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THE USE OF TRIFLUOROETHANOL FOR IMPROVED COUPLING IN SOLID-PHASE PEPTIDE SYNTHESIS

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The major problem in solid-phase peptide synthesis (1) has been the elimination or reduction of the impurities that arise from incomplete reactions on the peptide-resin. Previous work (2,3) has suggested that the primary cause is limited accessibility of the peptide chain to the reagents used in coupling amino acid derivatives to the peptide-resin. We report here the use of trifluoroethanol to increase the reactivity of the α-amino group in the peptide-resin.

The vehicle for this study was the synthesis of the model peptide H-Lys-Lys-Lys-Lys-Lys-Glu-Glu-Glu-Leu-Leu-Trp(Nps)-Phe-OH (I). The protected peptide resin corresponding to the sequence of Glu_3-Leu_2-Trp(Nps)-Phe was first synthesized by the procedure described below and the coupling efficiency of the lysine derivative, Boc-Lys(o-BrZ)-OH, was then investigated. Since we doubted the accuracy of the available analytical procedures for determining unreacted amine (see below), we judged that the most reliable measure of coupling inefficiency was the isolation of the error peptides arising thereby. The five lysine residues in I have the effect of magnifying coupling inefficiency since failure to incorporate any one of these residues in a given peptide chain would give the same peptide, H-Lys_4-Glu_3-Leu_2-Trp(Nps)-Phe-OH, the error peptide of I, hereafter called Ie.

To insure analytical accuracy authentic homogeneous samples of peptides I and Ie were prepared by the standard solid-phase procedure in the Beckman model 990 Peptide Synthesizer. Boc-phenylalanyl resin (0.64 g, 0.25 mmol) was treated according to the Table (4). Coupling of Boc-amino acids was achieved by use of their symmetrical anhydrides (5), preformed by reaction with dicyclohexylcarbodiimide (6). After coupling the fourth lysine, a portion of the peptide-resin was removed, and the last lysine residue was coupled to the remainder. Peptides I and Ie were obtained by treatment of the corresponding

peptide-resins with liquid HF (7,8) for one hour at 0°, and purified by chromatography on carboxymethylcellulose. Peptides I and Ie were homogeneous by partition chromatography (9) on Sephadex G-25 in n-butanol-ethanol-2.3 M aqueous ammonium acetate (4:2:5) with R_f values of 0.41 and 0.47, respectively, and in n-butanol-pyridine-0.1% aqueous acetic acid (5:3:10) with R_f values of 0.29 and 0.41. Each peptide gave a single spot on paper electrophoresis (400 V, pH 6.7) with R_f^{Lys} values of 0.44 and 0.34 for I and Ie, respectively. Both exhibited identical spectral characteristics (250-480 nm) in 80% acetic acid and in good agreement with published spectra for Trp(Nps)-containing peptides (10). Amino acid analyses of acid hydrolysates gave $Lys_{4.84}Glu_{3.08}Leu_{2.02}Phe_{0.98}$ for I and $Lys_{4.01}Glu_{3.01}Leu_{2.02}Phe_{0.98}$ for Ie.

Table

Schedule for Solid-Phase Synthesis

Step	Operation and Reagent	Mix time (min)
1	CH_2Cl_2, 12 ml (4 times)	1
2	55% trifluoroacetic acid/CH_2Cl_2, 10 ml (once)	1
3	55% trifluoroacetic acid/CH_2Cl_2, 7 ml (once)	15
4	CH_2Cl_2, 12 ml (3 times)	1
5	25% dioxane/CH_2Cl_2, 12 ml (3 times)	1
6	CH_2Cl_2, 12 ml (3 times)	1
7	2.5% diisopropylethylamine/CH_2Cl_2, 12 ml (once)	2
8	CH_2Cl_2, 12 ml (2 times)	1
9	Repeat step 7	
10	Repeat step 8	
11	Repeat step 7	
12	Repeat step 8	
13	Repeat step 7	
14	CH_2Cl_2, 12 ml (6 times)	1
15	Symmetrical anhydride, 3 equivalents in 6.5 ml of CH_2Cl_2 (once)	20 and hold
16	Diisopropylethylamine, 0.13 ml (once)	10
17	CH_2Cl_2, 12 ml (3 times)	1
18	33% ethanol/CH_2Cl_2, 12 ml (3 times)	1

Peptides I and Ie were found to be easily separable from each other by chromatography on carboxymethylcellulose while the Trp(Nps) moiety afforded an accurate measure of the amount of either. The percent of error peptide Ie present in synthesis of I was studied as a function of coupling conditions with the percentage error defined as (Ie)(100)/I + Ie. Under the conditions described in the Table the percentage of error peptide was 7.7%. Its identity

as Ie was established not only by its chromatographic behavior on carboxymethylcellulose but also by partition chromatography and paper electrophoresis. The average coupling efficiency of the lysine residues was therefore 98.3%. When 2,2,2,-trifluoroethanol (TFE) was added to the coupling reaction together with the base (step 16) so that the last 10 min of coupling proceeded in 20% TFE in CH_2Cl_2, the percentage of error peptide Ie was 1.6%. This corresponds to an average coupling efficiency of 99.7% for the lysine residues. The favorable effect of TFE on the coupling can be explained by the visual observation that 20% TFE in CH_2Cl_2 swells the peptide-resin to approximately twice the volume of the peptide-resin in CH_2Cl_2 alone. Phenol, which has a comparable swelling effect, also increased coupling efficiency; the use of 10% phenol as a coupling medium gave 2.2% of Ie in a synthesis of I. Since neither TFE nor phenol increased the swelling of the chloromethyl resin, the observed swelling in the peptide-resin must be caused by an interaction or solvation of the peptide by TFE and phenol (11). Probably this interaction increases the penetrability of the coupling reagent into the peptide-resin.

We have also observed an effect of TFE in the determination of free amino groups in the peptide-resin. The reaction of the fully protected peptide-resin corresponding to I (standard synthesis) with 1.5 M 2-hydroxy-1-naphthaldehyde (12) in CH_2Cl_2 for 20 hr gave an amine content of 0.8% with respect to total peptide content. Reaction with 0.5 M picric acid (13) in CH_2Cl_2 showed an amine content of 0.1%. Although the true efficiency of the last coupling could not be determined from the available data, these results are at variance with the observed error content of 7.7%, unless one assumes that the coupling efficiency of the fifth lysine is greater than the average coupling efficiency. Other experiments indicate that the coupling efficiency of the fifth lysine is no better than the average. When the reaction with 2-hydroxy-1-naphthaldehyde was conducted in 20% TFE in CH_2Cl_2 the amine content was 2.0%. The Boc group was not detectably removed by the latter reagent. Trifluoroethanol had no effect on the picric acid reaction.

The efficacy of TFE in promoting coupling of other amino acids was tested by utilizing the new amine detection procedure. After removal of the Boc group from the protected peptide resin corresponding to I, the coupling of the symmetrical anhydride of Boc-valine was carried out with three equivalents at 0.04 M concentration in CH_2Cl_2 for 10 min followed by 5 min in either CH_2Cl_2 or the test solvent. The percentage of amine remaining after reaction in CH_2Cl_2 was 6.2%; it decreased to 1.7% when the coupling was carried out in 10% TFE or 10% phenol in CH_2Cl_2.

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