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Note

Thin-layer chromatography of methylthiohydantoin amino acids

P. RABIN and A. DARBRE

Department of Biochemistry, King's College, Strand, London WC2R 2LS (Great Britain)

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Edman[1] introduced the method of sequencing of peptides or proteins in which the 3-phenyl-2-thiohydantoin (PTH) of the amino acid at the N-terminal end was obtained by stepwise degradation. This method was automated[2,3], with identification of the PTH amino acid using thin-layer chromatography (TLC) or electrophoresis on silica gel plates. TLC of PTH amino acids on polyamide-coated glass plates was used with an iodine–sodium azide spray reagent[4] and on polyamide-coated plastic sheets with a fluorescent indicator[5] for their detection. PTH amino acids could also be identified by gas–liquid chromatography (GLC)[6–8]. PTH arginine did not give a peak on GLC.

A sequencing method was described in which the 3-methyl-2-thiohydantoin (MTH) derived from the amino acid at the N-terminal end of the peptide was identified by GLC[8–10]. However, MTH arginine required prior treatment with cyclohexanedione followed by trimethylsilylation for GLC analysis[10].

Using manual methods for the sequencing of peptides[11], we used GLC for the identification of MTH amino acids. Because arginine could not be identified, we required an independent method, similar to that used for PTH amino acids, which were analyzed by two methods, *viz.* GLC in parallel with TLC[12]. It was stated[9] that TLC was a standard technique for identification of PTH and MTH amino acids (for reviews, see refs. 13 and 14). Stepanov and Lapuk[15] described four solvent systems for the one-dimensional TLC separation of many, but not all of the MTH amino acids on silica gel glass-coated plates. We describe here a separation achieved in less than 1 h using two-dimensional TLC on polyamide pre-coated plates.

EXPERIMENTAL

Materials

Reagent-grade toluene and glacial acetic acid were obtained from Fisons (Loughborough, Great Britain) and *n*-heptane and amino acids from BDH (Poole, Great Britain). Polygram polyamide-6/UV_{254} pre-coated (0.1 mm) plastic sheets (20×20 cm) and a Desaga UVIS lamp with emission at 254 nm were purchased from Camlab (Cambridge, Great Britain). The crystalline MTH amino acid derivatives were prepared from D,L-amino acids using the methods published[16–18]. MTH serine could not be crystallized and was prepared freshly from D,L-serine by adaption of the method of Peterson *et al.*[11].

Method

Two-dimensional ascending chromatography was carried out on a pre-coated polyamide plate (10×10 cm) cut from the larger sheets purchased. The coating incorporated a fluorescent indicator. The plates were sectioned as shown in Fig. 1 by removing 3-mm strips of coating at right angles to each other 8 cm from the starting edges to stop the solvent flow. The origins for the unknown sample and the standard MTH amino acids were 1 cm from the edges of their respective plates. Glycine was chromatographed as the marker with both solvents in the outer zones of each plate. It was better to restrict the spot at the origin to about 2 mm diameter to give a good separation, as reported previously[5]. Usually 0.5 to 1.0 μl solution of MTH amino acids in pyridine was spotted on to the plate with a disposable micropipette. A stream of cold air or nitrogen was used to evaporate the solvent. Chromatography was carried out in a small glass tank with glass lid.

The plate was developed with Solvent 1, toluene–*n*-heptane–glacial acetic acid (60:30:20), for approximately 15 min and dried with cold air from a hair-drier. After turning through 90°, the plate was developed with Solvent 2, 35% acetic acid. This required about 30 min. After hot air drying (quicker) the plate was viewed under UV irradiation at 254 nm. The MTH amino acids were seen as purple spots on a green background and their positions noted by circling with a pin. A clear plastic template showing the positions of all the derivatives was used to aid identification. This was lowered on to the unknown plate and its position adjusted to coincide with that of the MTH glycine.

TABLE I

$R_F \times 100$ VALUES FOR 19 MTH AMINO ACID DERIVATIVES IDENTIFIED BY TLC ON POLYAMIDE-COATED PLASTIC PLATES

MTH amino acid	*$R_F \times 100$ value*	
	Solvent 1	*Solvent 2*
Alanine	67	70
Arginine	02	92
Asparagine	26	82
Aspartic acid	19	70
Cysteine	45	45
Glutamic acid	33	70
Glycine	54	74
Histidine	08	93
Isoleucine	89	43
Leucine	86	43
Lysine	22	60
Methionine	74	52
Phenylalanine	80	35
Proline	90	63
Serine	55	61
Threonine	57	45
Tryptophan	42	21
Tyrosine	18	43
Valine	82	57

RESULTS AND DISCUSSION

Many different solvent systems were tried. Solvent 1 was a modification of Solvent III and Solvent 2 was identical to Solvent V described by Kulbe[4]. Improved separations were not obtained by pre-saturating the atmosphere as claimed by Summers *et al.*[5].

Table I shows the $R_F \times 100$ values for 19 MTH amino acids. These are the average values for six replicates. The spots were easily identified and reproducibility was good. Fig. 1 gives the schematic representation of a chromatogram with 19 MTH amino acids. The limit of sensitivity was 0.05 to 0.1 nmoles for each derivative[5]. Solvent 2 affected the appearance of the plate under UV light. Approximately one third of the lower end of the plate after development with Solvent 2 was very dark but only MTH tryptophan was found in this zone. All the derivatives were separated except for MTH isoleucine and MTH leucine, but these could be easily distinguished by GLC. We could not distinguish between MTH glutamic acid and MTH glutamine with any TLC solvent system. The amide may have been hydrolyzed to its corresponding acid during its preparation. It should be noted that TLC separations of PTH amino acids on polyamide-coated plates with the same solvent systems as published by Kulbe (Fig. 1b)[4] and by Summers *et al.* (Fig. 1)[5], disagree markedly over the relative positions of PTH glutamic acid and PTH glutamine, although they agree in most other respects.

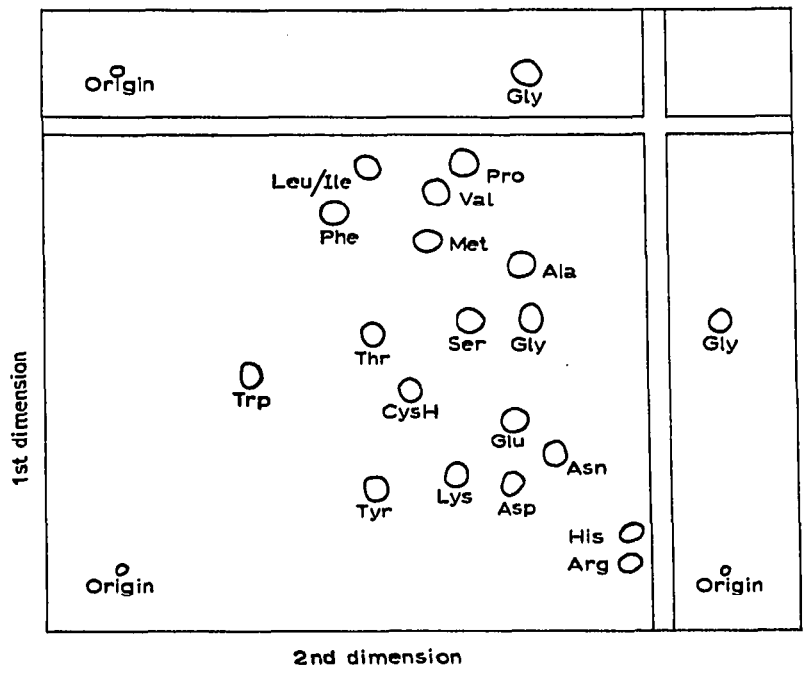

Fig. 1. Schematic representation of two-dimensional TLC chromatography of 19 MTH amino acids. For the first dimension Solvent 1 was used, for the second Solvent 2.

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