

Building Bonds

Peptides continue to grow in prominence among pharmaceutical manufacturers, and challenge the very ingenuity of pharmaceutical communities developing novel delivery methods in the future.

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With inherent abilities to block and/or enhance signal transfers in the human body, peptides, when harnessed as active pharmaceutical ingredients, can treat a host of metabolic diseases, cardiovascular and heart conditions, and neurodegenerative disorders. Peptide-based drug targets are being identified at an increasingly rapid pace, both in terms of recently introduced therapies and products in the development pipeline. In fact, a recent report by Frost & Sullivan indicated that more than 40 approved peptide-based drugs are in use today and approximately 400 are being developed to treat allergies, cancer, Alzheimer's, Huntington's and Parkinson's diseases. Peptide-based therapies tap into the direct hardwiring of human physiology, yielding substantial and far-reaching benefits to drug treatments and therapies. Moreover, developments in peptide manufacturing and implementation have made these amino acid compounds more accessible to the market in terms of cost, flexibility and effectiveness.

Compared to small molecule drugs, peptides offer lower toxicity, show higher

specificity, and demonstrate fewer toxicology issues, and in some cases lead to the development of new compounds that are otherwise unavailable. For example, two biotechnology firms are developing peptide-based therapies for cardiovascular ailments – specifically heart failure, which is a condition affecting 5.3 million Americans. One is a novel chimerical natriuretic peptide in clinical development for an initial indication of acute decompensated heart failure (ADHF). The other is a thrombin peptide that in preclinical studies has shown to minimise cardiovascular tissue damage by initiating a series of anti-apoptotic events. Despite their potential however, manufacturing peptides remains a complex process that requires careful considerations of process variables.

Chemical and Recombinant Synthesis

Chemical and recombinant peptide syntheses are the two basic amalgamations for these amino acids, each offering unique sets of advantages suited for different applications. The recombinant method, for example, is a

more natural process and can offer a price advantage on large production scales. It is also effective for longer sequences of more than 100 residues (residues are specific monomers within the polymeric chain of a polysaccharide, protein or nucleic acid). However, the development programme for a recombinant peptide may be costly and can involve complex production steps.

Chemical synthesis, in contrast, is more flexible and easier to scale. It can modify unnatural amino acids and is not constrained to naturally occurring amino acids. Chemical synthesis can be cost-effective from gram scale to multi-kilogram level depending on the synthesis route. Chemical synthesis is achieved by coupling the carboxyl group of an amino acid to the amino group of another amino acid.

Synthesis Techniques

For synthesis techniques, the two distinct methods are solid-phase and solution-phase, each with their own unique

Figure 1 (above): 12 inch reverse phase HPLC column used to purify peptides

application. Liquid- or solution-based peptide synthesis is the older technique, though most labs today use solid-phase synthesis. Solution-phase is better for shorter peptide chains and is useful in large-scale production greater than 100kg in scale. Solution-phase synthesis is still widely used in structure modification (peptide) synthesis, rare intermediates preparation and peptide/protein ligation and conjugation. In the peptide industry, solution-phase is more cost-efficient for large scale production of shorter chain peptides, such as luteinising hormone-releasing hormone (LH-RH) analogues.

Solid-phase synthesis allows for an innate mixing of natural peptides that are difficult to express in bacteria. It can incorporate amino acids that do not occur naturally and modify the peptide/protein backbone. In this method, amino acids attach to polymer beads suspended in a solution to build peptides, which remain attached to beads until cleaved by a reagent such as trifluoroacetic acid. This immobilises

the peptide during the synthesis so it can be captured during filtration, while liquid-phase reagents and by-products are simply flushed away. The benefits of solid-phase synthesis are that it greatly speeds production of peptides since it is a relatively simple process; it is easier to scale, and it is more suitable than solution-phase synthesis for longer sequences.

Within solid-phase, two different methods exist: (t)ert-(B)ut(o)xy(c)arbonyl, or t-Boc; and 9H-(f)luoren-9-yl(m)eth(o)xy(c)arbonyl, or Fmoc. T-Boc is the original method used in solid-phase synthesis and uses acidic condition to remove Boc from a growing peptide chain. This requires the use of small quantities of hydrofluoric acid, which is generally regarded as safe, but requires specialised equipment. This method is preferred for complex syntheses and when synthesising non-natural peptides.

Fmoc was pioneered later than t-Boc and makes cleaving peptides easier. It is also easier to hydrolyze the peptide

from the resin with a weaker acid. This eliminates the need for specialised equipment. Both methods are valuable and each suit different applications, however, Fmoc is more widely used because it eliminates the need for hydrofluoric acid.

Process Variables to Increase Yields

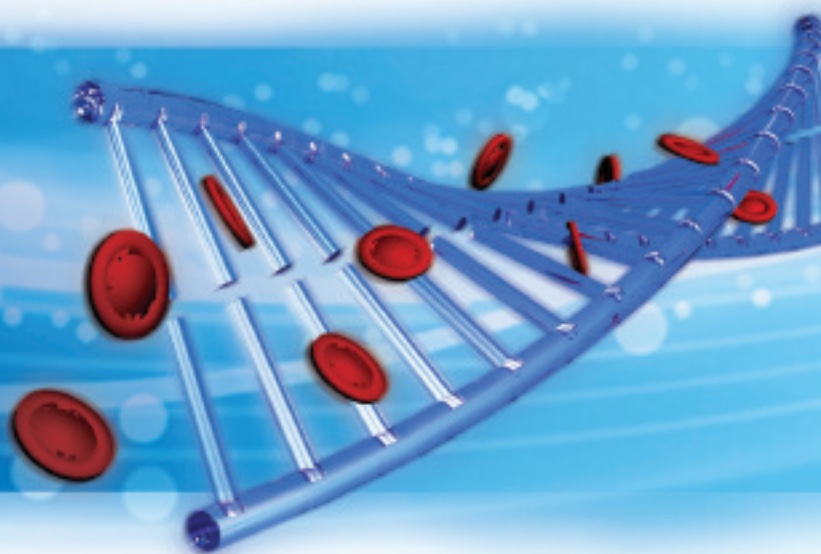
There are a number of process variables that can enhance yield in the peptide synthesis process. First of all, sequence analysis and synthesis strategy design are crucial for the whole process. In this initial step, a chemist will determine automatic synthesis or manual synthesis, suitable resin type, coupling/deprotection/cleavage method that could eliminate potential side reaction and minimise by-product content; and whether or not to insert special building blocks and positions to prevent aggregation in sequence assembly.

As peptide chemistry and technology continue to advance, it is possible to



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Figure 2: 200 litre glass peptide synthesis reaction vessel



All images: cGMP facility in Vista, CA

produce larger peptides in greater quantities. It is also possible to synthesise peptides that are more than 100 amino acids in length, although 10 to 50 amino acids long are more common for therapeutic peptides.

Optimising Purification

Purification is yet another important factor that must be carefully assessed among many methods to ensure desired results are achieved cost-effectively. Typically preparative high performance liquid chromatography (HPLC) is used to purify the peptide product. A common purification buffer is trifluoroacetic acid (TFA), which unlike other final salt form does not require an additional salt exchange step to convert the peptide to the desired salt form. Mass spectrometry data and amino acid analysis are obtained to confirm the identity of the target peptide.

Hydrophobic peptides can pose significant purification challenges because they are not readily soluble in typical purification buffers. Additionally, alternatives to freeze-drying of the purified peptides, such as large vessel precipitation and spray drying, are under consideration, but spray drying can pose problems since peptides can be thermally unstable. Another important purification technology is ion exchange, which is gaining importance in the purification of peptides. In addition, ultra performance liquid chromatography (UPLC) is becoming a useful analytical tool.

The Importance of Quality

As peptide-based therapies are increasingly becoming viable as drug discovery and development targets, the industry is paying more attention to the quality concerns that underlie peptide manufacturing processes. Peptide synthesis for pharmaceutical manufacturing can be tedious and time-consuming given the complexity of the product and the lengthy, intricate synthesis process. Regulatory compliance, quality control and assurance efforts are critical for the successful development and manufacture of peptides as active pharmaceutical ingredients (APIs). As a key element in the peptide production process, quality should be built into every step and thought of as a process parameter rather than a process outcome. This is required to assure the purity of the final product and effectively satisfy regulatory oversight.

Achieving product quality and purity requires a meticulous quality-centric approach from discovery to the final release of the product. While the notion of quality encompasses all activities designed to ensure adequacy of manufactured products, the protocols for the pharmaceutical industry are usually divided into two separate functions: quality assurance (QA), which oversees

the entire manufacturing process and is responsible for the final release; and disposition of the product and quality control (QC), which is responsible for analytical testing and characterisation of raw materials and finished products. Essentially, QC monitors the endpoints of a production run: what comes in and what goes out. QA, by contrast, is responsible for the entire process along the way.

The analytical chemists who are responsible for QC also ensure that analytical methods are developed and subsequently validated. Their assessment of structural integrity and purity of the peptide is critical during the development stages of a product. Without rigorous analytical characterisation and evaluation of potential impurities at the start of each manufacturing project, problems may be missed only to resurface at a later point in the process – often as product recalls and sometimes with devastating consequences.

Conclusion

The promise of peptides as active pharmaceutical ingredients will not only reinvigorate drug innovation and discovery, but it will also challenge the ingenuity of pharmaceutical communities to develop novel delivery methods for present and future therapies. In-depth knowledge of peptide production methods to optimise yield and purity is critical to enabling cost-effective and faster commercialisation of peptide-based therapies.

About the author



Gary Hu, Vice President of Sales and Marketing at American Peptide Company, oversees all non-GMP and GMP business units. Gary spearheads the Total Peptide Management™

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