application of the usual two dimensional paper chromatography involves the use of a butanol, acetic acid water mixture, in one dimension, followed by a phenol, ammonia, with traces of cyanide, in the other perpendicular direction. In our experience, the use of the phenol solvent system, has been a limiting factor in the resolution of amino acids in the media used for fermentations, and also in the analysis of mycelial hydrolysates. Many attempts have been directed recently to replace the phenol by other less objectionable solvent systems. However, none of the systems suggested were found suitable for routine analysis (Mizell & Simpson, 1961; Ambe & Tappel, 1961).

The use of electrophoresis in both dimensions, in different buffers, at different pH is probably incapable of sufficient resolution (Durrum, 1951). Application of high voltage electrophoresis, at acid pH, followed by chromatography, leads to excellent resolution (Pruslík & Keil, 1960). This technique, however, requires expensive apparatus, and is not economically suitable for routine analysis.

Therefore a technique was developed where electrophoresis under conditions not requiring excessively high voltages, and hence dispensing with the cooling system, was followed by rechromatography several times in the same solvent. Conditions for obtaining economically reproducible electrochromatography patterns, which are suitable for pursuing the study of changes in the amino acids during fermentation, are discussed in this paper.

MATERIALS AND METHODS

The hydrolysate of the mycelia of the submerged cultures of *Pleurotus ostreatus*. This was obtained by the hydro-
Lysis of the washed separated mycelia in 6N-HCl in sealed ampoules for 24 hours at 100°C. Excess acid was removed by repeated drying over sodium hydroxide under a vacuum, with several washings with distilled water. The pH was then adjusted to between 5.0–7.0, and finally the sample was centrifuged.

**Paper electrophoresis.** A horizontal type of apparatus was used (Grassman & Hanning, 1952). Between consecutive runs, the polarity was changed, thus saving greatly in the frequent change of the buffer in the electrode compartments. Whatman No 1 filter paper (W1) was used. Electrophoresis was carried out in veronal buffer, pH 8.6, μ 0.02.

**Technique.** The different amino acid mixtures and bromphenol blue dye solution are spotted alternatively in the centre of the paper as indicated in Fig. 1a. The paper is placed into the tank, and evenly wetted with the buffer, leaving the vicinity of the sample area dry, where it is wetted with the buffer creeping up evenly from both sides, and meeting simultaneously at the central axis of the paper, where the samples have been spotted. Voltage ranging from 500 to 1000 is then applied for a time long enough to permit a migration length of about 40 cm. for the bromophenol blue dye. This lasts between 3 hours, and 1.5 hours, depending on the voltage. The electrophoresis is then terminated, and the sheet dried in the oven, and further divided along the pencilled lines. Alternatively, the amino acid samples, could be applied to strips of paper from the start. A standard mixture of amino acids is then applied to one end of these strips (Fig. 1b).

**Paper chromatography.** Ascending chromatography was used, where the sheet was rolled as a cylinder, and allowed to stand upright, with the lower edge dipping in the solvent (Wolfson, Cohn & Devaney, 1949). For economy in the use of the chromatography solvents, a circular channel (width 1 cm., depth 1 cm.) was machined into a polyvinyl chloride disc (2 cm. thick). The diameter of this channel being enough to accommodate the rolled sheet of 50 cm. width. The capacity of this channel (30 ml.) proved sufficient for one chromatography. An inverted glass jar (45 cm. high, diameter 22 cm.) or, alternatively, a cylindrical frame of stainless steel, of the same dimensions, covered with a well stretched thin polyethylene sheet, served as a reliable and convenient cabinet for the chromatography over the PVC disc (Fig. 2). For chromatography, Whatman No 4 filter paper (W4) was preferred.

**Amino acids.** An aqueous solution containing 5 mole/ml. of each of the common amino acids as well as two mixtures (A, B) of these acids indicated below, were prepared (Keil — private communication). Before electrophoresis,