Using Site-directed Mutagenesis to Study Carboxypeptidase A: the Work of William J. Rutter

Use of Directed Mutagenesis to Probe the Role of Tyrosine 198 in the Catalytic Mechanism of Carboxypeptidase A


William J. Rutter was born in 1928 in Malad, a little town in southern Idaho. After hearing his grandfather’s descriptions of the tropical diseases he had observed as a British military officer in India, Rutter decided that he wanted to go to the School of Tropical Medicine in Calcutta to study parasitic diseases. He graduated from Harvard College with a B.A. in 1949 and planned to go to medical school, but after auditing medical school classes at the University of Utah, he decided that basic science was more interesting than practicing medicine. So he enrolled at the University of Utah Medical School and began working with Gaurth Hansen on metabolic physiology in rat tissue. Hansen moved to the University of Illinois and Rutter followed, earning his Ph.D. in 1952.

Next Rutter went to the Institute for Enzyme Research at the University of Wisconsin, Madison to study enzyme chemistry with Journal of Biological Chemistry (JBC) Classic author Henry Lardy (1). Rutter initially started working on the characterization of the malic enzyme, which was involved with carbon dioxide fixation in animals, but he soon picked up a side project studying the mechanism of aldolase. He discovered that aldolase catalyzed a stereospecific hydrogen exchange reaction and decided to investigate the mechanism in greater detail with future Nobel Prize recipient Hugo Theorell at the Nobel Institute in Stockholm.

In 1955, Rutter returned to the United States to take a tenure track position in the chemistry department at the University of Illinois, Urbana. He continued his research on enzyme mechanism, concentrating on aldolase. However, after the structure of DNA was published, Rutter turned his focus to the regulation of RNA transcription. To learn more biology to tackle this problem, he went to Stanford University in 1962 on a Guggenheim Fellowship and worked with developmental biologist Clifford Grobstein. At Stanford, Rutter began to define stages when enzyme expression was turned on in the early developing pancreas.

When Rutter returned to Illinois in 1965, he found his position in the chemistry department less attractive because his research no longer emphasized chemistry. He was more interested in genetics and biology. So he decided to leave, and he accepted a professorship in the departments of biochemistry and genetics at the University of Washington. He continued to work on pancreatic gene expression and development but also began to focus on mechanisms of gene transcription in yeast and sea urchins. He and his colleagues showed that in yeast, sea
urchins, and mammalian cells there are three distinct types of enzymes that transcribe the genes: RNA polymerases I, II, and III (2).

In 1969, Rutter became chairman of the biochemistry department at the University of California, San Francisco. With Vice Chairman Gordon Tomkins, Rutter recruited a large number of researchers who laid the foundation for a multidisciplinary approach to the biology of eukaryotes, using the rapidly developing tools of molecular biology. Under his leadership, the department made many important contributions to biotechnology, including the development of recombinant DNA techniques, which Rutter used in the first cloning of the gene for insulin (3).

Rutter also used recombinant DNA techniques to study the catalytic mechanism of carboxypeptidase A. By the mid-1980s, a wide variety of chemical, kinetic, and structural studies had investigated the enzyme’s catalytic mechanism, but a complete picture of the molecular events that occurred during catalysis remained elusive. In an attempt to clarify the roles of putative functional residues in the enzyme, Rutter turned to site-directed mutagenesis of rat carboxypeptidase A cDNA. He and his colleagues constructed a variant in which Tyr<sup>248</sup> was replaced by phenylalanine to analyze the role of this residue as a general acid catalyst, particularly in the hydrolysis of peptide substrates. His studies showed that the Tyr<sup>248</sup> hydroxyl was not obligatory for substrate hydrolysis, but it did play a significant role in ligand binding (4).

Next Rutter tested the functional significance of the Tyr<sup>198</sup> hydroxyl in carboxypeptidase A by analyzing the effects of a Tyr<sup>198</sup> to Phe replacement on catalytic activity. He also engineered a double mutant in which both Tyr<sup>248</sup> and Tyr<sup>198</sup> were changed to phenylalanine. As detailed in the JBC Classic reprinted here, Rutter found that the catalytic activity of carboxypeptidase A toward peptide and ester substrates was not abolished by the Tyr<sup>198</sup> to Phe replacement or the Phe<sup>248</sup>-Phe<sup>198</sup> double mutant. This showed that proton donation to the leaving group was not mediated independently by either tyrosine residue. However, the removal of Tyr<sup>198</sup> did result in significant decreases in <i>k</i><sub>cat</sub> values, indicating that the residue’s hydroxyl could facilitate substrate hydrolysis by participating in the stabilization of the rate-determining transition state. Although the use of site-directed mutagenesis is commonplace today, it was relatively new in the 1980s, and Rutter’s work was an early example of the power of site-directed mutagenesis in enzymology.

In the early 1980s, Rutter also began an analysis of the hepatitis B virus, and he eventually cloned the gene for the outer viral coat (5). In 1981, Rutter, along with Edward Penhoet and Pablo Valenzuela, founded Chiron Corp., with the idea of using the hepatitis work to develop a vaccine. Chiron became one of the major biotechnology firms in the San Francisco area, and eventually, in collaboration with Merck, it developed a vaccine for hepatitis B (this was also the first recombinant vaccine).

Rutter stepped down as chairman of the biochemistry department at UCSF in 1982, and in 1983, he became director of the UCSF Hormone Research Institute. He relinquished the directorship in 1989 but remained a member of the institute and head of a lab group. He retired in 1991 and became a spokesman for and developer of the biotechnology industry. In 1999, he founded Synergenics, LLC, a consortium of commonly owned but independent biotech companies organized to offer a cost-effective approach to startups in the life sciences industry in the form of financial support, shared lab space, management, and administrative services.

Rutter also served as treasurer of the American Society of Biological Chemists (1970–1976) and was on the editorial board of the <i>Journal of Biological Chemistry</i>. He was elected to the National Academy of Sciences in 1984 and the American Academy of Arts and Sciences in 1987. In 1996, he received the Heinz Award, and in 2003 the Chemical Heritage Foundation presented Rutter with the Biotechnology Heritage Award. In 2007, UCSF announced that Mission Bay’s newest building would be named the “William Rutter Center.”

Several of Rutter’s coauthors on his Classic paper have also had successful scientific careers. Stephen J. Gardell, a postdoctoral fellow in Rutter’s laboratory, has held senior positions at several large pharmaceutical companies, including Wyeth Laboratories where he was assistant vice president of cardiovascular and metabolic disease. He also directed obesity research at Bayer Research Center and biological chemistry at Merck Research Laboratories. Most

1 More information on William Rutter’s academic career and time at UCSF can be found in an oral history that can be accessed online at http://content.cdlib.org/ark:/13030/kt7q2nb2hm/.
recently, Gardell was named director of translational research resources and adjunct associate professor at the Burnham Institute for Medical Research at Lake Nona. Emil Thomas Kaiser, who was a professor at Rockefeller University at the time he collaborated with Rutter, is known for developing semisynthetic enzymes (enzymes with useful new catalytic activities formed by combining the binding properties of one enzyme with the catalytic activity of an unrelated coenzyme) and his work on amphiphilic peptides (in particular, showing the importance of secondary structure and the reasons why amphiphilic helices are essential to biological activity). Donald Hilvert, who was a postdoctoral fellow with Kaiser at Rockefeller, moved on to a faculty position at the Scripps Research Institute before joining the Swiss Federal Institute of Technology Zurich as Professor Ordinarius of Organic Chemistry in 1997. His current research focuses on directed evolution of enzymes, using the mammalian immune system to produce catalytic antibodies, the incorporation of selenocysteine into proteins, and semisynthetic enzymes.

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REFERENCES

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