From single molecules to whole organisms: the evolving field of mechanistic enzymology

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Modern biochemistry encompasses an ever-expanding universe of newly discovered organisms, pathways, genes and proteins, all churned out at a dizzying pace that can overwhelm even the ardent scientist. To find order and the opportunity for new knowledge in this chaos, biochemists have taken many different approaches. Some investigators choose to sort through vast genomic data from many organisms to find common structures and themes, while others explore the interconnections between proteins and pathways within a single cell-type or organism. Scientists that are interested in the chemical mechanisms of biochemical transformations share a reductionist approach to biology: the belief that a true understanding of any biochemical process must start with a detailed understanding of the chemistry catalyzed by individual enzymes and how the chemical structure of each enzyme contributes to catalysis. Incorporation of detailed knowledge about individual enzymes into a larger framework that includes interconnected metabolic pathways and whole organisms should eventually lead to a mechanistic understanding of cells, organisms, and perhaps at a very basic level, even life.

In pursuit of ever more detailed mechanistic understanding, enzymologists have become the ultimate scavengers of new scientific technologies. Genome sequencing has vastly expanded the range of organisms from which to clone a chosen target, enabling investigators to quickly clone, express, purify, and characterize an enzyme or a whole pathway from multiple sources. Obtaining the structure of an enzyme has become a standard starting point for mechanistic studies, and many enzymologists are trained in structural techniques or have a close and active collaboration with a structural biologist. Wherever possible, investigators routinely use sophisticated mass spectrometry and spectroscopic techniques to elucidate the structure and chemical reactivity of enzyme intermediates. At the same time, most enzymologists also incorporate many of the tried and true mechanistic techniques into their research, including, but not limited to, steady-state and transient kinetics, heavy atom and radioisotope labeling, kinetic isotope effects, and chemical model studies. Throughout the reviews featured in this issue, you will notice that the modern study of enzyme mechanisms involves a skillful blending of many techniques and approaches, attacking each intermediate and kinetic step with multiple tools until a consensus understanding is reached.

In this issue of *Current Opinion in Chemical Biology*, research at the forefront of enzymology is presented. As with the wide-ranging discipline itself, the studies presented here represent a large cross-section of topics ranging from novel coenzyme biosynthesis in *Mycobacteria* and natural product biosynthesis in plants, to the biosynthesis and catalytic mechanisms of novel protein-derived cofactors in microbes, to the trafficking of vitamins and the assembly of corresponding active enzymes in humans.

Enzymologists and structural biologists have long dreamed of being able to take ‘snapshots’ of enzymes in action, creating a slide-show of key enzyme states throughout catalysis. Towards this goal, one recent advance in the study of enzyme mechanism is the development and continued improvement of single molecule techniques. The review by **Gershenson** discusses how site-specific enzyme labeling and state-of-the-art time-resolved fluorescence technology is being used to study such topics as cooperativity in multi-subunit enzymes, the effect of ligand binding on enzyme motion, and protein-membrane interactions. In the latter category of membrane proteins, Gershenson describes how single molecule fluorescence techniques can be used to correlate different modes of protein-membrane interactions with the overall catalytic cycle.

X-ray crystallography is an invaluable technique for investigating enzyme structure and function, and enzymologists have forged strong collaborations with protein crystallographers to generate static views of enzymes and enzyme:substrate complexes. Whenever available, structures play a central role in informing and guiding mechanistic investigations. **Moran** describes investigations into the mechanisms of the structurally and mechanistically similar enzymes 4-hydroxyphenylpyruvate dioxygenase (HPPD) and hydroxymandelate synthase (HMS). Both are nonheme iron enzymes implicated in the conversion of L-tyrosine into important natural products in microbes and plants. A series of spectroscopic and kinetic studies have been integrated into a structural framework provided by crystal structures of enzyme:substrate and enzyme:inhibitor complexes to describe a mechanism that is reminiscent of the more well-known α -ketoglutarate oxygenases. However, both HPPD and HMS accumulate a series of reactive intermediates, which could provide a rich source for further spectroscopic studies of nonheme iron oxygenases. Taking a more extended pathway-based approach, the review by **Blanchard** describes the biosynthesis of the novel Mycobacterial biomolecule mycothiol. Generated by the condensation of cysteine with a modified disaccharide, mycothiol plays a role in cellular detoxification similar to the role of glutathione in other organisms, and inhibitors of the mycothiol biosynthetic pathway could lead to new therapies for combating tuberculosis. Blanchard describes a series of crystal structures and mechanistic investigations that define the complete biosynthetic pathway, and provide a basis for future inhibitor screening or design.

Occasionally, the solution of an enzyme structure by x-ray crystallography reveals unexpected features that drive mechanistic research in completely new directions. In recent years, several new enzyme cofactors have been discovered that are generated by the post-translational modification of one or more protein residues. Typically these cofactors are definitively assigned only through high-resolution structures. The review by **Bruner** describes insights into the 4-methylideneimidazole-5-one (MIO) enzyme family from recent crystallographic analysis. The MIO prosthetic group is a spectroscopically invisible catalyst

that is generated by spontaneous condensation of an Ala-Ser-Gly motif within the enzyme polypeptide chain. The electrophilic MOI catalyst was found initially in the aromatic amino acid ammonia lyases, where it plays a role similar to pyridoxal phosphate and is essential for the elimination of ammonia. More recently, MOI has been found in microbial aromatic amino acid aminomutases, which generate β -amino acids for incorporation into a variety of natural products. In a similar vein but a different system, the review by **Wilmot** and **Davidson** describes the biosynthesis of a novel quinone cofactor, tryptophan tryptophylquinone (TTQ), generated by the crosslinking and oxidation of two distal tryptophan residues in methylamine dehydrogenase. In this system, an operon encoding five proteins has been shown to be essential for cofactor biogenesis, and Wilmot and Davidson describe how MauG, a novel bis-heme peroxidase, is responsible both for crosslinking the two residues and for further oxidation to generate the active quinone cofactor.

New insights can sometimes be gleaned by revisiting well-studied enzymes and cofactors using the more powerful tools of modern chemistry. While enzymologists often refer to simple chemical model studies to understand the basis for chemical changes in the substrate, they rarely use these same studies to probe the importance of the enzyme cofactor. The pyridoxal phosphate (PLP) cofactor is a powerful electrophilic catalyst that can catalyze several different types of enzyme reactions, and **Richard** describes a series of model studies that probe the importance of the various chemical features of the cofactor in catalysis. Perhaps most surprising, the cumulative data suggest that the pyridine quinonoid intermediate, often described in textbooks as the catalytic electron sink central to all PLP mechanisms, is in fact not important for most PLP catalyzed reactions.

Understanding cofactor-based chemistry has long been topic of interest for enzymologists. The review by **Banerjee** focuses on the ever fascinating coenzyme cobalamin or vitamin B₁₂. With a series of genetic disorders associated with B₁₂ trafficking in humans, Banerjee describes the proteins involved in B₁₂ uptake, transport, tailoring, and insertion. While the chemistry of the enzymes that use B₁₂ has long intrigued scientists, recent work shows that the chemistry involved in preparing this cofactor for use in these enzymes is equally interesting.

One area of enzymology that has made a comeback in recent years is the mechanistic study of plant enzymes involved in natural product biosynthesis. While genome projects have provided rich information about proteins in natural product pathways in microorganisms, the relatively scarce genomic information about plant natural product pathways has hindered mechanistic studies. In the review by **O'Connor**, progress towards understanding the enzymology of the alkaloid pathway in periwinkle plants is discussed. Largely due to the efforts of O'Connor herself, the mysteries of this important pathway are starting to unfold.

Contrary to past predictions that the study of enzymes and enzyme mechanisms is a mature field that will inevitably decline in importance, enzymology has instead become an integral and inseparable part of mainstream biochemistry. As the reviews in this issue demonstrate, the study of chemical mechanism has been integrated into all areas of biology and medicine. With new organisms and new proteins being described seemingly every day, the field of enzymology is likely to be robust and productive for decades to come.

Biographies

Catherine L Drennan received her PhD in Biological Chemistry from the University of Michigan with Professor Martha L Ludwig. She completed her postdoctoral studies at the California Institute of Technology with Professor Douglas C Rees. Currently, she is a Professor of Chemistry and Biology at MIT, and an HHMI Investigator and Professor. Her current work involves the use of X-ray crystallography to solve the structures and understand the function of metalloenzymes and metallochaperones.

Joseph T Jarrett received a PhD in Biochemistry from the Department of Chemistry at the Massachusetts Institute of Technology, working with Professor Peter T. Lansury, Jr. on mechanisms of amyloid fibril formation. He conducted postdoctoral research at the University of Michigan with Professor Rowena G. Matthews, focusing on the mechanism of the vitamin B₁₂-dependent methionine synthase. Currently, he is an Associate Professor of Chemistry at the University of Hawaii at Manoa. His work focuses on the catalytic mechanisms of *S*-adenosylmethionine radical enzymes, and in particular, the novel use of iron-sulfur clusters as sulfur donors for the formation of carbon-sulfur bonds in the enzyme biotin synthase.