SDS-Subtilisin Catalyzed Coupling of Peptide Segments on the Solid Support

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Introduction

The segment condensation of long chain peptides is one of the most important problem in the fields of synthesis and semisynthesis of peptides and proteins. The use of enzymes as catalysts of the segment condensation of peptides make possible to remove some limitations which appear in the chemical synthesis of peptides, namely the risk of racemization and the need of side chain functional groups protection [1, 2]. We carried out SDS-subtilisin-catalyzed coupling of peptide segments on aminosilochrom-based support.

Results and Discussion

Aminosilochrom was used as a suitable insoluble support for the enzymatic synthesis. SDS-subtilisin complex solubilised in ethanol was chosen as a catalyst because of its effectiveness in organic solvents where the secondary hydrolysis of product is minimal [3, 4, 5]. The enzymatic reaction involved the coupling by means of SDS-subtilisin between N-protected peptide methyl esters and a peptide-spacer bound to the support. Tripeptides (Met-Gly-Gly, Met-Ala-Gly) and tetrapeptides (Phe-Met-Gly-Gly, Phe-Met-Ala-Gly), which have been joined to propylaminosilochrom by carbodiimide, were used as spacers. The data of enzymatic coupling of DNP-Ala-Ala-Leu-OMe to peptide- spacer bound to

Table 1. *Enzymatic coupling of DNP-Ala-Ala-Leu-OMe to peptide-spacer bound to aminosilochrom*[a]

Spacer composition	Spacer capacity, mkM/g	Peptide capacity after enzymatic coupling, mkM/g	Yield of enzymatic coupling, %
MGG	15	5	33
MAG	30	17	57
FMGG	40	13	33
FMGG	16	8	50
FMGG	12	7	58
FMAG	18	7	39

[a]Condensation conditions: 10-fold excess DNP-Ala-Ala-Leu-OMe (acylating component) to spacer (amino component), 52 μM SDS-subtilisin, ethanol/DMSO = 7/3 (v/v), 20°C, 72 h.

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Table 2. *SDS-subtilisin mediated [illegible] with amino-deficient [illegible]*

Peptide		Yield of enzymatic coupling, %
↓		
Boc-Ala-Ala-Leu-Met-Ala-Gly	(**1**)	30
↓		
Z-Ala-Ala-Glu(OMe)-Met-Ala-Gly	(**2**)	27
↓		
Z-Ala-Ala-Glu(OMe)-Ala-Ala-Leu-Met-Ala-Gly	(**3**)	78
↓		
Boc-Ala-Ala-Leu-Ala-Ala-Glu(OMe)-Met-Ala-Gly	(**4**)	75
↓		
DNP-Ala-Ala-Leu-Ala-Ala-Glu(OMe)-Ala-Ala-Leu-Met-Ala-Gly	(**5**)	57
↓		
DNP-Ala-Ala-Leu-Ala-Ala-Leu-Ala-Ala-Glu(OMe)-Met-Ala-Gly	(**6**)	83

[a]Condensation conditions are presented in the Table 1. [b]The arrows indicate the peptide bond formed. Yields are given as assessed by amino acid analysis or spectrophotometric method.

aminosilochrom are given in the Table 1. The best results were obtained when peptide Met-Ala-Gly or peptide Phe-Met-Gly-Gly with the smallest spacer capacity were used.

We carried out three-stepped enzymatic synthesis of nonapeptides (**5**, **6**) *via* (3+3+3) using peptide Met-Ala-Gly as spacer (Table 2). After coupling Boc-Ala-Ala-Leu-OMe or Z-Ala-Ala-Glu(OMe)-OMe to Met-Ala-Gly-aminosilochrom followed by deprotection of amino group we turned out well to realize coupling of second and then third tripeptides. It was shown that the second peptide coupling (peptide **3** or **4**) has better yield than the first peptide coupling (peptide **1** or **2**). The yield of 83% of the third step (peptide **6**) was greater than the yield of peptide **5**.

The product of the first peptide coupling was cleaved from support with BrCN/HCOOH. The yield of cleavage was 90%.

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