THE SEPARATION AND QUANTITATIVE IDENTIFICATION OF PTH-AMINO ACIDS BY HIGH PRESSURE LIQUID CHROMATOGRAPHY

J. X. de VRIES, R. FRANK and Chr. BIRR

Max-Planck-Institut für medizinische Forschung, Abteilung Naturstoff Chemie, D-69 Heidelberg, Jahnstr. 29, W. Germany

Received 21 March 1975

1. Introduction

The modified automatic stepwise degradation of peptides bound to a solid phase was described in a previous paper [1], and was used to monitor Merrifield type solid phase synthesis [2]. In this connection, it was desirable to use a method for the qualitative and quantitative analysis of the phenylthiohydantoin (PTH) derivatives of the amino acids. PTH derivatives have been previously identified by paper chromatography [3], thin-layer chromatography [4–6], gas liquid chromatography [7,8], mass spectroscopy [9,10] and, recently, high pressure liquid chromatography (HPLC) [11–14]; we have improved the separation and quantitative identification of the PTH derivatives [1,2], using the latter on inverse phase with aqueous organic solvent gradient elution.

2. Materials

2.1. *Apparatus*

A DuPont Model 830 Liquid Chromatograph was used, equipped with a gradient elution accessory (Du Pont) and a model SF 770 Spectroflow variable wavelength uv photometer (Schoeffel Instrument Corp.). Stainless steel columns (500 X 2.1 mm) were filled with the stationary phase using a Chromatronix Dry Column Packer. Sample injections were made through a silicone septum, with a Hamilton HP 305 syringe.

2.2. *Chemicals*

'Permaphase ETH' stationary phase was a product of Du Pont Instruments, and 'Bondapak C 18 Corasil' from Waters Assoc. The reference PTH-amino acids were obtained from Serva (Heidelberg), and some were synthesized in this department.

3. Methods

PTH-amino acids obtained directly from the automatic degradation were dissolved in acetonitrile (or methanol) (200 μl) and 5 or 10 μl aliquots were injected into the chromatograph. Analyses were performed in the linear gradient mode (see table 1), and the absorption recorded at 269 nm. Quantitative calibration of the HPLC system was performed for

Table 1
HPLC systems

System number	Stationary phase	Linear solvent gradient	Initial-final organic phase, conc. %	Gradient rate, %/min	Solvent rate, ml/min	Pressure, psi
1	Permaphase ETH	H_2O–CH_3CN	0–30	2%	0.7	500
2	Permaphase ETH	H_2O–CH_3OH	0–35	1%	0.7	550
3	Bondapak C 18/corasil	H_2O–CH_3CN	0–40	2%	0.6	600

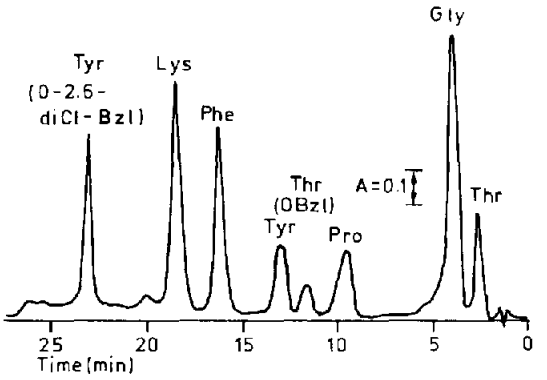

Fig.1. Analysis of a standard mixture of PTH-amino acids. The number of the PTH-amino acids corresponds to those of table 2. Conditions: see Materials and methods and table 1 (system 1); gradient (0–50%).

each PTH-amino acid; peak areas were evaluated by the height times width at half-height, method [15].

4. Results

PTH of simple amino acids, as well as those of protected ones, were eluted as shown in fig.1 and table 2, using system 1 (table 1); system 2 is similar. More polar PTH derivatives, such as Asp, Asn, Glu, Gln were analyzed with system 3 [14].

PTH-amino acids were identified using the retention time or capacity factor* (± 1%) and the relative amounts evaluated using peak areas and calibration curves. The amounts of the main PTH-amino acid were in the region of 2–10 nmol (± 5% relative error); peaks with areas 0.5% of the main peak could easily be detected without varying the sensitivity; varying the latter, quantitative results could be obtained with 20 pmol substance.

Elution modes in table 1 may be optimized for each particular mixture of PTH-amino acids, reducing further analysis times. Fig.2 is an example of HPLC

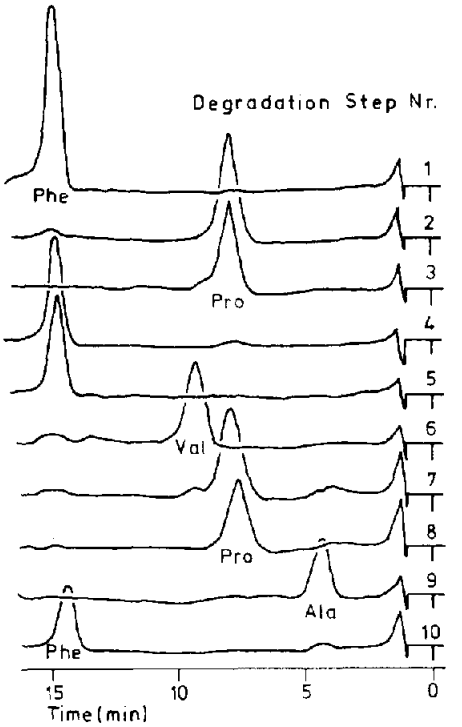

Fig.2. Elution diagrams of PTH-amino acids obtained from the degradation of antamanide. Conditions: same as in fig.1 (system 1).

* The capacity factor is defined by the relationship: $k' = \frac{t_R - t_0}{t_0}$, where t_R is the retention time of the sample (measured from the injection time = 0), and t_0 that of a non-retained component.

Table 2
Capacity factors (k') of PTH-amino acids

1. Thr: 0.65	2. Gly: 1.46	3. Ala: 2.60
4. Pro: 5.60	5. Val: 7.02	6. Met: 7.50
7. Tyr: 8.34	8. Leu: 8.36	9. Ileu: 9.10
10. Phe: 11.40	11. Lys: 12.42	12. Trp: 12.44
13. Cys(Acm): 1.0	14. Arg(NO_2): 2.80	15. Thr(OBzl): 7.83
16. Arg(Tos): 10.4	17. Tyr(2.6-diCl-Bzl): 16.15	18. Asp: 6.5*
19. Asn: 8*	20. Gln: 11.5*	21. Glu: 12*

The conditions are for system 1 (table 1); values marked* are for system 3 (table 1).

analysis for the control of the sequential uniformity of a peptide (a linear precursor of antamanide [16]) synthetized by the solid phase method and degraded as described in the preceding paper [1].

Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft.

References

[1] Birr, Chr. and Frank, R. (1975) FEBS Lett. preceding paper.
[2] Birr, Chr. and Frank, R. (1975) FEBS Lett. following paper.
[3] Schroeder, W. A. (1972) in: Methods in Enzymology (Hirs, C. H. W. and Timasheff, S. N., eds), Vol. 25, pp 298–323, Academic Press, New York.
[4] Edman, P. (1970) in: Protein Sequences Determination (Needleman, S. K., ed) p. 211, Springer Verlag, Heidelberg.
[5] Kulbe, K. D. (1970) Analyt. Biochem. 44, 548.
[6] Laursen, P. A. (1969) Biochem. Biophys. Res. Comm. 37, 663–667.
[7] Pisano, J. J. and Bronzert, T. J. (1969) J. Biol. Chem. 244, 5597–5607.
[8] Pisano, J. J., Bronzert, T. J. and Brewer, H. B. (1972) Anal. Biochem. 45, 43–59.
[9] Hagenmeyer, H., Ebbighausen, W., Nicholson, G. and Vötsch, W. (1970) Z. Naturforsch. B 25, 681–689.
[10] Lovins, R. E., Craig, J., Thomas, F. and Kinney, C. M. (1972) Anal. Biochem. 47, 539–549.
[11] Frankhauser, P., Fries, P., Stahala, P. and Brenner, M. (1974) Helv. chim. Acta 57, 271–277.
[12] Graffeo, A. P., Haag, A. and Karger, B. L. (1973) Anal. Letters 6, 505–511.
[13] Frank, G. and Strubert, W. (1973) Chromatographia 6, 522–524.
[14] Zimmermann, C. L., Pisano, J. J. and Appella, E. (1973) Biochem. Biophys. Res. Comm. 55, 1220–1224.
[15] Baumann, F. (1971) in: Basic Liquid Chromatography (Hadden, N., ed) p. 8–5, Varian Aerograph, USA.
[16] Wieland, Th. and Birr, Chr. (1972) Ann. Chem. 757, 136–146.