Journal of Chromatography, 115 (1975) 629–632

CHROM. 8671

Note

Improved separation of the methyl- and phenylthiohydantoins of arginine, cysteic acid and histidine by micropolyamide thin-layer chromatography

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(Received July 21st, 1975)

Since the Edman degradation has become automated[1,2], improvements in the methods for the rapid and sensitive identification of the thiohydantoin derivatives of split off amino acids remain one of the main goals in the determination of the primary structure of proteins and peptides. Apart from thin-layer chromatography, the most commonly used techniques for the separation and also for the quantitation of phenyl- (PTH) and methyl- (MTH) thiohydantoin derivatives obtained in sequential degradation of proteins are gas–liquid chromatography[3,4] and mass spectrometry[5,6].

The main problems with all of these methods are the separation and identification of the histidine and arginine derivatives. These derivatives either have low volatility, are thermally degraded during analysis or require large amounts for identification by chemical methods. Hydrolysis back to the free amino acids and their determination in an amino acid analyzer are both laborious and difficult to carry out on the micro-scale.

For high-sensitivity sequencing work, phenyl [^{35}S]isothiocyanate can be used[7]. However, this reagent doubles the costs of the sequencer chemicals in solid-phase work.

Based on our previous methods for very sensitive and rapid multi-sample identification of PTH- and MTH-amino acids[8–10], we have developed two solvent systems that are suitable for separating and identifying the arginine, histidine and cysteic acid ($CySO_3H$) derivatives on polyamide sheets in an extremely short time.

MATERIALS AND METHODS

PTH standards were products from Serva (Heidelberg, G.F.R.). The MTH derivatives of arginine and cysteic acid were purchased from Pierce (Rockford, Ill., U.S.A.). MTH-His was synthesized as described by Kulbe and Nogueira-Hattesohl[10]. Micropolyamide F-1700 sheets from Schleicher & Schüll (Dassel, G.F.R.) or from Cheng Chin Trading Co. (Taipeh, Taiwan) were used with similar results. Solvents of analytical-reagent grade were obtained from Riedel-de Haen (Seelze, G.F.R.) and Merck (Darmstadt, G.F.R.) (butanol-1, *tert.*-butanol).

The derivatives of the polar amino acids arginine, cysteic acid and histidine, which remain in the aqueous phase during extraction of methyl- or phenylthiohydantoins, were recovered by lyophilization and re-dissolved in methanol. After the

application of about 1 μl of sample on to the 5 × 5-cm sheets using disposable microcaps, ascending thin-layer chromatography was carried out in covered 250-ml glass beakers containing 2–5 ml of either solvent A or solvent B, which had the following compositions: A, ethyl acetate–butanol-1–glacial acetic acid (35:10:1); B, ethyl acetate–*tert.*-butanol–glacial acetic acid (35:10:1). Each of the solvents contained 250 mg/l of the fluorescent indicator 2-(4'-*tert.*-butylphenyl)-5-(4"-biphenylyl)-1,3,4-oxadiazole (butyl-PBD).

Chromatography was terminated after about 10 min. The sheets were removed, dried and examined under shortwave UV light (254 nm). The histidine derivatives are easy to identify by their yellow colour in the ultraviolet and visible range.

RESULTS AND DISCUSSION

Using one-dimensional chromatography, the MTH and PTH derivatives of arginine, histidine and cysteic acid were clearly separated in each of the new solvent systems (Figs. 1 and 2). The detection limit of this method varies between 50 and 300 pmoles, as demonstrated previously[8–10].

There are only minor differences in the separations obtained with polyamide sheets supplied by Schleicher & Schüll and Cheng Chin. A pre-run with the latter plates, however, is essential for good separations and improved contrasts. The R_F values for the MTH and PTH derivatives in solvents A and B for both types of sheets are given in Table I.

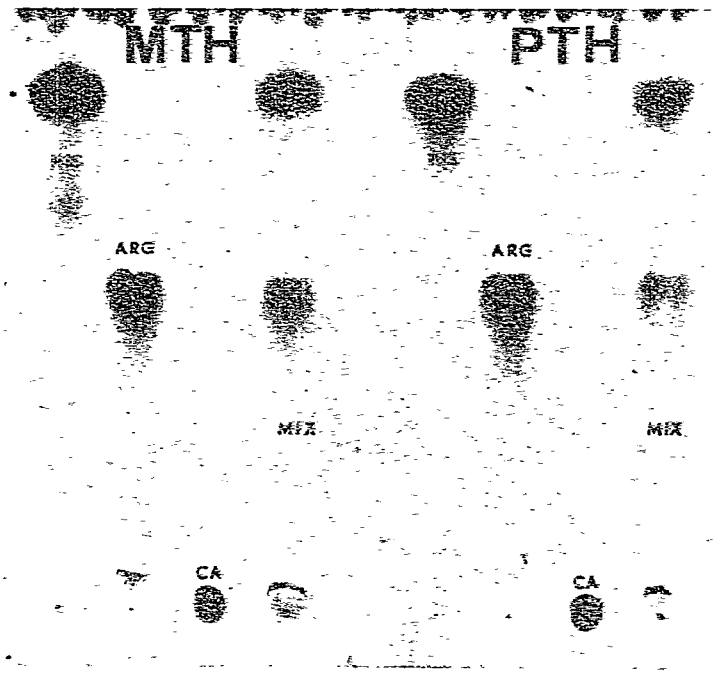

Fig. 1. UV photograph of an original chromatogram on 5 × 5-cm F-1700 polyamide sheet from Schleicher & Schüll in solvent A (ethyl acetate–butanol-1–glacial acetic acid, 35:10:1, containing 250 mg/l of butyl-PBD). CA refers to cysteic acid derivatives.

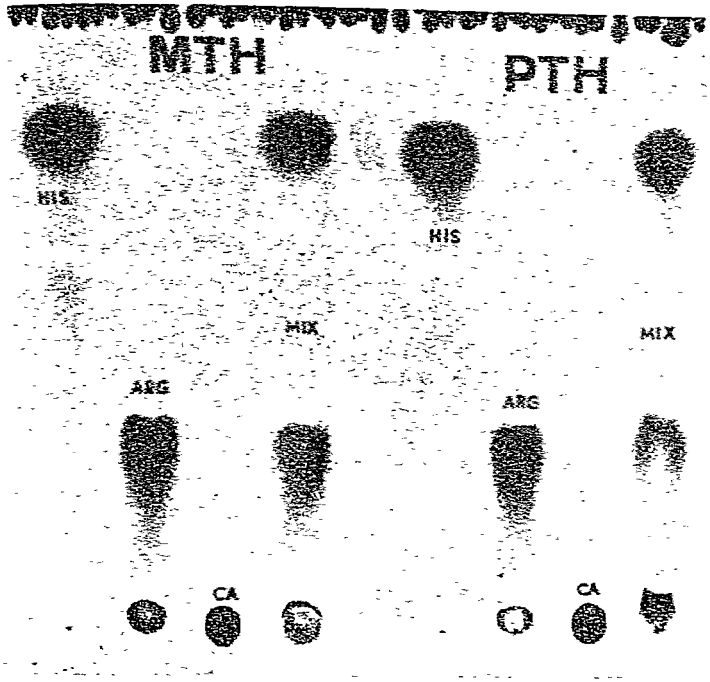

Fig. 2. UV photograph of an original chromatogram on 5 × 5-cm F-1700 polyamide sheet from Schleicher & Schüll in solvent B (ethyl acetate–*tert.*-butanol–glacial acetic acid, 35:10:1, containing 250 mg/l of butyl-PBD). CA refers to cysteic acid derivatives.

The results for the separation of arginine and histidine thiohydantoins obtained with the new solvent systems are much better than those previously reported by Rabin and Darbre[11] and by our group[8–10]. If both sides of a 5 × 5 cm plate are used for application of samples and standard, at least 12 individual derivatives can be processed within 10 min.

In the future, the more frequent use of methyl isothiocyanate instead of phenyl isothiocyanate in automated liquid- and solid-phase sequencing work is to be expected.

TABLE I

R_F VALUES FOR THE MTH AND PTH DERIVATIVES OF ARGININE, HISTIDINE AND CYSTEIC ACID ON SCHLEICHER & SCHÜLL AND ON CHENG CHIN POLYAMIDE SHEETS AFTER CHROMATOGRAPHY IN SOLVENTS A AND B

Solvent	*Amino acid*	*Schleicher & Schüll sheets*		*Cheng Chin sheets*	
		MTH	*PTH*	*MTH*	*PTH*
A	Arg	0.50	0.50	0.44	0.44
	$CySO_3H$	0.00	0.00	0.00	0.00
	His	0.85	0.83	0.82	0.82
B	Arg	0.29	0.28	0.30	0.30
	$CySO_3H$	0.00	0.00	0.00	0.00
	His	0.76	0.78	0.80	0.79

This reagent introduces a lower level of background impurities into the samples and its use therefore should allow the identification of products of more successive degradation cycles than before. Because in methyl isothiocyanate degradation conversion to the MTH amino acids occurs spontaneously, the time required for each cycle will be shortened[12].

To summarize, the new solvents offer a sensitive and rapid means for the identification of the PTH and MTH derivatives of arginine and histidine, which previously were very difficult to separate by other techniques such as gas–liquid chromatography, thin-layer chromatography and mass spectrometry. The method is very rapid and simple to carry out and can therefore be used for routine control of the aqueous extraction phase after the conversion to the thiohydantoin amino acids. It will be a valuable adjunct to the quantitative methods applied in manual and particularly automated sequence work.

ACKNOWLEDGEMENT

The skilful technical assistance of Mrs. Rose Keese is gratefully acknowledged.

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