Increasing the Efficiency of Peptide Synthesis

Microwave-Based Synthesis and Hybrid Strategies Show Promise

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During the past year, PolyPeptide Laboratories (PPL; www.polypeptide.com) has seen "a surge in demand for commercial peptide production--both in the number of peptides and the scale of manufacture," says Jane Salik, Ph.D., president of U.S. operations.

PolyPeptide Laboratories has peptide synthesis facilities worldwide including a facility in Torrance, CA, devoted mainly to solid-phase synthesis, at which the company can produce GMP peptides in lots of 250 g to 1 kg.

Facilities in Hillerod, Denmark, and Malmo, Sweden, produce industrial quantities of generic and custom peptides, with the Hillerod facility housing large-scale solution-phase manufacturing. The Hillerod, Malmo, and California sites are certified for GMP production of peptide APIs.

Meeting Demand

In response to the increasing demand, PPL is in the process of expanding its Torrance facility, more than doubling its manufacturing capacity. The expansion includes installation of a 200-L lyophilization unit, larger solid-phase reactors, and additional preparative HPLC systems.

Commenting on advances in solid-phase synthesis technology and reduced costs of raw materials, Salik says, "Long, complex peptides can be manufactured quickly and cost effectively on a scale of up to hundreds of kilograms or more."

"Capacity and price are the biggest challenges facing the peptide industry today, as peptide drugs move from smaller niche markets to more mainstream drug markets."

She points to the development of hybrid synthesis technology, in which peptide fragments produced using solid-phase synthesis are combined in solution or on a resin, as facilitating efforts in "large-scale synthesis of some very complex peptides that might not have been feasible even a few years ago."

HPLC-based purification and lyophilization of the final protein are still costly, labor-intensive processes; alternative technologies in development to increase scalability and cost efficiency include the use of spray drying for peptide isolation.


The company's PepSpot™ technology enables the synthesis of large numbers of peptides in parallel. Jerini combines its peptide sets with its PepStar™ peptide microarray platform to provide the tools for enzyme profiling and mapping protein-protein interactions or B/T-cell epitopes.

Jerini recently introduced Kinase Substrate Sets, which are ready-to-use peptide sets for kinase profiling comprised of 720 biotinylated kinase substrate peptides derived from human phosphorylation sites. The set comes in two 384-well microtiter plates, each of which contain 360 peptides, with 250 pmol per well.

The company's Phosphatase Substrate Set consists of a 384-well microtiter plate containing 360 phosphopeptides and 10 calibration standards. Jerini will soon introduce Protease Substrate Sets and PepMixes (custom or ready-to-use), which are defined peptide mixes for antigen-specific T-lymphocyte stimulation.

"The use of complete protein describes a significant increase in the demand for peptides at both extremes: for specialty peptides in bulk and for large numbers of screening peptides."

He also describes "a paradigm shift regarding the development of therapeutic peptides"—movement in interest from only a few specialized biotech companies to increasing activity among Big Pharma due to advances in overcoming some of the limitations of protein drugs, for example, regarding delivery and bioavailability.

Competition in Research Markets

Biopeptide (www.allpeptides.com) uses Fmoc chemistry, a combination of automated and manual peptide synthesis, and HPLC purification to produce mg- to kg-scale peptides for research use. During the past few months, Biopeptide introduced an express service that reduces delivery time from 3-4 weeks to 1-2 weeks.

Although the basic chemistry behind peptide synthesis has not changed in the past 25 years, advances enabling the synthesis of 96 peptides in microwell plates in a single run and the introduction of microwave-assisted synthesis are changing the field, according to Michael Freydkin, Ph.D., director of sales and marketing at Jerini Peptide Technologies. He
van Dijken says. Clients want help designing peptides specifically tailored for their applications.

Dr. van Dijken emphasizes the importance of being a partner with, and not just a supplier to clients, and of meeting project commitments.

When peptide companies oversell themselves and claim to be able to make large sets of complex working with peptides.

The industry has seen the price of simple, linear, short peptides drop dramatically in recent years to a recent low of US$35 per peptide at low milligram scale, according to Dr. van Dijken. He expects prices to continue to drop. For specialty peptides, however, companies continue to be willing to pay a higher price for consulting services and quality assurances.

**Deprotection and Speed**

The Odyssey™ System, developed by CEM (www.cem.com), employs microwave energy to drive deprotection and improve the speed and efficiency of solid-phase peptide synthesis.

Each step can be performed 10 times faster with microwave energy (compared to conventional solid-phase synthesis at room temperature) enabling production of a 10mer peptide in 2.5 hours, according to the company.

With microwaves, a large amount of energy can be delivered to a reaction without creating high temperatures. The increase in kinetic energy that results from microwave absorption results in decreased side chain aggregation, facilitating the synthesis of long peptides.

“The Odyssey System allows you to make up to 12 separate peptides automatically, at scales of up to 2-5 nmole, says Michael Collins, president and CEO of CEM. The company is just beginning to ship its first systems, and in the future plans to offer larger scale systems and peptide synthesis services.

Microwave-driven synthesis improves the overall efficiency of the process, yields purer peptides, and could enable the synthesis of peptides that previously could not be made, according to Collins.

“We have been making peptides as long as 50-60 amino acids with better results than with conventional methods,” claims John Collins, Ph.D., a scientist at CEM.

Peptides that would have taken five weeks to synthesize have taken only two days. The reactor’s single mode technology enables reliable energy delivery to the reactants.

At the core of the Odyssey System is CEM’s Discover® System, a single-mode applicator that provides a Focused™ Microwave field. This technology generates an energy field that is 30 times more intense than a typical multimode applicator, the company says.

The cavity automatically adjusts the field pattern to match a sample’s physical characteristics, geometry, and volume. The system can accommodate up to 25 amino acids, room for 20 naturally occurring amino acids, and up to five non-naturally occurring amino acids.

**Solution-Phase Approach**

Rapid Solution Synthesis of Peptides (DioRaSSP) is a novel method of solution-phase peptide synthesis developed by Diosynth (www.diosynth.com), a unit of Akzo Nobel. DioRaSSP combines the ability to use less costly amino acid derivatives and scalable characteristics of solution-phase synthesis with the speed and ease of development of solid-phase approaches.

Its advantages include minimal waste streams and high peptide purity and yield, according to Ralf van Dijk, Ph.D., product manager at Diosynth.

The main drawback with solution-phase synthesis is the long timeline to develop a synthesis protocol, due to the need for isolation and purification of intermediates with different physical properties at each step.

The difference with DioRaSSP is that “you do not isolate the intermediate product; the growing peptide stays in the organic phase,” says Dr. van Dijk. “You shield the growing peptide by anchoring it in the organic phase,” through hydrophobic interactions of the organic solvent with protecting groups placed on the amino acids.

“The system uses aqueous washings to remove unreacted products and reagents from the permanent organic phase containing the growing peptide, thus eliminating the need for solvent switches,” and the cost and environmental issues associated with them.

A single cycle of the DioRaSSP protocol consists of a coupling step, quenching of residual activated carboxyl group, aqueous extractive work-up, deprotection of the N-terminal amino function, and another aqueous extractive work-up. After completion of one coupling and before the coupling step of the next synthesis cycle, any residual activated carboxyl group compound, if hydrophobic, is quenched using an amion-forming amine.

This quenching step, combined with the work-up steps impedes the formation of insertion sequences according to the company.

Citing another advantage of the system, Dr. van Dijk points out that the impurity profile of a sample obtained from an early small-scale synthesis is the same as from a subsequent large batch.

The chemical protocol does not change with scale-up, so no new byproducts or intermediates are formed; thus new regulatory hurdles are introduced and no additional toxicology studies needed.

**CGRP Peptide Synthesis**

At its facilities in Brussels, Belgium, and Torrance, CA, Peptisyntha (www.peptisyntha.com) manufactures CGRP peptides for therapeutic use. Combining liquid-phase expertise in Brussels and solid-phase expertise in Torrance, the company selects the optimal synthesis strategy for each individual peptide.

Pierre Barthelemy, Ph.D., general manager at Peptisyntha, sees growth in both areas and, in particular, increasing importance of solid-phase synthesis, now that many barriers to large-scale synthesis have been overcome and solid-phase techniques can be used to produce tens of kilograms of peptide.

Remarkable on the growing interest in protein drugs, Dr. Barthelemy observes that people have no reluctance “taking complex, long peptides (25–40mers) into the clinic.” Synthesis efficiency has improved with the advent of hybrid technology and the availability of novel solid-phase resins.

More than two thirds of Multiple Peptide Systems’ (MPS; www.mps-sd.com) business derives from GMP production of peptides intended for therapeutic use. Customers are benefiting from the increasing competition in the peptide field through declining prices and value marketing by peptide providers, which are offering more options and services.

In order to compete, “companies have to show they are here and here to stay,” says Robert Hagopian, director of business development at MPS, noting that “there will be a big enough pie for everyone.”

MPS focuses on solid-phase synthesis, but also has solution-phase synthesis capabilities. “Hybrid chemistry will be the thing of the future,” says Hagopian, with peptide fragments made in solid phase and condensed in solution phase.

For now, though, he anticipates a continued focus on solid phase for use in synthesizing up to 50–100 kg quantities.

**Specific Needs**

Customers are increasingly requesting unusual syntheses, such as modified and dye-labeled peptides for high-throughput screening applications in drug discovery, and for phosphopeptides for use in kinase assays, according to Anita Hong, Ph.D., president of San Jose, CA-based Anaspec (www.anaspec.com).

The company manufactures its peptides according to GMP guidelines and is ISO 9001:2000 certified. Anaspec enables customers to check the status of their peptide projects online.

UCB-Bioproducts (www.ucbbioproducts.com) manufactures both custom non-GMP peptides for preclinical and research applications and bulk. The company also has CGMP manufacturing capabilities for producing active pharmaceutical ingredients.

New to Princeton BioMolecules (www.pbcbiotech.com) peptide portfolio are N15/C13 labeled peptides. The company produces crude to highly purified peptides in milligram to gram scale.

Invitrogen’s (www.invitrogen.com) EvoQuest™ custom peptide services is a source for peptides in a range of scales and purities, including labeled, modified, and conjugated peptides, and peptides containing specialty amino acids.

American Peptide Co’s (APC; www.americanpeptide.com) newest large-scale peptide manufacturing plant, located in Vista, CA, is producing grams to kilograms of APIs under CGMP conditions.

The company’s headquarters in Sunnyvale remain the R&D center for manufacturing research and GLP-grade peptides from milligram to multikilogram scale. APC has developed new products and services for PEGylation, quick labeled materials, fluorescent dye products, and other specialized reagents.

“APC is experienced in solid-phase and solution-phase peptide synthesis and organic conjugations to proteins, toxoids, and PEG,” says Gary Hu, VP of marketing and sales at APC.