CHROM. 4564

Rapid separation of some amino acids by ion-exchange paper electrophoresis

A number of different techniques have been tested for the separation of amino acids, but electrophoresis on ion-exchange papers has not yet been widely developed, except for a few limited explorations[1,2].

Amongst other usual methods, for example those using columns of ion-exchange resins[3], or those by ion-exchange paper chromatography reviewed by KNIGHT[4], chromatography on thin layers, generally associated with electrophoresis, has provided excellent results in the identification of protein hydrolysates. The work of MUNIER *et al.*[5] on cellulose-powder thin layers is an excellent example.

However, in general, these techniques are not very simple and somewhat sophisticated apparatus is necessary (*e.g.* for the preparation of the thin layer and a high voltage supply). Our aim here is to present a simple and relatively rapid technique using weakly acid ion-exchange paper and medium voltage electrophoresis (on commercially available papers). The one operation which is described, can, if desired, gain more efficiency by combination with chromatography.

Theory

Previously, SELEGNY AND FENYO[6,7] have studied and described the parameters which control the chromatographic and electrophoretic migration of inorganic ions on such charged papers. We have now conducted similar experiments with amino acids. The relevant details and the analytical aspects will be published elsewhere[8,9]. Here we give the general outlines, and show that a quick, simple and altogether interesting method has been evolved using these concepts.

Seventeen common amino acids and three types of ion-exchange papers were investigated. The papers are of the "heterogeneous" type, prepared by incorporating fine resin particles into the cellulose matrix (obtained from B.D.H. or Rohm and Haas). These were respectively:

A strongly basic ion-exchange paper SB-2 (containing particles of the quaternary ammonium type, Amberlite IRA-400);

A strongly acid exchange paper SA-2 (containing particles of the sulphonic type, Amberlite IR-120);

A weakly acid exchange paper WA-2 (with particles of the carboxylic resin IRC-50).

The first objective was the determination of the influence of the nature of the buffer, of its concentration, and of the pH. Experiments were conducted in acid buffers (phosphate or citrate) with the cation-exchange papers SA-2 and WA-2, and in basic media (borate or Tris) when the anion exchanger SB-2 was employed.

The charge on the amino acids is modified according to the pH used: an exchange occurs between an amino acid in solution and the counter-ion of the resin, that is to say the ions of the buffer. One has to remember that ionisation of carboxylic groups is also modified by the pH and by the ionic strength of the solution. These variables affect the distribution coefficients between "resin" and "solution" in a predictable manner.

If we take as an example a cation exchanger, we can consider the following equilibrium:

$$y\mathrm{M}^{x+} + x\overline{\mathrm{M}}'^{v+} \rightleftharpoons y\overline{\mathrm{M}}^{x+} + x\mathrm{M}'^{v+}$$

where M^{x+} corresponds to the cationic form of the deposited amino acid and M'^{v+} to the competitive counter-ion; symbols with a dash refer to the resin phase and those without it to ions in solution. We have already shown[6,7] that the electrophoretic mobility (u) of an ion in an ion-exchange paper–buffer solution system can be related to the so-called "limiting distribution coefficient" by an equation of the type:

$$u = \frac{k_1}{P^\circ + k_2} \text{ or } \frac{1}{u} = \frac{P^\circ}{k_1} + \frac{k_2}{k_1}$$

where k_1 and k_2 are characteristic constants which can easily be determined experimentally, and where the ratio k_1/k_2 is the mobility of the species on an inactive paper with the same porosity as the ion-exchange one ($P^\circ = 0$).

The chromatographic R_F and (u) are also interrelated:

$$u = \frac{k_1}{\frac{1}{R_F} + k_2} \text{ or } \frac{1}{u} = \frac{1}{k_1 \cdot R_F} + \frac{k_2}{k_1}$$

Although the complexity of the system with amino acids is evident, one can see that $k_1 = 0$ for molecules without a net charge.

Simultaneous chromatographic and electrophoretic measurements enabled us to understand and analyse more easily the slowing-down of the movement of the ions[3,10] in the presence of fixed ion-exchange groups, and allowed the semi-quantative prediction of mobilities with the help of the equations cited.

Electroosmotic migration could be correctly predicted for each paper by the shift of the neutral amino acids at pH 7 if the interactions of exchange groups and counter-ions were negligible. This point would need a more detailed analysis. The following paragraphs illustrate what currently seem to be the best experimental conditions.

Experimental

SB-2 paper. With an Na–K phosphate buffer ($C = M/20$), at pH 7.5 the neutral and basic amino acids are not absorbed by the exchanger $R_F = 1$. One hour of electrophoresis (14 V/cm) separates the mixture into three groups containing respectively the acidic, basic and neutral amino acids; separation inside these groups is very poor.

SA-2 paper. Again in phosphate buffer at pH 7, the acid and basic amino acids give individual spots after electrophoresis for 1 h at 14V/cm. The neutral amino acids remain grouped; their migration is small, showing little evidence of the effect of electroosmosis. By chromatography, $R_F = 1$ for neutral and acidic amino acids.

WA-2 paper. As is shown in Fig. 1, individual separation of the seventeen amino acids is obtained after 6 h in $M/20$ Na-citrate buffer at pH 2.3 (28.5 V/cm). Basic amino acids such as Lys, Orn, which do not appear on this photograph, as well as Arg

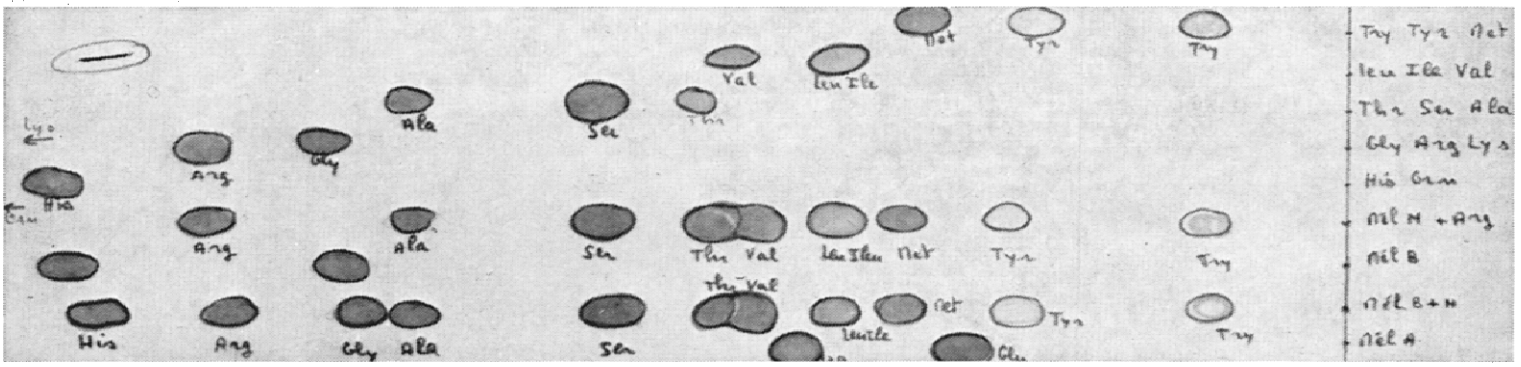

Fig. 1. The amino acids after electrophoresis for 6 h. The spots were resprayed as the colour fades with time. Lys and Orn do not appear here.

and Gly, have high mobilities. They are followed by the series of neutral and finally the acidic amino acids. Only the spots of Leu–Ile and Val–Thr are not separated.

Under these experimental conditions, the R_F values are not proportional to the mobilities.

Results

Our results permit the prediction of the position occupied by each spot after chromatography for 4 h followed by electrophoresis for 4 h in the same citrate buffer.

It was found that the neutral amino acids behave as cations at their isoelectric point or above. This can be attributed either to a large electroosmotic flow or to the interaction of these amino acids with the buffer or the resin.

With strongly charged papers, the spots remain small even after several hours of chromatography or electrophoresis; as a consequence complete separation of the spots is possible over relatively short migration distances. An uncharged paper gives higher mobilities but the spots are bigger and spread during migration. Strong exchangers can split the amino acids into groups; however, the weakly acidic paper is intermediate in charge at a sufficiently low pH and is consequently more selective. The citrate buffer is also of importance, for in acetic media at the same pH the mobilities obtained are different and separation less efficient.

Some dipeptides have been successfully separated by this method.

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First received November 11th, 1969; revised manuscript received December 21st, 1969