EFFECTIVE SOLVENT SYSTEMS FOR SEPARATION OF PTH-AMINO ACIDS ON POLYAMIDE SHEET

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ABSTRACT : *Two dimensional thin layer chromatography on polyamide sheets is a useful method for identification of PTH-amino acids which have very close Rf- values. The solvent systems described here are particularly effective and have an edge over other solvent systems used for the separation of PTH- amino acids. The solvent systems provide a clear separation of all PTH- amino acids except Ile / Leu in a relatively short time.*

INTRODUCTION :

Thin layer chromatography is a simple and rapid analytical method and has been employed for the identification of amino acids [1,2]. Two dimensional thin layer chromatography with two solvent systems is employed for the identification of amino acid derivatives [3]. However, the derivatives of hydrophobic amino acids have very close Rf values and are clustered

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[4,5]. The available solvent systems employed for PTH- amio acids [3-7], either do not separate all the amino acids or require more than two systems. Furthermore, the use of polyamide as thin layer support has not been explored well [8]. We are reporting two solvent systems for the separation of PTH- amino acids on polyamide plates by two dimensional thin layer chromatography. Both the solvent systems reported here provide complete separation of all PTH- amino acids except lle / Leu which overlap each other.
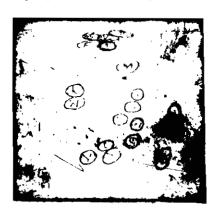
EXPERIMENTAL :
Materials

Standard PTH- amino acids and hexane were purchased from *Fluka* (Buchs, Switzerland). Propionic acid and acetic acid were from *E. Merck* (Darmstadt, W. Germany). Toluene was from BDH (Poole, England). All solvents used were analytical grade reagents. Polyamide plates were purchased from *Cheng Chin Trading* Co., *Taiwan.*

Methods

1 μl of a mixture of twenty PTH- amino acids (100 nmol / 100 μl) was applied on polyamide plates (5 × 5 cm) 0.5 cm away from the starting point. The plates were run in the first dimension with 40% propionic acid. The plates were then air dried. Two solvent systems (a) toluene, hexane, acetic acid (6 : 4 : 3), and (b)toluene, propionic acid (6 : 2) were used for separation in the second direction. The tanks were presaturated with the respective solvent systems and a temperature of 27°C - 30°C was maintained during these experiments.

The plates were developed by iodine vapours [9] for identification of PTH- amino acids. The plates were run till 0.5 cm short of the edge.

RESULTS AND DISCUSSION :

Fig. 1 shows the separation of PTH- amino acids in systems 1a and 1b, respectively and Table 1 and 2 provide the Rf values of the PTH-amino acids in these systems.

As can be seen from Fig. 1 all amino acids are well separated except for lle / Leu.

For PTH- amino acid which have very close Rf- values two dimensional thin layer chromatography on polyamide sheets is a quick qualitative method. The solvents systems described here are particularly effective and a clear separation of most PTH- amino acids can be achieved in a relatively short time. The detection limit in this case is 1 nmole. The system described is better than the available solvent systems normally used for separation of PTH amino acids on polyamide sheets [8].

a) 1st system: 40% Propionic acid
2nd system: Toulene: hexane: Acetic acid (6:4:3)

b) 1st system: 40% Propionic acid
2nd system: Toulene: Propionic acid (6:2).

Fig. 1 : Two dimensional Thin layer chromatography of PTH- amino acids.

Table 1 : Relative mobility (Rf) of PTH- amino acids in system 1a.

S. No	Amino acid	1st dimension Rf$_1$	2nd dimension Rf$_2$
1	Ala (A)	0.929	0.084
2	Cys (C)	0.516	0.205
3	Asp (D)	0.555	0.169
4	Glu (E)	0.613	0.289
5	Phe (F)	0.426	0.711
6	Gly (G)	0.697	0.361
7	His (H)	0.903	0.169
8	Ile (I)	0.419	0.807
9	Lys (K)	0.284	0.397
10	Leu (L)	0.419	0.807
11	Met (M)	0.645	0.506
12	Asn (N)	0.710	0.229
13	Pro (P)	0.600	0.843
14	Gln (Q)	0.697	0.301
15	Arg (R)	0.864	0.072
16	Ser (S)	0.684	0.181
17	Thr (T)	0.542	0.681
18	Val (V)	0.568	0.771
19	Trp (W)	0.297	0.331
20	Tyr(Y)	0.387	0.144

Table 2 : Relative mobility (Rf) of PTH- amino acids in system 1b.

S. No	Amino acid	1st dimension Rf$_1$	2nd dimension Rf$_2$
1	Ala (A)	0.857	0.326
2	Cys (C)	0.224	0.184
3	Asp (D)	0.469	0.102
4	Glu (E)	0.531	0.367
5	Phe (F)	0.326	0.888
6	Gly (G)	0.643	0.551
7	His (H)	0.898	0.408
8	Ile (I)	0.306	0.939
9	Lys (K)	0.214	0.571
10	Leu (L)	0.306	0.939
11	Met (M)	0.571	0.735
12	Asn (N)	0.643	0.337
13	Pro (P)	0.531	0.969
14	Gln (Q)	0.612	0.449
15	Arg (R)	0.816	0.102
16	Ser (S)	0.612	0.204
17	Thr (T)	0.449	0.877
18	Val (V)	0.449	0.939
19	Trp (W)	0.214	0.469
20	Tyr (Y)	0.306	0.102

Abbreviations

PTH: Phenylthiohydantoin   F: Phenylalanine
S: Serine   G : Glycine
I : Isoleucine   E : Glutamic acid
A : Alanine   L : Leucine
V : Valine   P : Proline
K : Lysine   H : Histidine
R : Arginine   Y : Tyrosine
D : Aspartic acid   C : Cystine
T : Threonine   W : Tryptophan

REFERENCES :

[1] Mesrob, B., and Holeysovsky, V., *"Differentiation of dimethylaminonaphthalene sulfonic derivatives (DNS) of valine, leucine, and isoleucine by thin- layer silica gel chromatography"*, *J. Chromatog.* 21 , 135- 137 (1966).

[2] Niederwieser, A., *"Methods in Enzymol".*, 25b 60 (1972).

[3] Chang, J. Y, Brauer, D., and Wittmann-Liebold, B., *Microsequence analysis of peptides and proteins using 4- N, N-dimethylaminoazobenzene, 4- isothiocyanate double coupling method"*, FEBS Lett., 93 , 205- 214 (1978).

[4] Trzaska, J., and Kowkabany, G. N., *"Paper chromatographic investigation of the homologous series of amino acids from glycine to α–amino- tetradecanoic acid",J. Chromatog.* 26 , 141- 150 (1967).

[5] Haurowitz, F., *"The Chemistry and Functions of Protein"*, Academic Press, New York, p. 31- 33 (1983).

[6] Edman, P., and Henschen, A., in: *"Protein Sequence Determination (ed. S. B.*

Needleman)". Springer Verlag, Berlin-Heidelberg- New York, p. 235- 248 (1975).

[7] Von Bahr- Lindstrom, H., Hempel, J., and Joernvall, H., *"Byproducts as an aid in residue identification during peptide sequence analysis with dimethylaminoazobenzene isothiocyanate"*, *J. Protein Chem.* **1** , 257- 262 (1982).

[8] Kulbe, K. D., and Konschewski, H., *"Rapid separation of phenylthiohydantoin (PTH) amino acids by thin layer chromato- graphy on polyamide glass plates"*, *Anal. Biochem.* **44** , 548- 558 (1971).

[9] Mangold, H. K., *"Synthesis of unsaturated fatty aldehydes"*, *J. Org. Chem,* **24** , 405- 407 (1959).