SOLID PHASE PEPTIDE SYNTHESIS

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Introduction

Peptide synthesis is the production of peptides which are organic compounds in which multiple amino acids are linked via peptide bonds (-CO-NH-) which are also known as amide bonds. The biological process of producing long peptides (proteins) is known as protein biosynthesis. Peptides are synthesized by coupling the carboxyl group or C-terminus of one amino acid to the amino group or N-terminus of another. Due to the possibility of unintended reactions, protecting groups are usually necessary. Chemical peptide synthesis starts at the C-terminal end of the peptide and ends at the N-terminus. This is the opposite of protein biosynthesis, which starts at the N-terminal end. Liquid-phase peptide synthesis is a classical approach to peptide synthesis. The classical approach to peptide synthesis has yielded impressive success in the preparation of several biologically active peptides. But these procedures are not ideally suited to the synthesis of long chain polypeptides because the technical difficulties with solubility and purification become formidable as the number of amino acid residues increases. A new approach to peptide synthesis has been investigated in an effort to overcome some of these difficulties by R.B. Merrifield and he introduced the solid phase peptide synthesis (SPPS) in 1963. Classical method of peptide synthesis has been replaced in most labs by solid-phase synthesis.

Since its inception in the early 1960s (Merrifield, 1963) solid phase peptide synthesis has become one of the most important methodologies in chemistry and biology. The fundamental premise of solid-phase peptide synthesis is that amino acids can be assembled into a peptide of any desired sequence while one end of the chain is anchored to an insoluble support. After the desired sequence of amino acid has been linked together on the support a reagent can be applied to cleave the chain from the support and liberate the finished peptide into solution. All the reaction involved in the synthesis can be brought to 100% completion, so that a homogeneous product can be obtained. The great virtue of using a solid support is that all the laborious purification at intermediate stages in the synthesis are eliminated, and simple washing and filtration of the product is substituted.

Principle

The principle of Merrifield’s solid phase peptide synthesis is illustrated in scheme 1. The Merrifield resin is basically non polar hydrophobic polystyrene (PS) cross linked with rigid divinyl benzene (DVB) and functionalized with a chloromethylated group. The first N-protected amino acid is bound by its carboxyl group to the polymer via a benzyl ester. The second step involves the deprotection of the amino group under conditions that do not cleave the resin-amino acid ester bond. In the third step, a second N-protected amino acid is coupled to the amino group of the polymer bound amino acid using dicyclohexyl carbodiimide (DCC) or through an active ester coupling.

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The N-deblocking and coupling steps are repeated until the desired sequence is formed. Finally, the resin peptide bond is cleaved by a suitable acid catalysed cleavage reaction which results in simultaneous N-deblocking and deblocking of most of the side chain functionalities. Since only the final peptide is obtained by cleavage from the polymer, the purity of the final product depends upon the coupling efficiency of each step.

(Scheme 1)
Over the years, Merrifield’s method of solid phase peptide synthesis has undergone many strategical improvements (Artherton, 1989). The rigidity, hydrophobicity and physicochemical incompatibility of the styrene-DVB resin necessitated the development of new polymer supports with optimum physicochemical characteristics and a number of polymer supports with different structural characteristics have been developed for peptide synthesis (Jaya TVarkey, 1998 and Ajikumar PK, 2000). Furthermore, the ‘linkers’ between the C-terminal amino acid and polystyrene resin have improved attachment and cleavage to the point of mostly quantitative yields (Wang, 1973 and Matsueda, 1981). The evolution of side chain protecting groups has limited the frequency of unwanted side reactions. In addition, the evolution of new activating groups on the carboxyl group of the incoming amino acid have improved coupling and decreased epimerization. Finally, the process itself has been optimized. In Merrifield’s initial report, the deprotection of the α-amino group resulted in the formation of a peptide-resin salt, which required neutralization with base prior to coupling. The time between neutralization of the amino group and coupling of the next amino acid allowed for aggregation of peptides, primarily through the formation of secondary structures, and adversely affected coupling. The Kent group showed that concomitant neutralization of the α-amino group and coupling of the next amino acid led to improved coupling (Schnolzer et al., 2007). Each of these improvements has helped SPPS become the robust technique that it is today. There are two majorly used forms of SPPS — Fmoc and Boc. Automated synthesizers are available for both techniques, though many research groups continue to perform SPPS manually.

### Comparison of BOC and FMOC solid-phase peptide synthesis

Both the Fmoc and Boc methods offer advantages and disadvantages. The selection of one technique over another is thus made on a case-by-case basis (Nilsson BL et al., 2005).

<table>
<thead>
<tr>
<th>Require special equipment</th>
<th>Boc</th>
<th>Fmoc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost of reagents</td>
<td>Lower</td>
<td>Higher</td>
</tr>
<tr>
<td>Solubility of peptides</td>
<td>Higher</td>
<td>Lower</td>
</tr>
<tr>
<td>Purity of hydrophobic peptides</td>
<td>High</td>
<td>May be lower</td>
</tr>
<tr>
<td>Problems with aggregation</td>
<td>Less frequently</td>
<td>More frequently</td>
</tr>
<tr>
<td>Synthesis time</td>
<td>~20 min/ amino acid</td>
<td>~20-60 min/ amino acid</td>
</tr>
<tr>
<td>Final deprotection</td>
<td>HF</td>
<td>TFA</td>
</tr>
<tr>
<td>Safety</td>
<td>Potentially dangerous</td>
<td>Relatively safe</td>
</tr>
<tr>
<td>Orthogonal</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Boc SPPS uses special equipment to handle the final cleavage and deprotection step, which requires anhydrous hydrogen fluoride. Because the final cleavage of the peptide with Fmoc SPPS uses TFA, this special equipment is not necessary. The solubility of peptides generated by Boc SPPS is generally higher than those generated with the Fmoc method, because fluoride salts
are higher in solubility than TFA salts. Next, problems with aggregation are generally more of an issue with Fmoc SPPS. This is primarily because the removal of a Boc group with TFA yields a positively-charged α-amino group, whereas the removal of an Fmoc group yields a neutral α-amino group. The steric hindrance of the positively charged α-amino group limits the formation of secondary structure on the resin. Finally, the Fmoc method is considered orthogonal, since α-amino group deprotection is with base, while final cleavage from the resin is with acid. The Boc method utilizes acid for both deprotection and cleavage from the resin. Based on this comparison, one sees that both methods possess advantages and disadvantages. Thus, several factors help to decide which method may be preferable.

**Conclusion**

Solid-phase peptide synthesis (SPPS), pioneered by Robert Bruce Merrifield, resulted in a paradigm shift within the peptide synthesis community. It is now the accepted method for creating peptides and proteins in the lab in a synthetic manner. SPPS allows the synthesis of natural peptides which are difficult to express in bacteria, the incorporation of unnatural amino acids, peptide/protein backbone modification, and the synthesis of D-proteins, which consist of D-amino acids.

**References**