Therapeutic Peptides and Proteins: Assessing the New Technologies

29

Banbury Report

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Therapeutic Peptides and Proteins: Assessing the New Technologies

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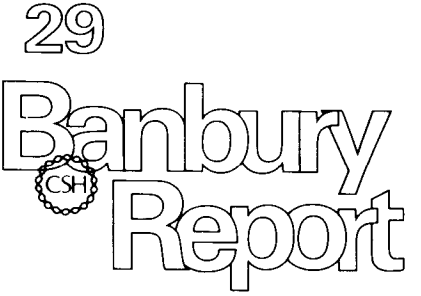

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Preface

Recombinant DNA methodology has spawned a new technology, biotechnology, and a corresponding industry. The goals of much of this infant industry are to manufacture therapeutic products for human health care. To this end, the actual products of cloned, recombinant DNA molecules are proteins, just as the molecular phenotypes of human genes are proteins. Therefore, recent attention in the industry has shifted from simply DNA cloning technology to protein engineering technology. Protein chemists are now in great demand to design, express, purify, and characterize the products of cloned genes. At every stage in production, the structure and function of the protein product is a concern. Therefore, it falls to the analytical protein biochemists to develop the appropriate techniques to evaluate the products.

Fortunately, in parallel with the recombinant DNA explosion, there has been a revolution in the characterization of the structure and function of proteins. This new technology consists not only of new instrumentation and automation, but of the proliferation of methods for protein analysis on the production line. Questions about renaturation of proteins, disulfide bond formation, post-translational modifications and protein degradation are no longer academic exercises. These are important concerns to the industry and to the regulatory agencies, because of the great financial investment in biotechnology and the great potential risk to patients receiving therapeutic protein products. As members of an emerging discipline throughout the world, it is the responsibility of the protein chemists to assess the new technology of protein production routinely, and to implement those methods necessary to evaluate the efficacy and safety of protein products.

In addition to the recombinant DNA-based products, there is a competing technology, that of chemical synthesis of peptides and proteins. Small peptide hormones are made by chemical synthesis routinely, and a variety of agonists and antagonists for small peptides have been produced as well. Several groups have succeeded in synthesizing biologically active molecules over 50 amino acids in length, including growth factors and plasma proteins. Therefore, chemical synthesis and recombinant DNA production must be evaluated together as competing and complementary approaches to constructing therapeutic peptides and proteins. Clearly, chemically synthesized proteins and protein products of cloned genes will share some of the same risks to patients, but will have many differences in their risks because of the widely different methods of preparation.

The meeting on Therapeutic Peptides and Proteins documented in this volume was organized to address the problems of production and evaluation of protein products. We hoped to ask questions concerning the state of the

technologies for production, the state of the methodologies for chemical and biological testing, and the views of the regulatory agencies on the use of proteins for therapeutics. Our concept was to bring together a small group of senior scientists from academics, industry, and the regulatory agencies to discuss these issues. The main goal was to develop a concensus of what the technologies are, where they are going, what the concerns are with therapeutic proteins, what we *can* do about the risks, and what we *should* do about the risks. Although these questions remain open, this meeting was timely in its approach to these problems.

The meeting was organized by four scientists, Darrell Liu of the Food and Drug Administration, Derek Bangham of the National Institute for Biological Standards and Control (U.K.), Alan Schechter of the National Institutes of Health, and myself at Cold Spring Harbor. Key to the organization of this meeting was the late Steve Prentis, director of the Banbury Center. Steve and I arrived at Cold Spring Harbor on the same day, March 31, 1986, and began discussing the idea of a therapeutic protein meeting immediately. Upon contacting Dr. Liu, we found that he and Dr. Bangham had conceived of a similar meeting some years earlier. Thus, Banbury Center provided an ideal setting for the culmination of the ideas of all of the organizers.

I would like to thank the organizers and participants of the meeting for the lively discussion and outstanding presentations. We are all indebted to Bea Toliver, Ralph Battey, and Inez Sialiano who carried on the administrative and editorial leadership for the meeting and this book. Finally, we honor Steve Prentis, who supported this cause with great efforts, but was taken from us in an untimely death on February 28, 1987. It is my hope that this volume will serve society in ways that will memorialize his life and work in science.

D.R. Marshak

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New Production Technologies

The Chemical Synthesis of Therapeutic Peptides and Proteins

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OVERVIEW

Methods for the chemical synthesis of peptides have been refined to the point where the total synthesis of polypeptide chains of 50 or more amino acids is routine. This brings to fruition the original goal of peptide chemists: the total chemical synthesis of proteins for investigation of the structural origins of their activities. We have developed a generally applicable chemistry for the automated assembly of protected peptide chains at the rate of 20 minutes per residue and with yields up to 99.7% per amino acid residue. Complete deprotection is achieved with strong acids (hydrogen fluoride [HF], sulfonic acids) without chain cleavage artifacts and with minimal side reactions due to the protecting groups. Purification of the synthetic products by reversed-phase high-performance liquid chromatography (HPLC) and their characterization by isoelectric focusing on immobilized pH gradient gels, quantitative Edman degradation, and mass spectrometry are described. The chemical synthesis of proteins (that is, long polypeptide chains folded into a precise three-dimensional structure) is further complicated by the requirement to fold the polypeptide chain to the precise three-dimensional structure of the protein. A key aspect of the success of this approach to the total chemical synthesis of peptides and proteins is automation that allows synthesis to be carried out in a rapid, reproducible fashion with quantitative documentation of the results. The chemical synthesis approach to the preparation of peptides and proteins complements the cloning approach and offers substantial advantages for the preparation of short peptides (up to 40 amino acid residues) and peptides containing α-carboxamide groups.

INTRODUCTION

The chemical synthesis of peptides has its origins in the work of Emil Fischer in the first decade of this century. Over the ensuing five decades, Fischer and his scientific descendants perfected the techniques of classical solution synthesis of peptides to the point where small proteins, such as insulin, were

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susceptible to this approach. The ultimate achievements of this approach and methodology were the total chemical synthesis of insulin and a series of disulfide mispaired analogs (Sieber et al. 1978) and the total chemical synthesis of ribonuclease by a fragment condensation approach (Fujii and Yajima 1979). However, these methods, despite their broad applicability, suffer from two serious limitations. The manipulations are difficult and demanding, requiring extremely skilled peptide chemists. In addition, the protected intermediate fragments are frequently insoluble, leading to low concentrations and poor coupling yields in the final stages of chain assembly. In 1963, Bruce Merrifield of the Rockefeller University introduced the revolutionary principle of stepwise solid-phase peptide synthesis (Merrifield 1963). In this method, the carboxy-terminal amino acid residue is covalently attached to an insoluble resin support. The N-α amino protecting group is removed, and the next amino acid in the sequence of the target peptide is introduced in carboxyl-activated form and allowed to react with the resin-bound growing peptide chain to form a new peptide bond. The repetitive cycle of deprotection, purification by simple washing and filtration of the insoluble peptide-resin, and introduction of the next activated amino acid, is continued until the target-protected peptide chain is built up in resin-bound form. Finally, all the protecting groups are removed and the peptide cleaved from the resin support by strong acid treatment giving the crude free peptide product which can be worked up and characterized.

The stepwise solid-phase method has virtually displaced the classic solution approach to the chemical synthesis of peptides and small proteins. Over the 25 years since Merrifield's original report of this method, the procedures have been improved to the point where the routine assembly of peptide chains of 50 amino acid residues or more in length has been accomplished. Similarly, methods for the protection of side chain functionalities and the subsequent removal of protecting groups have been refined to maximize yields and minimize side reactions. Here we describe a modern, optimized set of stepwise solid-phase synthetic chemistry that allows the rapid and efficient synthesis of long peptides and small proteins. This chemistry is carried out on a commercially available, fully automated peptide synthesizer which has been described earlier (Kent et al. 1984, 1985). This optimized high-efficiency solid-phase technology will be briefly described, together with the principles on which it is based. Automated protocols allowing the assembly of protected peptide chains at rates as fast as 20 minutes per amino acid residue will also be described (S. Kent and K. Parker, in prep.), together with methods for the deprotection and cleavage of the peptides from the resin and the purification and characterization of peptide products (Clark-Lewis and Kent 1988). A similar set of technologies adapted to the chemical synthesis of the long polypeptide chains that fold to form proteins will also be described (Kent and