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Note

Thin-layer chromatography on a chromatosheet coated with resin in different ionic forms for the separation of amino acid mixtures containing asparagine and glutamine

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Chromatography on serially connected thin layers combines the chromatographic features of the component layers, and this paper reports on chromatography on an ion-exchange chromatosheet coated with two different ionic forms of a resin on two areas of the sheet for the resolution of substances that are not separated by the individual ionic forms, and the determination of the ratio of the two ionic forms of the resin needed for the optimal separation of the mixture.

This approach was applied to the one-dimensional separation of asparagine and glutamine in amino acid mixtures, which is of great importance in sequence analysis and in the investigation of biological fluids.

The two-dimensional separation of asparagine and glutamine on a cellulose thin layer is possible[1], but in the one-dimensional separation of amino acids by ion-exchange thin-layer chromatography on resin-coated chromatosheets in the Na^+ form[2] the separation of asparagine and glutamine could not be achieved. This separation problem has been resolved by using an automatic analyzer technique with lithium citrate buffers[3,4].

EXPERIMENTAL

Strongly acidic cation-exchange resin-coated chromatosheets (20 × 20 cm; Fixion 50X8, Chinoin-Nagytétény, Budapest, Hungary, and Ionex 25SA-Na, Macherey, Nagel and Co., Düren, G.F.R.) were used. The chromatosheets were treated as follows in order to have one area of the resin in the Na^+ form and the remainder in the Li^+ form.

(a) The chromatosheets, which are available in Na^+ form, were converted completely into the Li^+ form by continuous development for 24 h with 1 *M* lithium chloride solution at room temperature.

(b) The sheets were then dried and the excess of lithium chloride was removed by continuous development with deionized water for 24 h at room temperature.

(c) The lower part of each thin layer was converted from the Li^+ form back into the Na^+ form by developing the lower 4 cm of the sheet with 1 *M* sodium chloride solution (2.5 cm above the starting line).

(d) The final equilibration of the chromatosheets was achieved by continuous development for 24 h with eluting buffer (see below) diluted 10-fold.

Sheets completely in the Li^+ form were prepared by treatments (a), (b) and (d), while thin layers completely in the Na^+ form were used after treatment (d). These sheets with resins in a single ionic form were used for comparative purposes.

The eluting buffer was Li^+ (0.20 *M*)–citrate (0.05 *M*)–formate (0.05 *M*), of pH 2.80 ± 0.02. It was prepared by mixing lithium citrate tetrahydrate (14.10 g), lithium chloride (2.12 g), 85% formic acid (2.30 ml) and 37% hydrochloric acid (8.0 ml), and making the volume up to 1000 ml with deionized water.

The chromatograms were developed with the eluting buffer at 37°. The sheets were fitted on to a glass plate and were developed for 4 h until the solvent front migrated into the filter-paper pad attached to the top of the sheets.

Ninhydrin spray reagent containing cadmium acetate and collidine was used for staining the chromatograms.

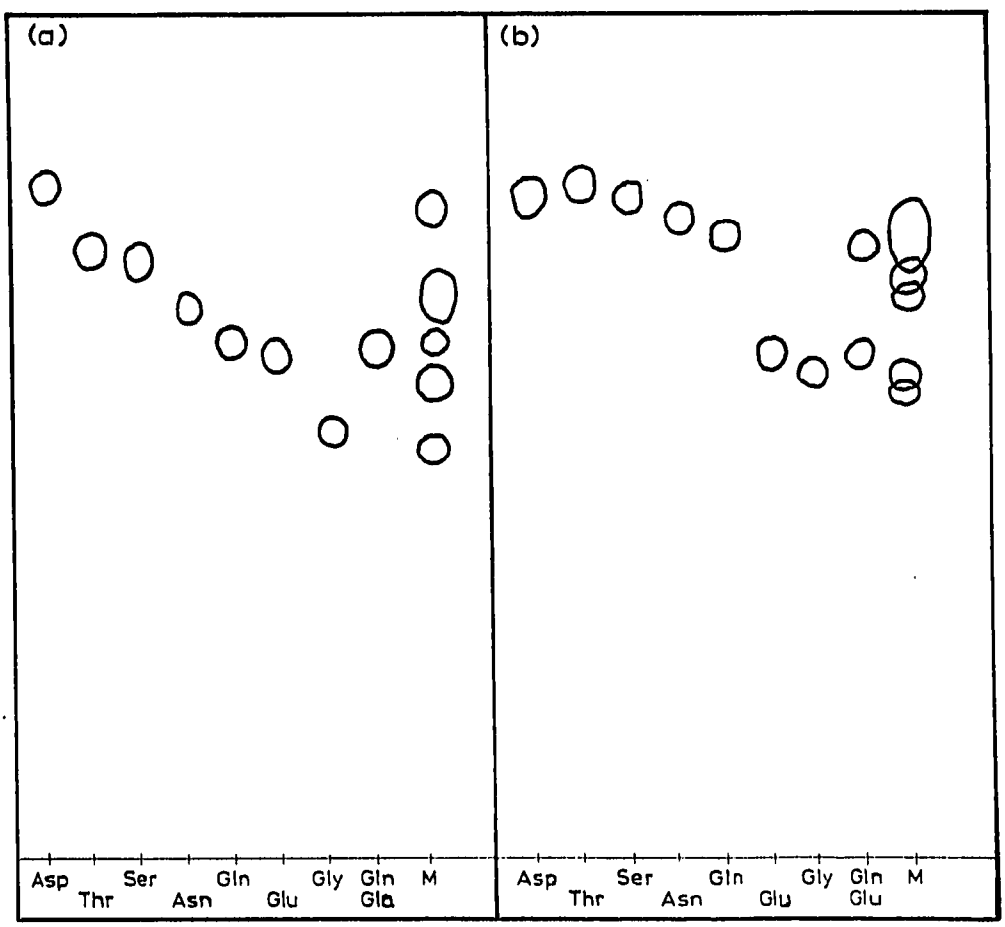

Fig. 1. Separation of amino acids on individual ion-exchange layers: (a) resin in Li^+ form; (b) resin in Na^+ form.

RESULTS AND DISCUSSION

For comparison, Figs. 1a and b show chromatograms obtained on sheets coated with resin completely in the Li^+ and Na^+ form, respectively. While in the case of the layer in the Li^+ form the amino acids are well separated with the exception of glutamine and glutamic acid, the reverse is true for the layer in the Na^+ form.

In general, it could be anticipated that if R_F values of three given compounds, (1), (2) and (3), on two different thin layers, (A) and (B), are:

$$R_{F(A)}^{(1)} = R_{F(A)}^{(2)} \neq R_{F(A)}^{(3)} \quad (1)$$

and

$$R_{F(B)}^{(1)} \neq R_{F(B)}^{(2)} = R_{F(B)}^{(3)} \quad (2)$$

then the separation of these three compounds might be achieved by the connection of (A) and (B).

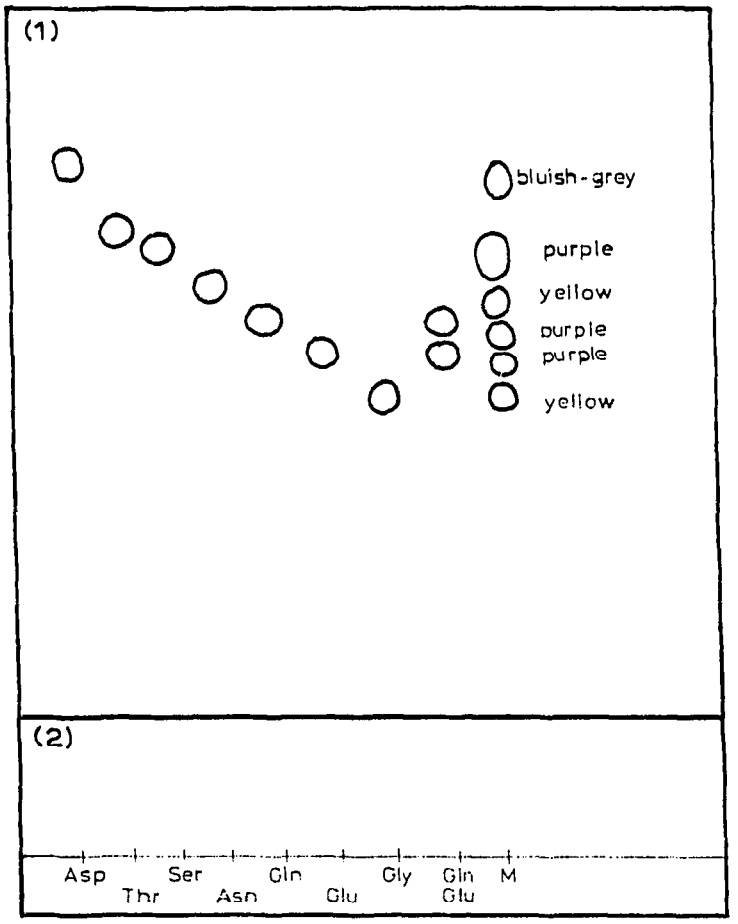

Fig. 2. Separation of amino acids on a layer of ion-exchange resin in the Na^+ and Li^+ forms in the areas (2) (2.5 cm) and (1) (16 cm), respectively.

Clearly, the R_F values of asparagine, glutamine and glutamic acid satisfy the conditions described in eqns. 1 and 2. Therefore, chromatography on a layer partly in the Na^+ and partly in the Li^+ form, combining the features of the two different ionic forms, might resolve this separation problem.

Fig. 2 shows a typical chromatogram on a layer consisting of resin in the Na^+ and Li^+ forms in different areas. Aspartic acid, threonine + serine, asparagine, glutamine, glutamic acid and glycine are separated from each other. The R_F values of other amino acids are much lower than the R_F values of glycine and therefore they do not interfere in the separation.

The optimal ratio of the heights of the Na^+ and Li^+ forms of the resin layer was determined by a simple procedure in which the height of the Na^+ form of the layer was varied from 0.5 to 5 cm. Fig. 3 shows that the best separation was achieved when the height of the Na^+ form of the layer was 2.5 cm.

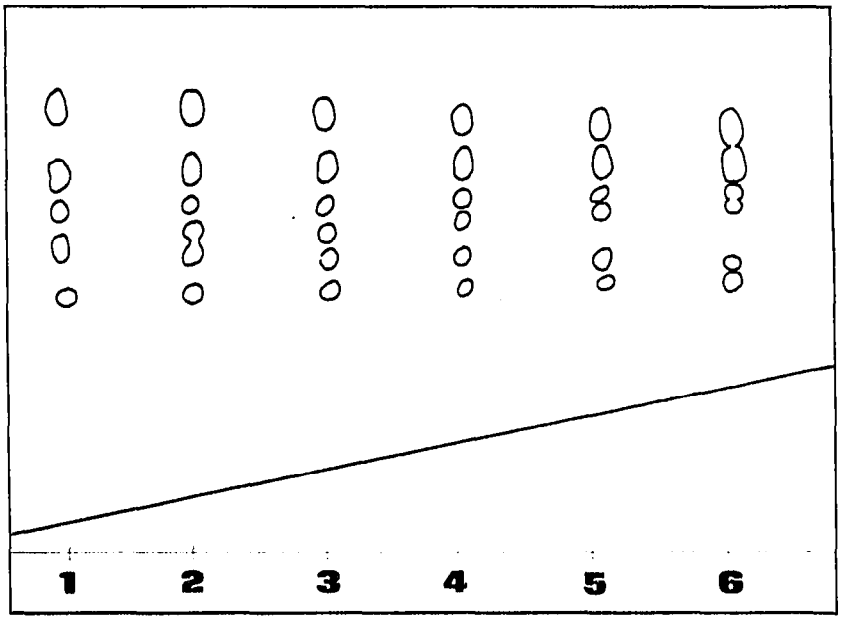

Fig. 3. Experimental determination of the ratio of the heights of the Na^+ and Li^+ forms of the resin layer required for the optimal separation of the amino acids listed in Figs. 1 and 2. The height of the Na^+ form of the layer varies from 0.5 to 5 cm. The best separation was achieved at position 3, where the height of Na^+ form of the layer is 2.5 cm.

The optimal ratio of the heights of A and B in the layer can be calculated as follows. The R_F value of a given compound (1) on the A + B layer, to a first approximation, is

$$R_{F(A+B)}^{(1)} = \frac{a}{SF} + R_{F(B)}^{(1)} - \frac{a}{SF} \cdot \frac{R_{F(B)}^{(1)}}{R_{F(A)}^{(1)}} \quad (3)$$

where SF is the height of the solvent front, a is the height of the layer A in the A + B layer and A refers to the lower layer.

The ratio expressed in terms of the pre-determined relative R_F value of two compounds ($r_{1,2}$) follows from eqn. 3:

$$\frac{a}{SF} = \frac{R_{F(B)}^{(1)} - r_{1,2}\, R_{F(B)}^{(2)}}{\left(1 - \frac{R_{F(B)}^{(2)}}{R_{F(A)}^{(2)}}\right) r_{1,2} + \frac{R_{F(B)}^{(1)}}{R_{F(A)}^{(1)}} + 1} \qquad (4)$$

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REFERENCES

1 E. von Arx and R. Neher, *J. Chromatogr.*, 12 (1963) 329.
2 T. Dévényi, I. Hazai, S. Ferenczi and J. Báti, *Acta Biochim. Biophys. Acad. Sci. Hung.*, 6 (1971) 385.
3 J. V. Benson, M. J. Gordon and A. J. Patterson, *Anal. Biochem.*, 18 (1967) 228.
4 P. L. Y. Lee, *Biochem. Med.*, 10 (1974) 107.