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Alternative carriers and methods in solid phase synthesis

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Introduction

Progress in peptide synthesis was always stimulated by the introduction of new techniques and methodologies. One of the quantum leaps was the solid phase approach introduced by Merrifield, who described already in his first paper [1] his attempts to use various solid carriers, including cellulose beads. However, peptide chemistry available in the sixties did not give satisfactory results using this carrier. Multiple peptide synthesis using cellulose paper disks was realized by Frank et al. [2] and by Eichler et al. [3].

Results and Discussion

It seemed obvious that the mechanical properties of paper are the limiting factor for its use in solid phase synthesis. Therefore we have suggested cotton as a replacement. Cotton is readily available, cheap, mechanically stable and apparently can be used to realize an old dream of peptide chemists – continuous solid phase synthesis [4]. Several papers describing the use of carriers in the form of thin foils have recently appeared [5,6]. We decided to compare the quality of the products obtained with hydroxypropyl acrylate-coated polypropylene membranes (kindly provided by Dr. Daniels, MilliGen) and with paper and cotton carriers. We have synthesized the test peptide ACP^{65-74}, decapeptide sequences of ACTH and prolactin on these carriers and in all cases we have obtained comparable results. However, for use in the continuous synthesis arrangement (when the carrier passes through the specific baths and all synthetic steps are performed simultaneously in various places of the carrier) cotton strip is superior due to its mechanical strength and higher capacity per area. Another comparison of suitability of cotton as a synthetic carrier has been performed by placing the classical polystyrene resin carrier and the cotton ribbon in a shaker vessel and performing all the synthetic steps of ACP^{65-74} simultaneously on both carriers. Again, both products were indistinguishable.

The carrier for continuous synthesis must be available as a very long strip and should have very good mechanical properties. Textile-like materials are obviously superior to membranes. Therefore we also decided to test probably the strongest available material in this field – glass fibers. However, in this case only the surface

of the glass threads is available for synthesis. We have covered the surface with aminopropyl groups (reaction with triethoxysilylaminopropane) and have achieved a substitution of 1.7 mol/g. Amino groups were substituted by a linker already functionalized with Fmoc-Met and the synthesis of Met-enkephalin was performed. The yield (0.26 mg/g) and quality (60% in a crude mixture after cleavage) did not show this carrier as a viable alternative to cotton.

Cotton is obviously the carrier of choice for multiple peptide synthesis since it can be easily handled and its size can be adjusted according to the desired synthetic scale. We have found that it is not necessary to shake the carrier in the solution of activated component, but because a substantial amount of solution can be soaked into the cotton carrier, as well as to the polypropylene membrane (1.1 g DMF/g cotton, 1.0 g DMF/g membrane, 0.4 g DMF/g fibre glass), we can use only this liquid for the coupling step. Using carriers of 0.1 mmol/g capacity and 0.5 mol/l solution of activated component, five molar excess of the reagent is present in the liquid soaked into the carrier, thus assuring fast coupling. The fact that no bath is needed either for the coupling or for the washing or cleaving steps simplifies the construction of the apparatus for multiple peptide synthesis.

The removal of the liquid after every step of the synthetic cycle can be realized either by squeezing the carrier together with the dry porous material (paper, textile), which can remove up to 76% of the liquid, or by centrifugation, which removes up to 94% of the liquid. Centrifugation is especially efficient and we have designed a multiple peptide synthesizer [7] utilizing this technique and bromophenol blue monitoring [8] for feedback control of the coupling. Due to the almost complete removal of the liquid after each step by centrifugation it was possible to decrease the number of washings. Using the model experimental setup, built from a small laboratory centrifuge, we were able to show that even the protocol using a single wash after protecting group cleavage and after coupling step did not influence negatively the purity of prepared peptides. Since the amount of solvent used for the washing is equal to the amount which is soaked into the carrier (cotton), its consumption is minimal. For preparation of 8 mg of ACP^{65-74} we used only 6.5 ml of dimethylformamide (for comparison: preparation of 114 mg of the same peptide on polystyrene resin consumed 750 ml of solvent).

Cotton is a carrier containing huge excess of free hydroxyl groups in relation to the synthesized peptide chain. At first sight it looked dangerous for the synthesis, but the results obtained on this carrier were found uninfluenced by these functional groups. On the other hand, possible hydrogen bonding of the growing peptide chain may prevent the self-association effects which complicate the synthesis on the classical carriers in several cases. This was shown for example in the synthesis of decaalanine sequence, where after the fifth or sixth step the synthesis performed on a polystyrene or polyamide resin usually collapsed. We were able to proceed with this synthesis up to the tenth step without observable slowing of the coupling rate. The only trouble was the cleavage of the synthesized peptide Ala^{10}-Val-Gly from the carrier. Aqueous sodium hydroxide (1M) treatment cleaved the peptide (bound directly to the carrier by an ester bond) from the cellulose, but no peptide

was released to the solution. It was necessary to extract it by trifluoroacetic acid/water mixture (95:5). The peptide is basically insoluble in 3M aqueous acetic acid. CD spectra of this peptide have shown high α-helical content. From the results obtained in the synthesis of alanine oligopeptide it is reasonable to conclude that the polysaccharide carrier is capable of protecting the peptide from either self-association or from intermolecular interaction.

For the successful synthesis on cotton the attachment of the first amino acid is extremely important. We have shown recently that direct attachment to the cotton by an ester bond can be used in cases where the basic treatment is not expected to cause any harm to the synthetic peptide. However, it is much simpler to use suitable 'handles' which allow to cleave the prepared peptide under mild conditions either in the form of free acid or as an amide. For this strategy it is necessary to modify the cotton by a suitable functional group first. The best for this purpose is an amino group. We have tried several approaches for the carbohydrate modification (cyanogen chloride chemistry or esterification of cellulose by a protected amino acid using either classical carboxyl activation – carbodiimide with dimethylaminopyridine or carbonyldiimidazole – or a rather unusual enzymatic reaction utilizing subtilisin, or methylester reesterification with the use of extremely high pressure [9]). However, the best results we have obtained with the application of ultrasonic bath (which probably utilizes the effect of local high pressure and temperature fluctuations) and diisopropyl carbodiimide activation in the presence of N-hydroxybenzotriazole and 4-N,N-dimethylaminopyridine.

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