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Editorial

Enzyme and coenzyme reaction mechanisms: Editorial overview

Enzymes play the primary role in the assembly of the complex molecules found in living systems, often with the assistance of small molecule coenzymes. It has been nearly fifty years since Bruice and Benkovic provided an elegant summary of early progress towards the determination of enzyme and coenzyme reaction mechanisms in their classic two-volume text *Bioorganic Reaction Mechanisms*. This field has expanded so fast, that in 2014 it would be difficult to provide a similar summary in even twenty volumes. This issue of *Bioorganic Chemistry* provides a modest sampling of the results of current studies on enzyme and coenzyme reaction mechanisms by chemists and chemical biologists working on some of the most interesting problems in the field.

Essential precursors to isoprenoids are generated in plants, protozoa, and bacteria by the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway. The enzymes in this pathway are absent from humans and therefore present interesting targets for the development of tight-binding inhibitors with potential applications as herbicides and antibiotics. The first committed step on this pathway is catalyzed by 1-deoxy-D-xylulose-5-phosphate reductoisomerase, which carries out the chemically distinctive conversion of 1-deoxy-D-xylulose-5-phosphate to 2-C-methyl-D-eryritol 4-phosphate. Andrew Murkin and coworkers summarize the results of recent studies on the mechanism of action of this enzyme, and emphasize the translation of this growing mechanistic insight into strategic plans for the synthesis of potent enzyme inhibitors.

Transglutaminases from mammalian tissue are calcium-dependent enzymes, which catalyze a variety of acyl transfer reactions. The activity of these enzymes was first detected by monitoring the incorporation of low molecular weight primary amines into proteins to form γ-glutamyl amides. It has since been demonstrated that tissue transglutaminases function physiologically to catalyze transimidation between the γ-carboxamide group of glutamine of one protein and the ε-amino group of a second protein to introduce isopeptide protein crosslinks. Jeffrey Keillor and coworkers present an overview of fifty years of studies on the mechanism for transglutaminase catalyzed hydrolysis and transimidation reactions. This paper highlights studies to determine the mechanism for enzyme activation by calcium dication, deactivation by GDP/GTP, and the development of fluorescence-based assays to examine the role of conformational changes in catalysis. The enabling role of this work in the determination of the physiological functions of tissue transglutaminases is emphasized.

The features of the structure of pyridoxal 5′-phosphate (PLP) that facilitate cofactor catalysis of the cleavage of the different bonds to the α-amino carbon of amino acids are well known to mechanistic enzymologists. The various proposals for the role of the protein catalyst in promoting these bond cleavages are currently being examined by X-ray crystallographic analyses and classical mechanistic studies. Tryptophan indole lyase and tyrosine phenol lyase utilize the PLP cofactor in catalysis of the reversible hydrolytic cleavage of L-tryptophan and L-tyrosine, respectively. Robert Philips and coworkers summarize the results of X-ray crystallographic analyses and site directed mutagenesis studies on these two enzymes. The studies provide strong support for the proposal that the enzymes introduce strain into the quinonoid reaction intermediate, which drives the aromatic ring 20° out of the plane of the C_β–C_γ bond of the amino acid fragment. This enzyme-induced strain is proposed to reduce to resonance stabilization of the intermediate by the aromatic ring, and favor protonation of the ring carbon (C_γ) by the phenol side chain of Try-71.

Biochemists and mechanistic enzymologists are interested in knowing the specific features of secondary and tertiary protein structure and of protein folds that contribute to enzymatic rate enhancements. In particular, it has been difficult to resolve the conflicting demands that enzymes provide precision in the placement of side chains at the transition state for their catalyzed reactions, with the placement of these side chains at flexible βα front loops of the TIM-barrel fold. Richard and coworkers describe the functions of the individual front loops of the eponymous TIM-barrel of triosephosphate isomerase. This discussion highlights the relationship between the structure of the inactive open form of TIM, and the active caged complex to substrate; and, the requirement for the utilization of dianion binding energy to drive the change in the conformation of the "plastic" TIM barrel. The authors emphasize that the substrate binding energy must be utilized to freeze the motions of the flexible catalytic side chains at the catalytically active caged complex.

Electron transfer between cationic metal ion centers is arguably the simplest reaction in chemistry. In biology, these electron transfers generally occur through or between proteins, which act to chelate the metal cation, or a cofactor host for the metal cation. The protein may act as a passive conduit for the transferred electrons, in which case there is good evidence that the protein structure has evolved to optimize the parameters that govern the rate of electron transfer – for example the electronic coupling term and the reorganization energy. Alternatively, the protein may play an active role in electron transfer by participating in steps that prime the system for either "coupled" or "gated" electron transfer reactions. Finally, the protein may play a direct role in electron transfer, by providing residence sites as electron to "hops" between the metal centers. Davidson and coworkers provide a lucid description of these different mechanisms for control of electron transfer reactions.

The side chains of methionine at the free amino acid, and at proteins are too readily oxidized to the sulfoxide. Boschi-Muller and

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Brantlant review the elegant mechanisms of three separate classes of methionine sulfoxide reductases, which repair this oxidative damage. Each class of enzymes features an assembly of acidic and basic side chains at the active site. The mechanism is discussed by which these side chains function in catalysis of the reduction of the methionine sulfoxide by a cysteine thiol, to form methionine and a protein sulfenic acid. The status of our understanding of the mechanism for reduction of this protein sulfenic acid to regenerate the active methionine sulfoxide reductase is also presented.

Members of the lysyl oxidase (LOX) family catalyze the oxidative deamination of the ε-amino group of lysines and hydroxylysines in collagen and elastin during the formation of protein crosslinks. These enzymes recruit Cu^{2+} and the tyrosine-derived quinone cofactor LTQ to assist in this oxidation. The lysyl oxidase like-2 (LOX2) has been proposed to play a role in the regulation of extracellular and intracellular cell signaling pathways. Breakdowns in the regulation of LOX2 have been linked to many diseases, including cancer, pro-oncogenic angiogenesis, fibrosis and heart disease. Mure and coworkers discuss the results of experiments to test different hypotheses about the many physiological roles of LOX2.

3-Deoxy-D-arabino-heptulosonate 7-phosphate synthase (DAH7P synthase) catalyzes the aldol addition-like reaction between phosphoenolpyruvate and erythrose 4-phosphate to form DAH7P. This enzyme sits at the first committed step in the shikimic acid pathway, which branches to aromatic amino acids and a variety of aromatic metabolites. *Neisseria meningitidis* is the causative agent of pyogenic meningitis and of meningococcal septicaemia: the DAH7P synthase from this organism has been identified as a drugable enzyme target. Parker and coworkers recently reported the X-ray crystal structure of DAH7P synthase from *Neisseria meningitidis*. These investigators now report crystal structures for complexes between DAH7P synthase and three competitive inhibitors, the most potent of which shows a K_i of 3.9 μM. These X-ray crystal structures show the presence of a key active site water molecule, which is situated in a manner that suggests a direct role for water in the DAH7P synthase-catalyzed reaction.

The coenzyme thiamine diphosphate (TDP) features prominently in two articles. This cofactor promotes a wide range of reactions, including simple decarboxylation and oxidative decarboxylation of α-keto acids, transfer of the α-keto group between sugar donors and acceptors, and phosphorolytic cleavage reactions of α-keto sugar phosphates to form acyl phosphate and sugar phosphate products. These enzyme-catalyzed reactions proceed through a plethora of protein-bound cofactor reaction intermediates, which have been characterized in careful structural and kinetic studies carried out in solution and in the solid-state. Prominent among the work reviewed by Frank Jordan and Natalia Nemeria are X-ray crystallographic studies, rapid chemical quench studies monitored by solution NMR, and circular dichroism studies. These last studies provide critical assignments of the ionization and tautomerization states of the 4-aminopyrimidine group of TDP.

Chemists, who struggle to find solutions to difficult problems in organic synthesis, can only gaze with rapturous awe at the diverse efficient solutions to these problems uncovered after billions of years of natural selection of living systems. Kai Tittman reviews strategies that Nature has evolved for the reversible cleavage of ketose phosphosugars. His article features TDP-mediated transketolase and phosphoketolase catalyzed reactions, and examples of class 1 aldolases, which utilize the amine side chain of an active site lysine for the reversible cleavage of fructose-1,6-bisphosphate or fructose-6-phosphate or as a shuttle for 3-carbon units between various phosphosugars (transaldolases). There is a lucid presentation of the structural basis for the different chemical fates and lifetimes of central enamine intermediates that form in five different classes of enzyme-catalyzed reactions.

These ten papers provide a sampling of the best work in mechanistic enzymology, a field of continued vibrancy and importance. *Bioorganic Chemistry* will continue to promote studies on the mechanism of enzymes action, by providing a forum for publication of the best work in the field.

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