INCREASED EFFICIENCY IN SOLID-PHASE EDMAN DEGRADATION OF SYNTHETIC PEPTIDYL-RESINS USING AN OXYMETHYLPHENYLACETAMIDOMETHYL-LINKAGE

Gary R. MATSUEDA and Michael N. MARGOLIES
Cellular and Molecular Research Laboratory, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA

Received 8 June 1979
Revised version received 6 August 1979

1. Introduction

The solid-phase Edman degradation procedure as proposed by Laursen [1] is ideally suited to the evaluation of peptidyl-resin products resulting from Merrifield solid-phase synthesis [2,3] of peptides. Incomplete deprotection and incomplete coupling reactions which occur during synthesis have been detected using quantitative Edman degradation [4,5]. However, extended sequencing experiments were precluded, because the repetitive yield for the Edman procedure averaged 80% [5]. We have also observed repetitive yield values of 80–85% for other peptides synthesized on the same types of benzyl ester resin. In contrast, however, for recent sequence analyses of peptides which were synthesized using a resin with the -oxymethylphenylacetamidomethyl-linkage [6] repetitive yields averaged 95 ± 3% (n = 11). In order to study this difference more critically, two model peptides were synthesized using two different peptidyl-resin linkages. Quantitative sequence analysis of these four peptidyl-resins provided direct comparisons of the influence of:

(1) The chemical linkage between the peptide and the solid support;
(2) The amino acyl residue attached directly to the support, on the efficiency of the Edman degradation.

Abbreviations: Boc, *tert*-butyloxylcarbonyl; PTH, phenylthiohydantoin; TFA, trifluoroacetic acid; TEA, triethylamine; DCC, *N,N'*-dicyclohexylcarbodiimide; HOBt, hydroxybenzotriazole hydrate; Tos, *p*-toluenesulfonyl

2. Materials and methods

2.1. *Amino acid analysis*

Peptidyl-resin samples (~10 mg), were hydrolyzed in vacuo for 4 h with proprionic acid : concentrated HCl (1 : 1) as described [7]. The hydrolysates were analyzed using a Durrum D-500 amino acid analyzer (Dionex, Sunnyvale, CA).

2.2. *Solid-phase peptide synthesis*

Esterification [8] of Cl–CH_2–resin (Lab Systems, San Carlos, CA) provided Boc-Arg(Tos)-oxymethyl-resin with a 0.18 mmol/g substitution. Esterification with DCC : HOBt (1 : 1) [9] was used to prepare: Boc-Gly-oxymethyl-resin (0.47 mmol/g) starting from hydroxymethyl-resin [3], Boc-Gly-oxymethylphenyl-acetamidomethyl-resin and Boc-L-Arg(Tos)-oxy-methylphenylacetamidomethyl-resin (0.20 mmol/g) starting from hydroxymethylphenylacetamidomethyl-resin [10].

Starting with 1.5 g Boc-Gly-oxymethyl-copoly(styrene–1%-divinylbenzene)-resin the general procedures in [3] were used to synthesize Phe–Ala–Phe–Ala–Gly-oxymethyl-resin (peptidyl-resin A). The resin was washed 6 times with 30 ml CH_2Cl_2 before the addition of each reagent. Treatment for 30 min with TFA : CH_2Cl_2 (1 : 3) was used to deprotect the Boc-α-amino protecting group. TEA : CH_2Cl_2 (1 : 9) for 10 min was used for the neutralization step. A 3-fold excess of Boc-amino acid (Peninsula Laboratories, San Carlos, CA) and *N,N'*-dicyclohexylcarbodiimide (1 : 1) were used for each coupling step. Completeness of coupling was monitored using the ninhydrin reagent [11].

Following the coupling of the N-terminal Phe residue, the resin was treated with the TFA reagent, as described above, washed with CH_2Cl_2 and MeOH, and dried to constant weight in vacuo over P_2O_5. A sample of peptidyl–resin was weighed and submitted for hydrolysis and amino acid analysis. Similarly, peptidyl–resins B, C, and D (see table 1) were synthesized using Boc-L-Arg(Tos)-oxymethyl resin, Boc-Gly-oxymethylphenylacetamidomethyl-resin, and Boc-L-Arg(Tos)-oxymethylphenylacetamidomethyl resin, respectively.

2.3. *Solid-phase peptide sequencing*

TFA (Halocarbon, Hackensack, NJ) which was used as a cleavage acid during Edman degradation was distilled before use (71–72°C). This acid contained 0.06 ± 0.01% water as determined by near-infrared spectrophotometry [12] using a Cary 14 Spectrophotometer equipped with a high-sensitivity (0–0.1 o.d.) slide-wire.

Peptidyl-resins (2-7 mg) were subjected to automatic solid-phase Edman degradation (Sequemat Inc., Watertown, MA). Two sequencing protocols were used. Using the 'standard' sequencing protocol (as recommended by Sequemat Inc.), 5 cycles of Edman degradation were performed at 46°C. In the 'modified' sequencing protocol, following 2 automatic cycles of Edman degradation, the peptidyl-resin was treated in situ with cleavage acid by pumping TFA for 300 min in the manual mode at 46°C. Automated sequencing was resumed for the 3 remaining amino acids. Anilinothiazolinone amino acids were converted manually to the corresponding PTH-derivatives before quantitation by either gas–liquid chromatography or high-pressure liquid chromatography as described [13,14].

After each sequencing experiment, the entire contents of the reaction column were collected directly into a hydrolysis tube using methanol washes. The excess methanol was decanted and the sample dried prior to hydrolysis of the remaining peptidyl-resin.

3. Results and discussion

The solid-phase syntheses of the 4 model pentapeptides produced peptidyl-resins which upon hydrolysis yielded the expected amino acid compositions, as shown in table 1. The sequence, Phe–Ala–Phe–Ala–X–, was chosen to provide PTH-derivatives which are stable and easily quantified.

Each peptidyl-resin was sequenced by the 'standard' and 'modified' protocols as defined in section 2.3. The initial yields of PTH-Phe obtained for the 8 sequencing experiments averaged 314 ± 65 nmol. The results are summarized in table 2 by comparing the average recovery of PTH-Phe and PTH-Ala from Edman cycles 3 and 4 relative to the recovery of PTH-Phe and PTH-Ala in cycles 1 and 2, respectively. This comparison demonstrated that the effect of the prolonged acid treatment on the yield of PTH-Phe and PTH-Ala at cycles 3 and 4 was resin-dependent. Prolonged acid treatment used in the 'modified' sequencing protocol dramatically diminished relative yields when the -oxymethyl-linkage was used. By contrast, the -oxymethylphenylacetamidomethyl-linkage afforded undiminished yields of PTH-amino acids after prolonged acid treatment.

The relative yields in table 2 indicated that the influence of the amino acid attached directly to the resin was minimal. The increased stability provided by Arg(Tos) as compared to Gly (peptide A versus B)

Table 1
Amino acid analysis of peptidyl-resins

Peptidyl -resin	Chemical linkage between peptide and resin	Peptide sequence	Amino acid composition				Substitution of peptidyl-resins (mmol/g)
			Phe	Ala	Gly	Arg	
A	$-OCH_2-$	Phe–Ala–Phe–Ala–Gly	2.00	2.00	1.01	–	0.41
B	$-OCH_2-$	Phe–Ala–Phe–Ala–Arg	2.19	1.96	–	0.90	0.25
C	$-OCH_2C_6H_4CH_2CONHCH_2-$	Phe–Ala–Phe–Ala–Gly	2.05	1.92	1.03	–	0.23
D	$-OCH_2C_6H_4CH_2CONHCH_2-$	Phe–Ala–Phe–Ala–Arg	2.03	1.93	–	1.03	0.20

Table 2
Solid-phase amino acid sequence analysis of peptidyl-resins

Peptidyl -resin	Chemical linkage between peptide and resin	Peptide sequence	Relative yields (%) during Edman degradation	
			Standard protocol	Modified protocol
A	$-OCH_2-$	Phe–Ala–Phe–Ala–Gly	83.1	37.5
B	$-OCH_2-$	Phe–Ala–Phe–Ala–Arg	85.1	49.3
C	$-OCH_2C_6H_4CH_2CONHCH_2-$	Phe–Ala–Phe–Ala–Gly	99.5	99.2
D	$-OCH_2C_6H_4CH_2CONHCH_2-$	Phe–Ala–Phe–Ala–Arg	94.5	98.4

was significant only after prolonged acid treatment. Thus, for a few Edman degradation cycles this amino acid specific effect is negligible, but does become significant during extended sequencing experiments. For the -oxymethylphenylacetamidomethyl-resin, an amino acid specific effect was not observed (peptide C versus D, table 2).

It is likely that prolonged acid treatment caused diminished relative yields by acidolysis of the peptide-to-resin bond. The -oxymethylphenylacetamidomethyl-linkage has been demonstrated [10], to be more acid-resistant than the -oxymethyl-linkage. This was confirmed in our experiments by the determination of the amount of amino acids remaining on the Phe–Ala–Phe–Ala–Arg-resins after sequencing. If an amino group blocking reaction was responsible for lower yields observed at cycle 3 following acid exposure, one would have expected to detect amino acids corresponding to the Phe–Ala–Arg tripeptide still attached to the resin. As shown in table 3, only small quantitities of amino acids remained on these resins (peptidyl-resin B and D) after Edman degradation. This was consistent with acidolysis of the peptide-to-resin bond rather than a blocking reaction of amino groups.

An average sequencing efficiency (repetitive yield) of 80% was reported [5] for a synthetic peptide anchored by an -oxymethyl-bond. They proposed that 10% of the peptide was cleaved from the resin at each cycle by acidolysis. In our experiments, the acidolytic effect was totally responsible for lowering the efficiency of the Edman degradation.

In a series of synthetic peptidyl-resins ranging in length from 18–117 residues (details of synthesis to be published elsewhere), employing the oxymethyl-phenylacetamidomethyl-linkage the repetitive yields in solid-phase Edman degradation averaged 95% (range 91–98%) as shown in table 4.

Table 3
Amino acid analysis of peptidyl-resin B and D after sequence analysis using modified protocol

Peptidyl -resin	Resin substitution (mmol/g)			
	Amino acid	Before sequencing	After sequencing	% Amino acid remaining after sequencing
B	Phe	0.55	0.01	2
	Ala	0.49	0.02	4
	Arg	0.23	0.01	4
D	Phe	0.39	0.01	3
	Ala	0.37	0.01	3
	Arg	0.20	0.01	5

Table 4
The repetitive yield of the Edman degradation of synthetic peptides attached to solid support via oxymethylphenyl-acetamidomethyl-linkage

Length of peptide[a] (amino acyl-residues)	No. Edman degradation cycles performed	Repetitive yield[b] (%)
18	18	97
31	17	92
43	17	95
67	18	91
99	15	95
117	14	98

[a] Peptides represent C-terminal fragments related to variable region of rabbit antipneumococcal antibody-3374 [15] starting with Arg, residu[illegible]18
[b] Calculated as in [13]

4. Conclusion

Acidolysis of the peptide-to-resin bond during Edman degradation of synthetic peptidyl-resins has been demonstrated to be a side-reaction during TFA cleavage of anilinothiazolinone amino acids. This side reaction contributed to the lowering of the relative yields of PTH-amino acids and thus decreased repetitive yields significantly. The effect of this side-reaction was abrogated when an acid-resistant -oxymethylphenylacetamidomethyl-linkage was used.

The possibility that this side reaction contributes to the lowering of repetitive yields for other peptide-to-support bonds remains untested. However, the sequence protocols described here may be used advantageously to measure this effect. We suggest the use of more acid-resistant peptide-to-support bonds in order to take advantage of this high efficiency of the solid phase Edman degradation.

Acknowledgements

The authors thank Dr Edgar Haber for his support and counsel; Stephan Gaehde, John Wechsler, Andrew Brauer and Leslie Dotson lent skillful technical assistance. Supported by NIH grant HL 19259.

References

[1] Laursen, R. A. (1971) Eur. J. Biochem. 20, 89–102.
[2] Merrifield, R. B. (1963) J. Am. Chem. Soc. 85, 2149–53.
[3] Stewart, J. M. and Young, J. D. (1969) Solid Phase Peptide Synthesis, Freeman, San Francisco.
[4] Fankhauser, P., Fries, P., Stahala, P. and Brenner, M. (1974) Helv. Chim. Acta 57, 271–277.
[5] Birr, C. and Frank, R. (1975) FEBS Lett. 55, 68–71.
[6] Mitchell, A. R., Kent, S. B. H., Englehard, M. and Merrifield, R. B. (1978) J. Org. Chem. 43, 2845–2852.
[7] Scotchler, J., Lozier, R. and Robinson, A. B. (1970) J. Org. Chem. 35, 3151–3152.
[8] In [3], p. 32.
[9] Chang, J. K., Shimizu, M. and Wang, S. S. (1976) J. Org. Chem. 41, 3255–3258.
[10] Mitchell, A. R., Erickson, B. W., Ryabtsev, M. N., Hodges, R. S. and Merrifield, R. B. (1976) J. Am. Chem. Soc. 98, 7357–7362.
[11] Kaiser, E., Colesott, R. L., Bossinger, C. D. and Cook, P. I. (1970) Anal. Biochem. 34, 595–598.
[12] Meeker, R. L., Critchfield, F. E. and Bishop, E. T. (1962) Anal. Chem. 34, 1510–1511.
[13] Brauer, A. W., Margolies, M. N. and Haber, E. (1975) Biochemistry 14, 3029–3035.
[14] Margolies, M. N. and Brauer, A. W. (1978) J. Chromatog. 148, 429–439.
[15] Margolies, M. N. and Haber, E. (1978) Fed. Proc. FASEB 37, 1852.