LAWRENCE
LIVERMORE
NATIONAL
LABORATORY

UCRL-PROC-231884

An Appreciation of the Scientific Life and Acheivements of Bruce Merrifield

Mitchell, A. R.

June 18, 2007

An Appreciation of the Scientific Life and Acheivements of Bruce Merrifield
Montreal, Canada
June 26, 2007 through June 30, 2007

An Appreciation of the Scientific Life and Achievements of Bruce Merrifield

Alexander R. Mitchell

Energetic Materials Center, Lawrence Livermore National Laboratory, University of California, 7000 East Avenue, L-282, Livermore, CA 94550, USA.

Introduction

Bruce Merrifield's scientific biography, "Life During a Golden Age of Peptide Chemistry: The Concept and Development of Solid-Phase Peptide Synthesis," provides a history of solid phase-peptide synthesis (SPPS) from 1959 to 1993 [1]. While many readers will be familiar with SPPS literature after 1963, the inclusion of unpublished material from Merrifield's early laboratory notebooks opens a fascinating window on the development of SPPS from the formulation of concept in 1959 (p. 56, ref. 1) to the synthesis of a tetrapeptide four years later [2]. This early period was characterized by slow progress interrupted by numerous setbacks that led Bruce to later record (p. 90, ref. 1): "*At the end of the first two years the results were so poor*, *I wonder what made me think that this approach would ever succeed. But from the outset I had a strong conviction that this was a good idea, and I am glad that I stayed with it long enough"*. Garland Marshall, Bruce's first graduate student (1963-1966), as well as later colleagues, were essentially unaware of the many highways, byways and dead ends that Bruce had explored in the early years [3].

From Concept to Ribonuclease A Synthesis (1959 -1969)

The use of an insoluble polymer covalently linked to a growing peptide chain was without chemical precedent when Bruce began his studies on SPPS in 1959. The search for an acceptable polymer support and appropriate chemistry was especially challenging with Bruce later writing (p. 89, ref. 1): *"When I look back at my old notebooks, I am amazed at how inefficient the early developmental work was. I seemed always to choose the wrong reaction to do first and was not able to identify the most important parameters as the work was progressing."* The synthesis of Leu-Ala-Gly-Val on a polystyrene support was a watershed event that provided the proof of concept needed for SPPS [2]. A series of increasingly larger, biologically active peptides were prepared over the next three years beginning with bradykinin (1964) and culminating with bovine insulin (1967). The arrival of Bernd Gutte, Bruce's first postdoctoral fellow, from Germany (1967) provided the opportunity to push existing SPPS methodology to the limit and undertake the synthesis of the 124-residue enzyme RNase A. In early 1969 Bernd Gutte and Bruce Merrifield published the use of SPPS to achieve the total synthesis of an enzyme with RNase A activity [4]. This achievement, coupled with a similar effort by the Merck group using classical solution chemistry [5] attracted global attention in the scientific and popular press.

Critical Assessments of SPPS

Garland Marshall has recalled the early "vehement and vitriolic" critics in his discussion of SPPS as a paradigm shift [3]. Some of the most vehement tirades surfaced at meetings of the European and American Peptide Symposia in the late 1960s and early 1970s. Brenner essentially summarized early criticism of SPPS in stating: *"The invention of the solid-phase method looked like an ingenious trick to overcome some of the unpleasant features of the classical methods. As we know today, the ingenuity of the trick remains, but only a large investment of heavy real effort will eventually, if ever, work it into a real progress over the classical approach* [6]." Wünsch, upon reflecting on the problems of synthetic peptide research, concluded in 1971 that SPPS exhibited "inborn defects" and was "unsuitable for the satisfactory synthesis of higher natural peptides (with more than 15 amino acid residues) [7]." Bruce Merrifield, a man modest in demeanor but strong in character, persevered. The rest, of course, is history [1].

Onward and Upward (1970-1984)

By the early 1970s it had became apparent that the solid-phase synthesis of RNase A could not be generalized. Consequently, virtually every aspect of solid-phase peptide synthesis (SPPS) was reexamined and improved during the decade of the 1970s (pp. 151-179, ref. 1). The sensitive detection and elimination of possible side reactions (amino acid insertion, N^{a}-trifluoroacetylation, N^{ae}-alkylation) was examined. An optimization of the HF cleavage reaction based on an understanding of the mechanism was developed. The quantitation of coupling efficiency in SPPS as a function of chain length was studied. A new and improved support for SPPS, the "PAM-resin," was prepared and evaluated. In addition to considerable methodological work on SPPS, parallel synthetic efforts on biologically active peptides such as glucagon, thymosin α_1, epidermal growth factor and antimicrobial peptides were undertaken (pp. 180-195, ref.1). These and many other studies from the Merrifield laboratory and elsewhere increased the general acceptance of SPPS.

The success of SPPS dramatically influenced the chemical synthesis of DNA [8]. The chemical synthesis of DNA had been extremely laborious and time-consuming prior to the development of solid-phase syntheses of DNA. For example, the preparation of a *lac* operator (a 21 base paired DNA duplex) required the equivalent of four years of highly skilled and intense effort. When the appropriate chemistry (phosphoramidite method of DNA synthesis) and support were discovered, the rapid preparation ($\leq$ 1 day) of deoxyoligonucleotides the size of a *lac* operator became possible. Use of automated DNA synthesis machines now leads to very high yields of relatively pure polynucleotides having 100 or more mononucleotides [8]. In retrospect, it seemed inevitable that Bruce would receive a call from Stockholm. The decisions of the Nobel Committee are not always obvious, however. To the great delight of friends and colleagues the call came on October 17, 1984 and we must take Bruce at his word when he states (p. 241, ref.1): "Some are dubious when I say I did not ever expect such a thing, but it is surely true. And I still do not know how it happened, but I am grateful."

Bruce Merrifield

How to best describe the man and his science? Garland Marshall has assessed the scientific impact of Bruce's work in his incisive review "Solid-Phase Synthesis: A Paradigm Shift" [3]. Solid-phase synthesis as used for the synthesis of biopolymers (peptides, proteins, nucleic acids), synthesis of natural products, chemical ligation and materials development has indeed provided a paradigm shift in the molecular biology, biotechnology and chemistry communities. The man who emerges from the pages of *Life During a Golden Age of Peptide Chemistry,* and the man his colleagues knew and respected, was tough and dedicated but also caring and modest. He deeply cared about his two families, the family at home and the family in the laboratory (pp. 208-227, ref. 1). Libby Merrifield, his wife, friend and colleague for over 55 years provided the bedrock for his career. He did not voice anger when the early critics maligned him and his work, just as he did not complain about a long-term progressively invasive skin cancer and the increasingly draconian medical treatments. It would have been out of character and a waste of energy that could be better used in the laboratory. Early in 2003, prior to the final combinations of treatments (surgery, antibiotics, and radiation), I asked Bruce if he had considered retirement as an option. He smiled and said, "sure, I think I'll retire just about 2 minutes before I drop dead." Bruce, thank you for your life, your work and your inspiration to all who knew you.

Acknowledgments

This paper was prepared under the auspices of the U.S. Department of Energy by the University of California, Lawrence Livermore National Laboratory, under Contract No. W-7405-Eng-48.

References

1. Merrifield, B. *Life During a Golden Age of Peptide Chemistry : The Concept and Development of Solid-Phase Peptide Synthesis*. American Chemical Society: Washington, DC, 1993.
2. Merrifield, R. B. *J. Am. Chem. Soc.* **85**, 2149–2154 (1963).
3. Marshall, G. R. *J. Pept. Sci.* **9**, 534-544 (2003).
4. Gutte, B. and Merrifield, R. B. *J. Am. Chem. Soc.* **91**, 501-502 (1969).
5. Hirschmann, R., Nutt, R. F., Veber, D. F., Vitali, R. A., Varga, S. L., Jacob, T. A., Holly, F. W. and Denkewalter, R. G. *J. Am. Chem. Soc.* **91**, 507-508 (1969).
6. Fankhauser, P. and Brenner, M. in "The Chemistry of Polypeptides," Katsoyannis, P. G., Ed., Plenum Press, New York, 1973, p 389-411.
7. Wünsch, E. *Angew. Chem. Int. Ed. Engl.* **10**, 786-795 (1971).
8. Caruthers, M. H. *Acc. Chem. Res.* **24**, 278-284 (1991).