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SOLID-PHASE PEPTIDE SYNTHESIS

—Bruce Merrifield—

○ Arthur M. Felix

Bruce Merrifield, the 1984 Nobel laureate in Chemistry and inventor of solid-phase peptide synthesis, was born in Fort Worth, Texas on July 15, 1921. Merrifield's childhood years were influenced by the Great Depression; by the time he started Montebello High School in Southern California, his family had moved 25 times and he had attended 11 different schools. After completing a 2-year period of study at Pasadena Junior College, he enrolled at the University of California at Los Angeles and received his Bachelor's degree in 1943. Merrifield worked as a laboratory technician for 1 year at Philip Park Research Foundation and decided to attend graduate school.

His doctoral studies were carried out at the University of California at Los Angeles and his Ph.D. studies, under the direction of Max Dunn, featured microbiological assays for nucleic acid constituents. Following his graduation in June 1949, he married Elizabeth (Libby) Furlong and accepted a postdoctoral position at Rockefeller Institute for Medical Research (now The Rockefeller University) in New York City. Merrifield's entire academic career unfolded on the Rockefeller campus, starting as an Assistant with D. W. Woolley, a pioneer in vitamin research, authority on anti-metabolites, and leading researcher on serotonin. Merrifield was promoted to Associate Professor in 1958 and full Professor in 1966.

Merrifield's first introduction to peptide synthesis took place in Dr. Wooley's laboratory and featured the synthesis of a family of small pentapeptides with streptogenin activity. This laborious undertaking was time consuming and resulted in only low yields. It made clear to Merrifield the need for simpler, faster, and more efficient procedures of peptide synthesis. In the late 1950s and early 1960s it was apparent to most peptide

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chemists that the preparation of peptides with more than 20 amino acid residues was a major undertaking requiring teams of investigators. The assemblage of even small proteins, a major goal of peptide chemists, could not be routinely achieved by available chemical methods at that time.

In 1959, Merrifield conceived the idea of carrying out synthesis on a polymeric support. The fundamental idea was to make use of an insoluble solid support, which holds the peptide chain while it is being assembled. The terminal amino acid is linked covalently at the COOH-terminus to the solid support. Subsequent attachments of additional NH_2 -protected amino acids could be coupled by a suitable activation procedure in a stepwise fashion until the desired peptide sequence was assembled. An important advantage of this process is that excess reagents and byproducts can be removed simply by thoroughly washing the insoluble support with an appropriate solvent. The tedious purification of intermediates is not required in this procedure. Merrifield realized that stepwise peptide synthesis by this method would be simpler, more rapid and could result in better yields since the entire synthesis could be carried out in a single vessel without the need for isolating intermediates or transferring product.

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However, the methodology would require the development of a suitable solid support, efficient coupling methods, mild deprotection conditions, and a procedure for the ultimate removal of the target peptide from the solid support.

Merrifield initially thought that the entire procedure could be worked out in 3 months, but there were unforeseen challenges at every stage. Numerous solid supports, including cellulose, poly(acrylamide), poly(methylmethacrylate) and sulfonated polystyrene were evaluated until poly(styrene) cross-linked with divinylbenzene was found to be ideal. As shown in Fig. 1, functionalization of this resin by chloromethylation enabled the attachment of the COOH-terminus amino acid as the benzyl ester anchor bond. *N*^α-*t*-butyloxycarbonyl-amino acids [Boc-amino acids] were ideally suited for each coupling step since they could be deprotected by mild acidolysis with trifluoroacetic acid (TFA) and neutralization with a tertiary amine. Different coupling conditions were evaluated and ultimately coupling with dicyclohexylcarbodiimide (DCC) proved to be the method of choice since the reaction proceeded rapidly to completion. Anhydrous HF, initially introduced by Shumpei Sakakibara, was used for the cleavage of the peptide from the support.

Four years later, Merrifield published his seminal paper¹⁾ that provided the experimental documentation of a successful synthesis of a tetrapeptide, Leu-Ala-Gly-Val, by this new procedure which he called 'solid-phase peptide synthesis.' This novel approach to peptide synthesis was met with skepticism from many of the established peptide groups throughout the world. Criticism focused on the fact that intermediates are not isolated or characterized and that final products were potentially contaminated with a multitude of impurities. These were difficult times for Merrifield. Some laboratories had difficulty reproducing the solid-phase methodology. His work was openly criticized, even ridiculed, at symposia. Merrifield recognized the limitations of the procedure, including the necessity that coupling reactions must proceed to nearly 100% at each stage of synthesis in order to avoid the formation of deletion peptides. Merrifield has devoted much of his research efforts to developing methods to improve solid-phase

peptide synthesis.

This work was followed with syntheses in the Rockefeller laboratories of biologically active peptide hormones including bradykinin,²⁾ angiotensin,³⁾ and numerous analogs. In 1966, Merrifield and Arnold Marglin published a paper on the solid-phase synthesis of human insulin; a peptide consisting of an A-chain and a B-chain of 20 and 31 amino acids, respectively. Each chain was synthesized by the solid-phase procedure, recombined by oxidation and shown to have comparable biological activity to human insulin prepared by classical methods.⁴⁾

At an early stage, Merrifield recognized the feasibility of automating this procedure and, together with John Stewart at Rockefeller University, constructed the first automated synthesizer.⁵⁾ Merrifield and his coworkers also developed methods for feedback control based on picrate titration of uncoupled *N*^α-amino-function after each coupling. This instrument laid the groundwork for later commercial machines including batch and continuous flow synthesizers. From the outset, Merrifield made a decision not to patent the new solid-phase methodology. This enabled solid-phase peptide synthesis to be available to the entire peptide community without the need for licensing fees. This decision placed this new methodology in research laboratories worldwide and an entire generation of peptide chemists soon recognized the importance of solid-phase peptide synthesis.

Merrifield tested the limits of the methodology with

Fig. 1. The scheme for solid-phase peptide synthesis.

the total synthesis of the entire 124-residue enzyme bovine pancreatic ribonuclease A. This work, undertaken with Bernd Gutte using a combination of manual and automated solid-phase synthesis, is a milestone achievement and was published in 1969. They reported that the synthetic ribonuclease A (1–124) had 80% of the specific enzymatic activity.⁶⁾ The synthesis of ribonuclease A proved unambiguously Anfinsen's hypothesis that the primary sequence of a protein can determine its tertiary structure. Proteins that have been synthesized by solid-phase peptide synthesis by other groups include Inhibin, HIV Protease, Chaperonin 10, Integrin, Colony Stimulating Factor, Migration Inhibitor Factor, and Leukemia Protease.

Continued efforts by Merrifield and coworkers from the 1970's to the present day have had a dual focus on extending and optimizing the basic solid-phase chemistries and applying them to furnish compounds that are used to solve important biological problems associated with peptide and protein science. Merrifield recognized the importance of using solid-phase chemistry to facilitate and direct organic synthesis. His pioneering work revolutionized the field of peptide synthesis and this same solid-phase synthetic philosophy is currently being implemented in the latest revolution of organic synthesis. Solid-phase techniques have been developed for the preparation of classes of biomacromolecules beyond peptides, including carbohydrates and nucleic acids.

Merrifield's career has shown the importance of having a good scientific idea, believing in it, overcoming the criticisms of respected colleagues, optimizing and refining the methodology, and demonstrating its relevance through application to important biological problems. He revolutionized chemistry with his discovery of a novel method to make peptides and he has developed one of the truly innovative ideas of chemistry, the solid-phase principle. The solid-phase methodology has laid the groundwork for the synthesis of combinatorial libraries, which are being used as a flourishing new prototype for drug discovery for the next generation of therapeutics.

Bruce Merrifield's contributions were recently recognized by his colleagues with a special satellite symposium in San Diego, California in June, 2001 (following the 17th American Peptide Symposium/2nd International Peptide Symposium) in his honor in celebration of his 80th birthday. This symposium featured presentations by many prominent scientists in the field and the proceedings have recently been published.⁷⁾

Bruce and Elizabeth Merrifield recently celebrated their 53rd wedding anniversary with their 6 children and 14 grandchildren. They continue to reside in their modest home in Cresskill, New Jersey; the town has recognized their distinguished citizen by renaming the street on which they live to Merrifield Way. A favorite activity of the Merrifield family is camping which has included trips to most of the national parks including Grand Canyon, Bryce, Zion, Mesa Verde, Grand Tetons, Black Canyon of the Gunnison, Carlsbad Caverns, and Yellowstone National Parks. The Merrifield family has also been camping in Alaska. This trip featured a successful effort of panning for gold in the Yukon Territories and traveling 1,500 miles over the gravel roads of the Alcan Highway that included nine flat tires en route to Mt. McKinley.

Over the years, Bruce Merrifield has trained and nurtured numerous graduate students and postdoctorals that have become leaders in academia and industry and serve as the backbone of modern peptide science. He is warmly beloved and admired for his grace, modesty, integrity, and courage. Bruce Merrifield organizes a dinner during the biennial American Peptide Symposium for all his former associates and students. This is a special tradition that continues to this day and is a major feature at the symposium. Another tradition has been the annual picnic at the Merrifield home in Cresskill. All former associates are invited to this special event that occurs in the late fall. This features hamburgers that are cooked on an open fire with all the trimmings, and of course the traditional volleyball game. The more experienced associates have learned to arrive in the afternoon, after all the younger associates have finished raking the leaves on the Merrifield property. Bruce Merrifield continues to pursue his laboratory research at Rockefeller University and serves as an inspiration to the next generation of peptide scientists.

REFERENCES

- 1) R. B. Merrifield: *J. Am. Chem. Soc.*, **85**, 2149 (1963)
- 2) R. B. Merrifield: *J. Am. Chem. Soc.*, **86**, 304 (1964)
- 3) G. R. Marshall, R. B. Merrifield: *Biochemistry*, **4**, 2394 (1965)
- 4) A. Marglin, R. B. Merrifield: *J. Am. Chem. Soc.*, **88**, 5051 (1966)
- 5) R. B. Merrifield, J. M. Stewart: *Nature (London)*, **207**, 522 (1965)
- 6) B. Gutte, R. B. Merrifield: *J. Am. Chem. Soc.*, **91**, 501 (1969)
- 7) G. Barany, A. M. Felix (Eds.): "Crossroads of Chemistry and Biology," *Biopolymers (Peptide Science)*, Volume **60**, No. 3 (2001)