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THE INFLUENCE OF THE CHAIN LENGTH ON THE COUPLING REACTION IN SOLID PHASE PEPTIDE SYNTHESIS

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The size of peptides which can be synthesized by the Merrifield solid phase method (1) is limited by incomplete coupling reactions (2). According to Merrifield the coupling reactions with all BOC-amino acids go so nearly to completion, that it is unneccessary to introduce a blocking step for free amino groups (3). For a number of peptide sequences (e.g. Bradykinin (3) oxytocin (4,5,6,7), deaminooxytocin (8), S,S'-dibenzyl-serin^4-oxytocin (9) and antamanid (10,11)) it was found that the yield in every coupling reaction was very close to 100 %. In - vestigations from our laboratory (2) show, however, that due to in - complete coupling reactions failure sequences can be detected by suitable experiments.

During the last year, methods for the quantitative determination of the yield in the coupling reaction have been proposed (12,13,14). Tri- and tetrapeptides were synthesized, and free amino groups determined during or after the coupling reaction. All three laboratories found that peptide bonds involving amino acids with bulky side chains are not formed as readily as others.

In order to investigate the influence of chain length and steric hindrance on the yield of the coupling reaction, we have synthesized the dodecapeptide $(Leu\text{-}Ala)_6$. The method of Dorman (14) was selected for the determination of amino groups. The methods proposed by Weygand and Obermeier (12) and Esko et al. (13) are too time -

consuming for routine analysis. Steric hindrance involved in the use of voluminous reagents in these two methods, may in addition lead to erroneous results.

For the synthesis of the dodecapeptide, the following solid support was used: Polystyrol copolymerized with 2 % divynylbenzene (200-400 mesh) was chloromethylated as described by Stewart and Young (15) with the exception that n-hexane was used as solvent. 3.4 millimoles of chloride were found per g of chloromethylated resin. In the esterification step, 1 g (5.25 mM) of BOC-alanine, 0.7 ml (5 mM) of triethylamine and 1.5 g of chloromethylated resin (5.1 mM chloride) were stirred in 50 ml of ethanol for 24 hours at 78° C. Deprotection was carried out with 1N HCl in acetic acid. Neutralization occurred with 10 % of triethylamine in N,N'-dimethylformamide. For the coupling, 10 millimoles of BOC-alanine or BOC-leucine were used, with a reaction time of usually two hours. Acetylation was carried out with a mixture of one part acetic acid anhydride in two parts of 10 % triethylamine in N,N-dimethylformamide; reaction time 20 min. Determination of free amino groups was carried out after each coupling reaction, acetylation reaction and deprotection reaction. Chloride was determined potentiometrically.

The results of the chloride titration are given in the table. The first coupling step proceeds with a yield of 99,5 %, and with a yield of 100 % when the coupling is repeated with fresh BOC-amino acid plus dicyclohexylcarbodiimide. The next five coupling steps also give almost quantitative yields after repetition of the coupling reaction. In later coupling steps the following becomes obvious: a) The yield in peptide bond synthesis decreases steadily with increasing chain length. This is more pronounced for leucine as carboxyl component than for alanine which is explained by the greater steric hindrance in the case of leucine. b) Carrying out of the coupling reaction for a third time does not increase the yield. One has to assume that the unreactive amino groups are somehow buried. c) Acetylation of these unreactive amino groups is of value only when amino groups of alanine have to be blocked. If alanine does not react with the free amino groups of leucine, as is the case in the tenth coupling step, acetic acid anhydride does not react either. Again steric hindrance must be the explanation.

Determination of free amino groups during the synthesis of $(Leu\text{-}Ala)_6$

Amino acid sequence	Ala	Leu	Ala	Leu	Ala	Leu	Ala	Leu	Ala	Leu	Ala	Leu
coupling step	1	2	3	4	5	6	7	8	9	10	11	
Free amino groups after cleavage of protecting groups (m Mole = 100 %)	2,05	1,96	2,05	2,04	1,92	1,85	1,85	1,52	1,55	1,08	1,01	
Free amino groups after 1. reaction in mMole	0,01	0,08	0,08	0,08	0,48	0,09	0,77	0,137	0,79	0,29	0,535	
in %	0,5 %	4 %	4 %	4 %	25 %	5 %	42 %	9 %	51 %	27 %	53 %	
Free amino groups after 2. reaction in mMole	0	0	0,01	0,01	0,08	0,037	0,388	0,076	0,543	0,26	0,23	
in %	0 %	0 %	0,5 %	0,5 %	4 %	2 %	21 %	5 %	35 %	24 %	23 %	
Free amino groups after 3. reaction [a] in mMole							0,388			0,26	0,21	
in %							21 %			24 %	21 %	
Free amino groups after 1. acetylation in mMole					0,015		0,037		0,052	0,19		
in %					0,8 %		2 %		3,5 %	17,5 %		
Free amino groups after 2. acetylation in mMole										0,104		
in %										9,6 %		

a) reaction time 8 hours

The dodecapeptide was formed with a yield of about 40 %. The product obtained after treatment of the peptide resin with hydrogen bromide in trifluoroacetic acid was subjected to partial hydrolysis and the failure sequences Leu-Leu and Ala-Ala determined (2). About 4 % Leu-Leu and no Ala-Ala were found. If the coupling reaction proceeds with a low yield, repetition of the reaction as well as subsequent acetylation are only of limited value. Since the acetylation reaction may in addition give rise to side reactions, acetylation can not be recommended for the effective blocking of amino groups. Results similar to those described were obtained when the dodecapeptide was synthesized on a resin with a capacity of about 0,7 millimole chloride per g of resin (16). Our investigations show, that it cannot be taken for granted that "for any desired sequence all reactions can be brought to 100 % completion (15)".

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