SOLID-PHASE EDMAN DEGRADATION: ATTACHMENT OF CARBOXYL-TERMINAL HOMOSERINE PEPTIDES TO AN INSOLUBLE RESIN

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1. Introduction

Attachment of peptides to insoluble supports prior to sequencing by solid-phase Edman degradation [1] has been achieved by the use of peptide amino [2] and carboxyl group [1, 3] coupling agents. However, neither of these procedures is very useful for cyanogen bromide peptides containing C-terminal homoserine, because the former requires a side chain amino group near the C-terminus, and the latter causes side reactions of the carboxyls of aspartic and glutamic acid. Stimulated by the findings of Knobler et al. [4] and of Offord [5], we have found a simple procedure for selectively attaching cyanogen bromide peptides to resins by their C-terminal homoserine residues. The method involves lactonization of the homoserine residue with trifluoroacetic acid [6] and subsequent aminolysis of the lactone with an amino resin (fig. 1).

2. Experimental

2.1. *Triethylenetetramine sequencing resin*

Ten grams of chloromethyl polystyrene (prepared from BioBeads S-X1, minus 400 mesh; cf. ref. [1]) was stirred with 125 ml of triethylenetetramine (TETA) for 30 min at room temperature, and the mixture was then heated on a steam-bath for 1.5 hr. The resin was

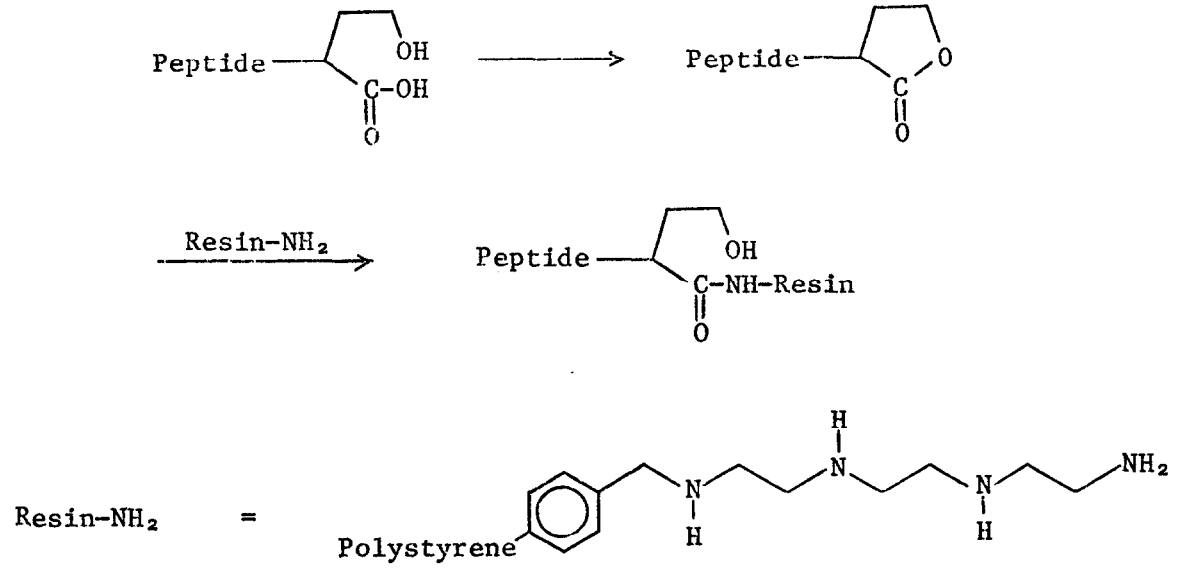

Fig. 1.

filtered, washed thoroughly with methanol, stirred with 20 ml of triethylamine, and filtered. After washing with copious amounts of methanol, water, then methanol again, the resin was dried under vacuum at 60°C overnight; yield, 11.5 g of white resin.

2.2. *Attachment of peptides using the homoserine lactone method*

A solution of the C-terminal homoserine peptide (usually 20–200 nmoles) was evaporated to dryness in a small vial, and the residue was redissolved in 1.0 ml of anhydrous trifluoroacetic acid. The solution was kept at room temperature for 1 hr, and the solvent was evaporated over KOH in a vacuum desiccator. The peptide sample was dissolved in 100–150 μl of dimethylformamide (DMF), and the solution was added to a 13 × 100 mm test tube containing 40 mg of TETA sequencing resin previously swollen in 200 μl of DMF. The first test tube was rinsed with 100 μl of DMF, which was added to the resin mixture. (In cases where the peptide was poorly soluble in DMF, up to 20% the total DMF volume of water could be added to promote solution.) A 50 μl aliquot of triethylamine was added to the resin mixture, which was then stirred at 45°C for 2 hr. To block excess amino groups on the resin, 100 μl of CH_3NCS–CH_3CN (1:1) and 100 μl of sequencing buffer [*N*-methylmorpholinium trifluoroacetate at pH 8.1 and pyridine (2:3)] were added to the reaction mixture cf. [ref. 1], and stirring was continued for 75 min longer. After washing with methanol (3 × 8 ml) by centrifugation, the resin was dried under vacuum; weight approx. 50 mg.

2.3. *Degradation of peptides and analysis of phenylthiohydantoins*

The resin sample from the above procedure was mixed with glass beads and was degraded using a automatic sequencer and procedures described earlier [1, 2]. The radioactive phenylthiohydantoins and unlabeled standards were chromatographed on silica gel-coated aluminum sheets, and the sequence was determined by comparing the mobility of the unknowns with the knowns. For quantitation, the sheets were cut into pieces and counted [1, 2].

3. Results

Table 1 shows the results of sequencing a cyanogen bromide peptide from rabbit muscle actin attached to TETA resin by the homoserine lactone procedure. The peptide had the composition Thr_1, Ser_1, Glx_2, Pro_2, Gly_1, Leu_1, Ile_1, Phe_2, $AminoethylCys_1$, Hse_1, and had resisted attempts at sequencing by conventional means [8]. The data in table 1 give the sequence AECys–Pro–Glu–Thr–Leu–Phe–Gln–Pro–Ser–Phe–Ile–Gly–Hse. Since the excess resin amino groups had been blocked with methyl isothiosyanate, the N-terminal amino acid was recovered as a non-radioactive methylthiohyndantoin. It was identified as the ε-methylthiocarbamyl methylthiohydantoin of aminoethylcysteine by comparison of its R_f with standards. Sequence analysis of this peptide required about 51 hr for lactonization, attachment, automatic Edman degradation and phenylthiohydantoin analysis.

4. Discussion

Attachment of the peptide to the resin can be accomplished without prior blocking of the peptide amino groups, because the resin amino groups are present in great excess. The methyl isothiocyanate used to block excess resin amino groups [1] also reacts with the unprotected peptide N-terminal amine, resulting ultimately in formation of a methylthiohydantoin for the first amino acid. As discussed in the Results section this can generally be identified without difficulty.

A number of peptides have been attached to the TETA resin in yields of 80–100% and sequenced. A 37-residue peptide was attached with a loading of 4 nmoles per mg of resin, and smaller peptides have been attached in greater amounts. Very large peptides tend not to be soluble in dimethylformamide and do not couple efficiently.

Early fears that peptide attached to resins by homoserine amide bonds would be cleaved in acid by attack of the homoserine hydroxyl proved to be groundless. However we have noted, as exemplified by the 85% yield that can be calculated from the data in table 1, that the degradative yields for homoserine peptides are generally 5–7% lower than for other peptides. This

Table 1
Degradation of 230 nmoles of a peptide attached by the homoserine lactone procedure.

Cycle	cpm minus background							
	Origin	Thr Hse	Gly	Δ-Thr Phe	Ile	Leu	Pro	Amino acid
Solvent I								
1	0	0	0	0	0	0	0	(AECys)
2	91	60	4	65	0	0	1512	Pro
3	1631	24	64	0	0	16	0	–
4	210	611	0	364	8	41	0	(Thr)
5	75	23	0	4	166	841	0	Leu
6	17	0	0	1021	22	88	23	Phe
7	706	82	0	93	0	9	0	–
8	76	0	10	59	7	0	604	Pro
9	152	33	37	0	10	0	39	–
10	0	0	2	437	0	0	21	Phe
11	0	0	0	13	283	13	13	Ile
12	0	0	287	0	80	25	0	Gly
13	0	248	29	0	9	7	0	–

Cycle	Glu	Gln	Ser	Hse	Thr	Amino acid
Solvent II						
3	1132	0	0	0	0	Glu
4	27	0	1	68	547	Thr
7	186	365	19	5	0	Gln
9	0	15	3	0	10	(Ser)
13	0	0	0	147	1	Hse

Thirty percent of each phenylthiohydantoin sample was chromatographed on silica gel in solvent I,$CHCl_3$–EtOH (98:2). Spots which remained at the origin were chromatographed in solvent II,$CHCl_3$–MeOH (9:1). For quantitation, areas corresponding to phenylthiohydantoin spots were cut out and counted. N-terminal aminoethylcysteine was identified as described in the text. The serine derivative (cycle 9) decomposed to products which remained at the origin in solvent I, but were not located in solvent II.

may be due to slow hydrolysis of the homoserine peptide bond. Nevertheless it has been possible to carry out as many as 27 degradative steps on peptides attached by this procedure.

The TETA resin has proved to be generally superior to earlier resins [1, 8], in terms of capacity and chemical properties, for attachment of peptides by carboxyl activation [1, 3, 8]. This may be because the reactive amino groups are farther away from the polystyrene backbone, and because there are more of them. The 1,2-diamine structure may also be important since Jencks [9] has shown that 1,2-diamines are much more reactive towards activated carboxyls than are monoamines.

One problem associated with the TETA resin is an increased background observed on thin layer chromatography of the phenylthiohydantoins, specifically, a strong ultraviolet-absorbing spot migrating near threonine, as well as weaker spots near Glu and Asp. These appear to be breakdown products of the methylthiocarbamyl resin, since the spots are not radioactive (i.e., when tritiated phenyl isothiocyanate is used). Preliminary results indicate that acetylation of the resin with acetyl imidazole (the peptide amino groups having previously been protected) reduces the background.

The reactivity of homoserine lactone may have other applications in peptide chemistry, for example, separation of the C-terminal peptide from a cyanogen bromide digest of a protein by selective attachment of the homoserine peptides to a resin. Furthermore, preliminary experiments suggest that homoserine peptides can be labeled, e.g. with [^{14}C]ethylene diamine, thereby converting them to single ionic species

which can readily be located because of their radioactivity.

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