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Note

Miniature thin-layer chromatography of phenylthiohydantoin amino acids Application of automatic Edman degradation

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Amino acid sequence determinations can be carried out by automatic Edman degradation[1,2]. In order to obtain quantitative data, the most suitable method is gas chromatographic (GC) analysis[3], but often it is best simultaneously controlled by thin-layer chromatography (TLC).

In this paper, we describe an adaptation of previous methods[1,4], involving 1/3-size silica gel sheets, which is very sensitive (at the 10^{-10} *M* level) and can be carried out in 15 min.

MATERIALS AND METHODS

Automatic Edman degradation

A normal human haemoglobin α-chain was prepared[5] and submitted to automatic Edman degradation with a Beckman 890 B sequenator following the procedure of Edman and Begg[6,7].

Identification of phenylthiohydantoin amino acids (PTHs)

Silica gel thin-layers of dimensions 20 × 20 cm on plastic sheets were obtained from Merck (Kieselgel 60 F_{254}). Before use, the layers were soaked in a 1% (w/v) solution of soluble starch and dried at 80° for 30 min. For conventional TLC, the procedure of Boigné *et al.*[1] was followed. For the micro-TLC technique, the sheets were cut with small scissors and the edges scraped with a scalpel. A glass holder to keep the sheets vertical was designed. Development was carried out at room temperature in a cylindrical glass jar, height 17 cm, diameter 10 cm.

Standard mixtures of PTHs and the chromatographic solvents were as described by Brenner *et al.*[8] and Boigné *et al.*[1]. Table I summarizes the procedure followed.

The PTHs were detected by using a starch–iodine spray[1]; PTH-Arg and PTH-His were identified by specific staining[2,9].

RESULTS AND DISCUSSION

Micro-TLC is presently used for the separation of dansyl amino acids[10,11] but not for PTH. The main difficulty is related to the mechanical properties of the silica

TABLE I

PROCEDURE FOLLOWED FOR TLC OF PTHs

After ascending chromatography in one solvent, the layers were dried and then another ascending chromatography was carried out in the same direction in a different solvent.
It is well known that the PTHs can be resolved and identified by using two different combinations of chromatographic solvents. For instance, in solvents V plus I, PTH-Val and PTH-Met are separated but not PTH-Phe; PTH-Phe is resolved in solvent VII.

Procedure	*Solvent*			*Conventional technique[4] on 20 × 20 cm plates**		*Micro-scale technique on 6.3 × 6.3 cm plates***	
	No.	*Components*	*Proportions (v/v)*	*Distance of solvent front (cm)*	*Time of migration (min)*	*Distance of solvent front (cm)*	*Time of migration (min)*
Standard technique for screening	V	Chloroform	90				
		Methanol	10	8	60–90	2.7	3–5
	I	Chloroform	100	15	120–135	5.0	6–8
For Asn, Gln, Glu, Asp, His, Arg***	VI	Chloroform	80	8	90–120	2.7	6–7
		Methanol	20				
	VI	Chloroform	80	15	240–270	5.0	8–10
		Methanol	20				
For Val, Phe, Leu, Ile, Trp, Tyr	VII	*n*-Heptane	70	15	90–120	5.0	10
		Pyridine	30				
	VII	*n*-Heptane	70	15	90–120	5.0	10
		Pyridine	30				

* Sensitivity 1–0.5 nmole.
** Sensitivity <0.3 nmole.
*** PTH-His and PTH-Arg are more easily identified by specific staining-.

gel layer. If it is cut without caution, the edges will crack and the spots will not migrate in a straight line. We found that a size of 6.3 × 6.3 cm gave the best results; thin layers of smaller size (5 × 5 cm, for instance) failed to give satisfactory results, most probably because it is very difficult to obtain undamaged edges.

The starch–iodine spray permits the detection of less than 0.5 nmole of PTH (0.3 nmole in routine use), and the substances appear immediately as white spots on a brownish background.

In order to test our procedure, we degraded the α-chain of a normal human haemoglobin in the sequenator for 39 cycles; the PTHs were identified in parallel by GC[3], conventional TLC[1] and micro-TLC. Fig. 1 gives an example of the results obtained, and shows that the quality of separation of PTHs is similar by the conventional method and by our procedure.

By using two different combinations of solvents, we were able to identify at least eight PTHs in half an hour; in the same time, we can identify only one PTH by GC. Micro-TLC was found to be the most rapid method for the determination of amino acid sequence with a sequenator. Nevertheless, GC is the only method suitable for quantification, and we therefore believe that the two techniques must be carried out together.

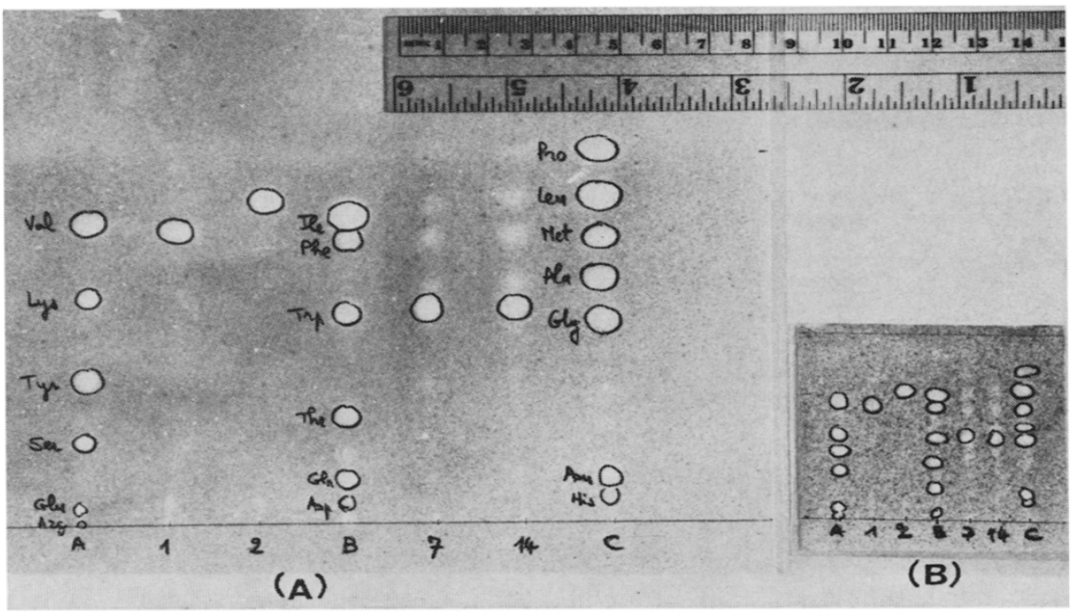

Fig. 1. Comparison of the results obtained by the conventional TLC of PTH on 20 × 20 cm plates (A) with our procedure using 6.3 × 6.3 cm TLC plates (B). The solvents used are V and I. Three PTH standard mixtures (1 nmole/μl in ethyl acetate) were used: A, Arg, Glu, Ser, Tyr, Lys, Val; B, Asp, Gln, Thr, Trp, Phe, Ile; and C, Asn, His, Gly, Ala, Met, Leu, Pro (in ascending order). Fractions obtained with the sequenator are numbered 1, 2, 7 and 14.
The PTHs were detected after spraying under a hood with a mixture of a 10% (w/v) solution of sodium azide–0.1 N I_2–water, 15:10:25 (v/v/v). The figure shows that the quality of the separation of the PTHs is as good in the micro-TLC form (B) as in the conventional form (A).

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