DIRECT CONVERSION TO PURE PTH-AMINO ACIDS WITHOUT MANIPULATIONS IN THE SOLID PHASE EDMAN DEGRADATION (LAURSEN VARIANT) OF PEPTIDES

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1. Introduction

The Edman degradation of peptides and proteins [2] in solution and in heterogeneous phase can be precisely performed automatically at the present time [3]. Usually the automatic process on each stage of degradation ends with the cleavage and extraction of an amino acid thiazolinone, which subsequently has to be manually converted into the characteristic phenylthiohydantione of the amino acid (PTH) before identification. The quantity and purity of each PTH derivative, which determine the accuracy of PTH identification and therefore the reliability of sequence analysis, both depend upon completion of all particular chemical reactions during degradation and conversion and of their entire protection from oxygen. Therefore each saved manipulation leads to a methodical advantage.

Except for direct conversion of thiazolinones to PTH-amino acids the Laursen method [4] of Edman degradation on peptides bound to a solid phase satisfies best the demands for precise sequence determination. We succeeded in eliminating these remaining shortcomings of manual conversion and purification: Pure PTH-amino acids are directly obtained from automatic degradation of peptides on solid phase by injecting H_2O to the solution of the thiazolinones in TFA and passing the mixture through an additional heated reaction coil for conversion. The quantitative identification of the PTHs is performed by high pressure liquid chromatography (HPLC), which in a single run permits the separation of all PTH-amino acids cited below in the sequences degraded [5].

2. Materials

The peptides degraded [6] had been synthesized on polystyrene gel phases [7] by the Merrifield method [8] and therefore are already attached to insoluble polymers. The sequences investigated, usually bound to the support by a benzylester-type of linkage are: Phe–Pro_2–Phe_2–Val–Pro_2–Ala–Phe–*O*-polystyrene [2% divinylbenzene (DVB)], linear precursor of antamanide [9]; Arg–Gly–Phe_2–Tyr–Thr–Pro–Lys–Thr–*O*-polystyrene (0.5% DVB), human insulin B 22–30 [10]; and Arg–Leu–Glu–Arg–Leu_2–Gln–Gly–Leu–Val–NH-benzhydrylpolystyrene (1% DVB), secretin 18–27 [11].

The degradations were performed on a Sequemat apparatus (Sequemat Inc., Watertown, Mass. USA) modified by us for direct automatic conversion (see fig.1). Trifluoroacetic acid (TFA), phenyl isothiocyanate (PITC), *N*-methylmorpholine, pyridine, 1,2-dichlorethane (DCE), methanol (MeOH), acetonitrile and H_2O were distilled under N_2 and stored in brown bottles under N_2.

Parts for direct conversion modification: A second thermostat, a Teflon solenoid valve (Valcor Engineering Corp., Kenilworth, N.J., USA); a water-circulated screwed reaction bath (100 ml; brass) containing a reaction coil (Teflon tubing, 1.8 mm outer diameter, 1.0 mm inner diameter, 320 cm length); a T-junction (Kel F); a peristaltic pump (LKB Perpex 10200); H_2O reservoir; PTH sample collector rebuilt for 12 conical bottom flasks (25 ml); N_2-line (protective

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* [1] Part of diploma work (1975) Heidelberg.

gas), H_2O-line, and by-pass (Teflon tubing, 1.8 mm outer diameter, 1.0 mm inner diameter).

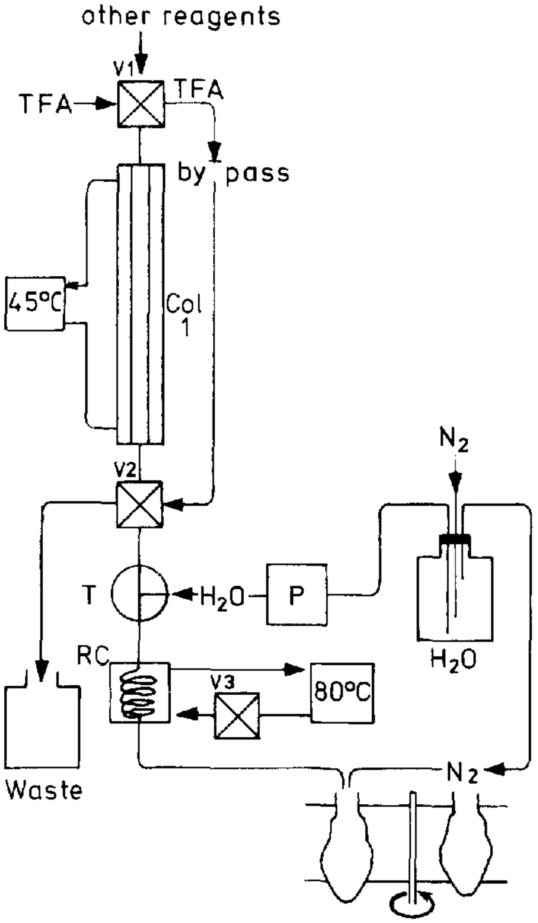
3. Methods

3.1. *Apparative modifications*

The dual column system of the sequemat is used; column 2 (Col 2) is skipped. Partition valve V_1 (Col 1–Col 2 change-over) instead of Col 2 is directly connected by Teflon tubing (by-pass) to partition valve V_2 (waste-collection change-over). The T-junction (T) for H_2O injection is set into the line for sample collection directly behind V_2. The second position on T is connected to the H_2O-line (from peristaltic pump P and H_2O reservoir), the third one combines T to reaction coil RC. The circulation of water in the screwed reaction bath of RC (from extra thermostat, 80°C) is controlled by Teflon solenoid valve V_3. The flow out of RC ends on top of the PTH sample collector, mounted in a side by side parallel position to a N_2 outlet (protective gas first bubbles through H_2O reservoir). The valve for partition of sample collection (dual column program) is skipped.

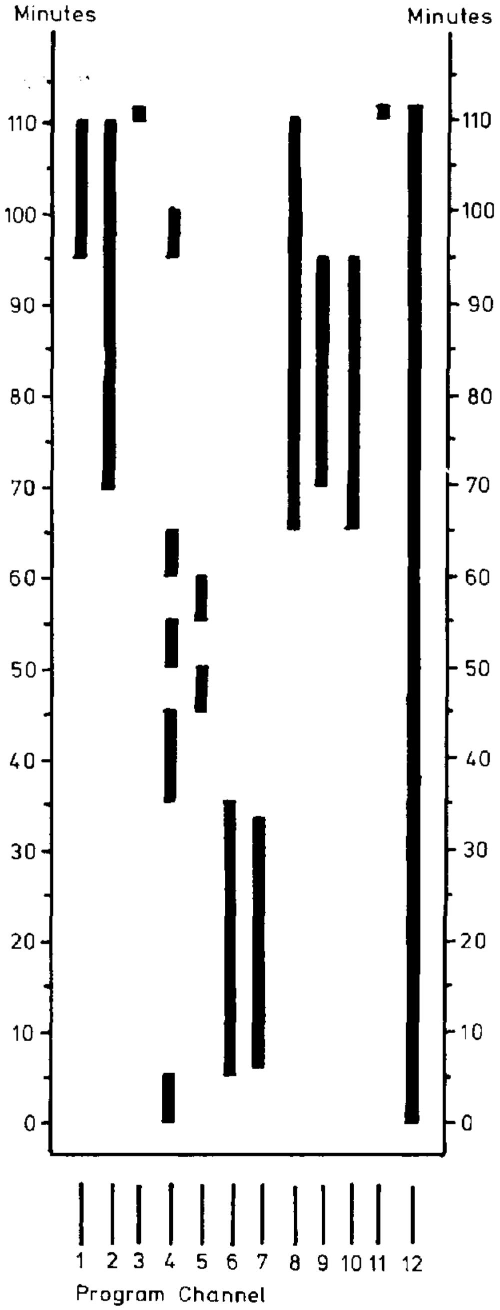
3.2. *Operation of the modified system*

Col 1 is washed for 5 min. with MeOH before thiazolinone formation. The cleavage of thiazolinones in Col 1 (45°C) is performed as usual. The signal for pumping of TFA is used to open valve V_3 for heating of RC (80°C). Program channel 2 is used to activate peristialtic pump P, which injects H_2O in T (0.2 ml/min). After TFA (0.05 ml/min) for 30 min is passed through Col 1 (45°C)–T(H_2O injection)–RC (80°C, PTH formation) to sample collector, V_1 switches TFA from top of Col 1 to the by-pass position and connects MeOH-line to Col 1. Simultaneously V_2 switches the outlet of Col 1 to waste and combines the by-pass line to T (H_2O-injection continued)–RC (80°C)–sample collector. In this position TFA for an additional 15 min is pumped by-passing Col 1. Col 1 is rinsed simultaneously with MeOH for 5 min. Termination of TFA-flow shuts V_3 (reaction bath of RC cools down). Pump P is stopped simultaneously. RC remains filled with TFA/H_2O. When partition valve V_1 switches the TFA-line back to top of Col 1 the by-pass is not rinsed with solvent. The other operations of degradation are programmed as usual. The PTH samples in the conical bottom flasks (20% TFA in H_2O) are frozen, lyophilized and dissolved in 200 μl acetonitrile; 5 μl are injected into HPLC for quantitative PTH identification. With remaining PTH solutions the uv spectra are recorded to determine the ratio of min–max absorption at 245 and 269 nm ($\frac{\varepsilon_{245}}{\varepsilon_{269}}$ [12], test for purity of PTHs).



V1: partition Col 1/by-pass to TFA/other reag.
V2: partition Col 1/by-pass to collector/waste
V3: valve for heat control of RC
RC: reaction coil
T : T-junction for H_2O injection
P : peristaltic pump

Fig.1. Modification of the Sequemat apparatus.



Channel function		Timing (min)
1	By-pass (TFA)	96–110
2	H_2O injection (P)	70–110
3	Start of next cycle	111
4	MeOH	1–5; 36–45; 51–55; 61–65; 96–100
5	DCE	46–50; 56–60
6	Buffer	6–35
7	PITC	7–33
8	TFA/V_3 (heating of RC)	66–110
9	V_2 (change-over Col 1 to collector)	70–95
10	V_1 (change-over TFA to Col 1)	66–95
11	Stop (collector)	111
12	Tape transport	1–111

Fig.2. Punched tape program for direct conversion to PTHs in the Laursen variant of Edman degradation.

4. Results

The PTH-amino acids obtained by this method do not need any purification by filtration on ion exchanger as recommended by Laursen. The average value for $\frac{\epsilon_{245}}{\epsilon_{269}}$ of pure PTHs is about 0.4 [12]. Our $\frac{\epsilon_{245}}{\epsilon_{269}}$ values are shown in table 1. The separation and quantitative PTH identification on HPLC elution diagrams are published in the following paper.

The modification of the Sequemat apparatus for direct conversion of thiazolinones to PTH-amino acids is possible with low effort and cost. Since no manipulations of the PTH samples are necessary, which would cause losses of material we can check quantitatively the uniformity of peptides synthetized on solid phase by subsequent degradation and quantitative identification of PTH-amino acids.

Table 1
Ratio of min–max absorption of PTH-amino acids at 245 nm (min) and 269 nm (max)

PTH	$\frac{\epsilon_{245}}{\epsilon_{269}}$ found	$\frac{\epsilon_{245}}{\epsilon_{269}}$ Edman [12]
–Phe	0.37	0.37
–Pro	0.65	0.67
–Val	0.38	0.38
–Ala	0.38	0.39
–Arg (Tos)	0.55	–
–Tyr	0.4	0.42
–Thr	0.39	0.46
–N$^{\epsilon}$PTC-Lys	0.65	0.72
–Leu	0.36	0.36
–Gly	0.61	0.42
–Glu	0.36	0.38

Acknowledgements

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