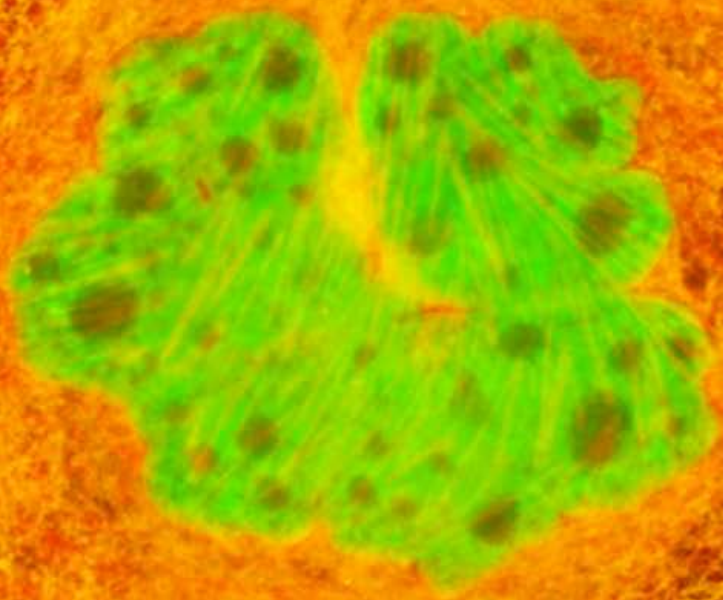
A publication of the BioProcess Technology Network

Fall 2009
ISSN 1538-8786

BioProcessing JOURNAL

Trends and Developments in BioProcess Technology



Vol. 8/No. 3

www.bioprocessingjournal.com

Peptide Synthesis in Pharmaceutical Manufacturing

By GARY HU

Amino acids are the most basic of building blocks in physiology, which is why peptides (oligomers of amino acids) are continuing to grow in prominence among pharmaceutical manufacturers.

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 market and technology research firm 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.[1]

Peptide-based therapies tap into the direct hard-wiring 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 working with California-based American Peptide Company, a manufacturer of these active pharmaceutical ingredients (APIs), to develop peptide-based therapies for cardiovascular ailments, specifically heart failure; a condition affecting 5.3 million Americans.[2]

One such therapy 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 minimize cardiovascular tissue damage by initiating a series of anti-apoptotic events.

The examples are just two of the many life sciences companies pursuing the development of peptide-based therapies. However, manufacturing of peptides can be a complex process and requires careful consideration of manufacturing and process variables.

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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 at large production scales. It is also effective for longer sequences of more than 100 residues (specific monomers within the polymeric chain of a polysaccharide, protein or nucleic acid). However, the development program 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

There are two distinct methods: solid-phase and solution-phase; each with unique applications. 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 100 kg 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 luteinizing 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 (TFA). This immobilizes 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: 1) it greatly speeds production of peptides since it is a relatively simple process; 2) it is easier to scale; and 3) it is more suitable for longer sequences than solution-phase synthesis.

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 employs an acidic condition to remove Boc from a growing peptide chain. This requires the use of small quantities of hydrofluoric acid (generally regarded as safe) and specialized equipment. This method is preferred for complex syntheses and when synthesizing non-natural peptides.

Fmoc, pioneered later than t-Boc, makes cleaving peptides simpler. It is also easier to hydrolyze the peptide from the resin with a weaker acid, eliminating the need for specialized equipment. Again, both methods are valuable and they each suit different applications. However, because Fmoc eliminates the need for hydrofluoric acid, it is more widely used.

Process Variables to Increase Yields

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

As peptide chemistry and technologies continue to advance, larger peptides made in greater quantities are possible. It is also possible to synthesize peptides that are more than 100 amino acids in length although 10 to 50 amino acids long are more common for therapeutic peptides.

Optimizing Purification

There are numerous methods necessary for ensuring that desired results are achieved cost-effectively. Among them, purification is a particularly important factor that must be carefully assessed.

Typically, preparative high performance liquid chromatography (HPLC) is used to purify the peptide product. A common purification buffer is TFA. However, when the final salt form is not TFA, an additional salt exchange step is required 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.

Conclusion

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

ABOUT THE AUTHOR

Gary Hu oversees all non-GMP and GMP business divisions. He has been with APC for 18 years and he is tasked with keeping the organization profitable and meeting growth objectives year after year.

Mr. Hu spearheads the Total Peptide Management™ program, American Peptide Company's flagship initiative, designed to help the pharmaceutical and life sciences industries bring new and innovative drugs to market faster. Specializing in business development, he emphasizes a team approach to bring value and integrity to customers' projects.

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