SIMULTANEOUS PEPTIDE SYNTHESIS USING CELLULOSE PAPER AS SUPPORT MATERIAL

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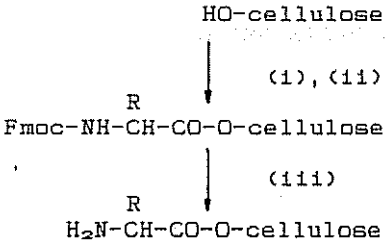
Introduction

Simultaneous solid phase peptide synthesis by support segmentation has been developed in order to meet the growing need of synthetic peptides as tools for the investigation of protein - ligand interactions (1,2,3).
Cellulose paper was esterified with Fmoc-amino acid chlorides (4) providing a mechanically and chemically stable support material. The applicability of this support for simultaneous peptide synthesis was demonstrated by the synthesis of model peptides following different synthetic strategies.

Results

The functionalization of the paper (Whatman 540) is outlined in Scheme 1. The substitution of the support with amino groups was determined by photometric measurement of the Fmoc-cleavage product (dibenzofulvene-piperidine adduct) and comes to 1 to 2 $\mu mol/cm^2$.

Peptides 1988



R = -H, $-CH_3$

Scheme 1: Functionalization of the paper with Fmoc-amino acid chlorides; (i): 1 M NaOH 15 min, (ii): 0.25 M Fmoc-amino acid chloride in toluene/acetone 1:1 (v/v) 30 min, (iii): 20% piperidine/dimethylformamide 10 min.

The cellulose ester bond is sufficient stable under usual reaction conditions of solid phase peptide synthesis. After 3h treatments with 25% trifluoroacetic acid/dichloromethane and 20% piperidine/dimethylformamide, respectively, no substantial loss of substitution was detectable.
On this paper support, penta- to undecapeptides were synthesized. (For experimental detail see Ref.7)
Two sets of model peptides, synthesized following Boc/Benzyl- (set A) and Fmoc/Bu^t- (set B) strategies, are demonstrated here:

A: I: Tyr-Val-Pro-Lys-Ahx-Ala-OH
II: Tyr-Glu-Gly-Thr-Ahx-Ala-OH Ahx=ε-aminocaproic acid
III: Tyr-Lys-Gln-Ile-Ahx-Ala-OH

B: I: Tyr-Pro-Thr-Lys-Phe-Leu-Gly-Lys-Ala-Phe-Val-OH
II: Tyr-Pro-Ala-Gly-Val-Leu-Ala-Thr-Pro-Phe-Val-OH
III: Tyr-Leu-Ala-Lys-Val-Pro-Gly-Thr-Ala-Phe-Leu-OH
IV: Tyr-Leu-Thr-Gly-Phe-Pro-Ala-Lys-Pro-Phe-Leu-OH

Amino acids were coupled batchwise as their preactivated HOBt-esters. Course and completion of couplings were checked by bromophenol blue-monitoring (5). Benzyl side chain groups of set A were cleaved by boron tris(trifluoroacetate).

The first residues of set B were coupled bound to a p-alkoxybenzyl ester handle (6). The peptides were removed from the paper by alkaline cleavage of the cellulose ester (set A) and by acidic cleavage from the handle (set B), respectively. HPLC analysis of the crude peptides showed main peaks of 70 to 80% on average, the identity of which was confirmed by amino acid analysis (set A and B) and FAB mass spectrometry (set B).

The reported paper support offers following advantages:

- Peptide synthesis on it is possible using both Boc and Fmoc for temporary N^{α}-protection.
- The synthesized peptides can get either support-fixed immunologically tested or cleaved and isolated.
- Cellulose paper is an inexpensive and convenient to handle material. The size of the support segments can be individually chosen by variable cutting.

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