Continuous solid-phase synthesis

Michal Lebl[a], Vladimír Gut[a] and Jutta Eichler[b]

[a]Institute of Organic Chemistry and Biochemistry, Flemingovo 2, 16610 Prague 6, Czechoslovakia

[b]AdW der DDR, Institut für Wirkstofforschung, Alfred-Kowalke-Str. 4, 1136-Berlin, G.D.R.

Introduction

In SPPS, material containing the starting functional group, usually the first amino acid, is placed into the reactor, and all steps of the synthesis are performed without isolation of intermediates. However, at the end of synthesis, the product must be taken out and worked up separately. Obviously, a system allowing for continuous input of starting material and reagents and continually producing the desired peptide would be very useful.

The idea of continuous SPPS may have come to the mind of peptide chemists after the introduction of SPPS by Merrifield [1], since it represents the next logical step in the development of this technology. However, its verification was possible only after continuous carriers became available. We have proven the soundness of continuous peptide synthesis with the use of cotton strips. However, the use of carriers modified only at the surface layer, allowing for a very high coupling rate, would be optimum.

Results and Discussion

The key component of the continuous synthesizer is the continuous carrier, which allows simultaneous performance of all synthetic steps at different locations of the carrier. During all operations, the carrier moves from one compartment to another, and the time of exposure to a particular bath is determined by the path length through this bath and the velocity of carrier movement. The carrier is led through the system of stirred or shaken baths in which washing is performed. To increase the effectiveness of washing, the carrier is, before reaching the next solution, compressed between two cylinders along with porous material (paper, textile), which removes the liquid from the previous wash. Use of the Fmoc protecting group requires less washing solution than using Boc protection. After deprotection in a bath containing the cleavage solution (in our case 20% piperidine in dimethylformamide) and thorough washing, the carrier is introduced to the solution of activated amino acid, and coupling proceeds. The exposure in this compartment must be determined experimentally to assure complete coupling. The most convenient method is continuous monitoring by bromophenol blue [2] which consists of observing decolorization of the blue carrier during coupling. After the coupling, the carrier again undergoes washing

and it is ready to enter the next segment in which another amino acid is coupled to the peptide chain. In the last compartment of the synthesizer, the carrier is introduced to the cleavage solution, which releases the peptide from the carrier.

The search for a suitable carrier led us to use cotton, which had shown the most promising mechanical and chemical properties. Our experimental set-up on which we tested continuous peptide synthesis consisted of only one set of rollers and shaken baths; therefore, all the transfers had to be made manually. We used cotton strips 3 cm wide onto which Boc-glycine or Boc-alanine is coupled by the action of dicyclohexylcarbodiimide in the presence of dimethylaminopyridine. It is necessary to pretreat the cotton with trifluoroacetic acid and diisopropylethylamine before the coupling. Without this pretreatment, the coupling of protected amino acid onto the cotton is much less efficient. This carrier has a capacity of 3.1 μmol/cm^2 or 0.1 mmol/g. After cleavage of the Boc protecting group by TFA, we continue the synthesis with Fmoc-protected amino acids or attach onto the first amino acid the acidolytically cleavable handle (oxymethylphenoxypropionic acid) and perform the synthesis on this handle. The time required for complete coupling is determined by bromophenol blue monitoring, i.e., until the cotton strip looses its blue color. After the last step, the carrier is treated either with the 50% solution of trifluoroacetic acid and 5% dimethylsulfide in dichloromethane (for syntheses performed on the handle) or with a 1 M solution of sodium hydroxide. The products are purified by RPHPLC, and characterized by FABMS, then elemental and AAA. We have prepared the following peptides: Tyr-Gly-Phe-Met, Tyr-Gly-Gly-Phe-Met, Tyr-Gly-Gly-Phe-Met-Gly, Pro-Leu-Gly-Ala, Leu-Pro-Gly-Ala, Leu-Phe-Pro-Val-Ala, Leu-Phe-Pro-Val-Gly-Ala, Cys(Acm)-Tyr-Ile-Gln-Asn-Cys(Acm)-Pro-Leu-Gly, Cys(Acm)-Tyr-Ile-Met-Asn-Cys(Acm)-Pro-Leu-Gly.

References

1. Merrifield, R.B., J. Am. Chem. Soc., 85 (1964) 2149.
2. Krchňák, V., Vágner, J., Šafář, P. and Lebl, M., Coll. Czech. Chem. Commun., 53 (1988) 2542.