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Separation of acidic amino acids by high voltage paper electrophoresis and paper chromatography

Many methods for the separation of amino acids on paper[1], or using thin layers of cellulose[2–4], starch[5], silica gel[6], or mixed cellulose–silica gel[7], have been recorded, and have been recently reviewed[8]. Although these methods usually separate common amino acids, many specific groups of amino acids require modified procedures, *e.g.*, imino acids[9], iodo-amino acids[10], substituted tryptophans[11] and sulphur- and seleno-amino acids[12,13]. During investigations into the soluble amino acids present in various fern genera in New Zealand, a method was required to separate the numerous acidic amino acids encountered.

High concentrations of sugars, organic acids and salts present in some extracts interfere with amino acid separation during chromatography. To overcome this, high voltage electrophoresis was used in the first dimension, followed by chromatography in the second dimension. A chromatographic step was used in preference to another electrophoretic step because of the structural data that can be gained from the R_F value of the amino acid. This paper reports the most useful separation procedures investigated.

Experimental

Electrophoresis was carried out using Whatman 3MM paper, 43 cm × 53.5 cm in a high voltage apparatus (Miles Hivolt Ltd, Shoreham-by-Sea, Sussex). The paper was dipped in buffer solution, uniformly blotted and the extract in a volume of 10 μl, applied as a narrow 2 cm streak. Amino acid loading of up to 50 μg of each amino acid gave rise to the best separation. Electrophoresis was carried out at pH 3.4 (acetic acid 10 ml, pyridine 0.6 ml in 1 l water) for 30 min at 7.5 kV and 100 mA.

After drying for 1 h in a current of warm air, the paper was folded and descending partition chromatography carried out in a single phase solvent *n*-butanol–acetic acid–water (12:3:5 v/v) for 20 h at 20°.

Amino acids were located by spraying with 1 % ninhydrin solution (in 95 % ethanol with 1 % 2,4,6-collidine added) and the colours allowed to develop at room temperature for several hours before being recorded.

Prior to electrophoresis at pH 3.4, a preliminary separation into neutral, basic and acidic amino acids could be carried out at pH 5.3 (pyridine 10 ml, acetic acid 4 ml in 1 l water) for 10 min at 5.5 kV and 300 mA. A small strip was sprayed with ninhydrin solution as location reagent and the amino acid bands eluted, concentrated and electrophoresed at pH 3.4.

A two-dimensional separation employing electrophoresis at pH 5.3, 30 min, followed by chromatography could also be employed but resolution of many compounds was poor. Electrophoresis at this pH did however yield some structural data.

Results and discussion

All of the compounds listed could be separated by pH 3.4 electrophoresis followed by chromatography in *n*-butanol–acetic acid–water as is shown in Fig. 1. Two of these compounds, α-aminopimelic acid (L) and γ-ethylideneglutamic acid

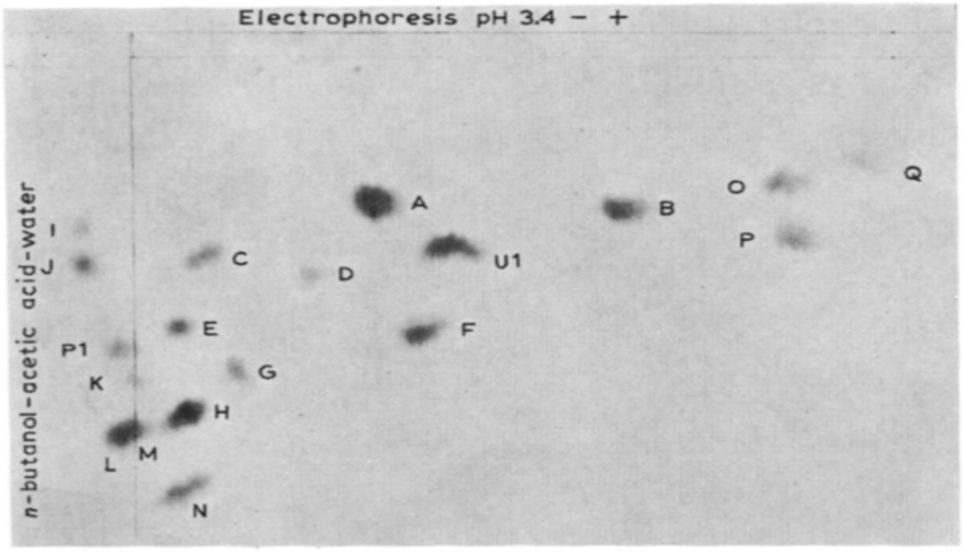

Fig. 1. A two-dimensional separation of various amino acids using pH 3.4 high voltage electrophoresis for the first dimension followed by chromatography in *n*-butanol–acetic acid–water. The key and relative movement data are listed in Table I. β,γ-Dihydroxyglutamic acid is not shown in this figure.

TABLE I

R_{GLU} VALUES, ELECTROPHORETIC MIGRATION DISTANCES (cm)* AND NINHYDRIN COLOURS FOR VARIOUS AMINO ACIDS

Key	*Compound*	*Electrophoresis*		R_{glu}	*Colour*
		pH 3.4	*pH 5.3*		
D	Aspartic acid	9.4	22.7	0.81	grey-blue
B	*threo*-β-Hydroxyaspartic acid	22.3	22.5	0.57	green-grey
A	*erythro*-β-Hydroxyaspartic acid	11.9	21.4	0.56	green-grey
E	Glutamic acid	3.9	19.6	1.0	blue
C	β-Hydroxyglutamic acid**	5.2	18.9	0.72	brown-blue
C	β-Hydroxyglutamic acid**	4.5	18.9	0.76	brown-blue
U1	*threo*-γ-Hydroxyglutamic acid	15.2	20.6	0.72	blue
–	β,γ-Dihydroxyglutamic acid***	9.8	19.5	0.35	blue
H	*erythro*-γ-Methylglutamic acid	4.4	18.7	1.30	blue
H	*threo*-γ-Methylglutamic acid	3.5	18.7	1.34	blue
G	γ-Methyleneglutamic acid	6.1	20.2	1.15	blue
F	*erythro*-γ-Methyl,γ-hydroxy-glutamic acid	13.8	19.7	0.99	grey-blue
M	γ-Ethylideneglutamic acid	1.9	16.9	1.36	grey-blue
N	*erythro*-γ-Ethylglutamic acid	4.4	17.2	1.56	blue
N	*threo*-γ-Ethylglutamic acid	3.2	17.2	1.61	blue
K	α-Aminoadipic acid	1.9	16.7	1.19	blue
L	α-Aminopimelic acid	1.0	14.7	1.42	blue
P1	γ-Hydroxy-α-aminopimelic acid***	1.4	14.9	1.08	blue
Q	Cysteic acid	32.4	25.5	0.39	blue
O	Homocysteic acid	29.2	23.3	0.48	blue
P	Cysteine sulphinic acid	29.4	24.1	0.66	blue
I	Asparagine	0.0	0.0	0.63	brown
J	Glutamine	0.0	0.0	0.78	blue

* As electro-osmotic flow of buffer takes place during electrophoresis, the migration distances recorded have been measured from the position of the common amide asparagine.
** Synthetic, configurations not known.
*** Natural isolate, configuration unknown.

(M) run close together, but the different ninhydrin colour reaction (Table I) allows accurate identification.

During chromatography, lateral diffusion of the spots does take place so the diastereo-isomers which were clearly separate as narrow bands after pH 3.4 electrophoresis have in some cases merged together. Electrophoresis alone at this pH could therefore be a useful method to separate these isomers. At pH 5.3, however, no useful separation of the isomers was found.

Electrophoresis at pH 5.3 showed that although many of the compounds did not separate from one another, they could be considered in groups. The sulphur amino acids were the fastest, although an increase in chain length decreased the migration distance. Likewise as the straight chain length increased from aspartic acid to α-aminopimelic acid, the migration distance decreased. Aspartic acid and the substituted aspartic acids behaved similarly. A hydroxyl-, methyl-, or methylene-substitution of glutamic acid only slightly altered the migration rate. Substitution of an ethylidene-, or ethyl-group however slowed the migration rate appreciably to values approaching α-aminoadipic acid. The substituted α-aminopimelic acid behaved in a similar manner to α-aminopimelic acid. Hence we can use the position of migration of an unknown compound after electrophoresis at this pH as an aid in tentatively arriving at its carbon skeleton. This is certainly the case when considered in conjunction with the chromatographic data.

From the chromatographic data also, structural correlations could be noted. Increase in chain length increased R_F value, while addition of a hydroxyl-group as a side chain decreased the R_F value. Addition of two hydroxyl-groups further reduced the R_F value. Addition of a methyl-group increased the R_F value, and an ethyl-group substitution increased it further still. The respective unsaturated compounds had slightly lower R_F values than the saturated compounds.

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