Chemical and Computational Probes of Biological Mechanism
Editorial overview

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Scientists with interests in mechanism at the chemistry-biology interface share a fundamental curiosity for understanding the chemistry underpinning biological processes from molecular to more macroscopic levels. Understanding mechanism in this area often requires a marriage of well-established kinetic methodology, traditionally associated with physical organic chemistry, and state-of-the art structural and computational methods. The ‘tried and tested’ methods towards understanding chemical mechanism include structure-activity studies such as linear free energy relationships (LFERs), isotopic labelling and chemical modelling. The eleven reviews in the 2014 ‘Mechanisms’ issue of Current Opinion in Chemical Biology were specifically chosen to represent the broad ranging expertise of their authors on the application of these traditional and contemporary methodologies towards the determination of mechanism. The topics covered include the mechanistic study of small molecule biomimetic compounds, through different enzymes or biocatalysts, to more complex biological systems such as the ribosome. Based on recent developments, this issue collects opinions on broader long-standing questions such as the origin of different aspects of enzymatic catalysis, and more discrete topics including the current understanding of mechanisms of phosphoryl transfer in DNA and RNA systems and the origins and implications of DNA damage.

The alteration of the microenvironment of a polar substrate, upon movement from a more chaotic structure in bulk solution to an organized environment when bound at an enzyme active site, is an important source of catalysis of polar reactions. John Richard reviews how being in a protein cage rather than water can enhance enzymatic catalysis of polar reactions, based on the wealth of experimental kinetic and structural data available in recent years for several prototypical enzymes, as well as studies with substrate analogues or mutant substrates. In the case of amino acid racemases that catalyse the reaction of two polar substrates of opposite charge to form a zwitterionic product, the reaction is favoured by the decrease in the effective dielectric constant in the less polar medium of the protein catalyst. The destabilization of charge at the initial ground-state enzyme-substrate complex relative to the transition state can result in a decrease in the reaction barrier as long as the cost of desolvation of the transition state is smaller than that of the reactants. However, the requirements for harnessing of such desolvation effects, and the mechanism by which they are achieved, differs between enzyme-substrate systems. The author provides an overview of several key enzyme systems in this regard, including ketosteroid isomerase, triosephosphate isomerase and DAP epimerase.

Dan Tawfik discusses the broader issue of the mechanistic and structural base of selectivity, or specificity, in enzymatic systems of different complexities, and the associated trade-offs in catalytic efficiency. DNA methyl transferases and the ribosome are highlighted as examples of systems utilizing Ground State Discrimination to achieve specificity via exclusion of undesirable substrates at the
level of substrate binding. The origins of the intrinsically larger speed-accuracy trade-offs that result from this mechanism of ‘negative selection’ are discussed. The smaller specificity-efficiency trade offs of the less well-understood mechanism of Transition State Discrimination are also evaluated. It is suggested that this latter mechanism of achieving selectivity is predominantly applicable to enzymatic reactions of small, rigid substrates, which utilize the same or proximal active site residues for both binding and chemical reaction events.

In the case of highly promiscuous enzymes, the main challenge is not the rate enhancement itself, but the chemical control of a given reaction towards appropriate product outcomes. Dan Major, Yehoshua Freud, and Michal Weitman discuss this problem in relation to the terpene cyclases, which achieve a diverse array of carbocation chemistry in the syntheses of specific target terpenes. The terpene cyclases have not achieved ‘catalytic perfection’, and the enzymes remain inherently promiscuous. By reviewing multi-scale simulation studies on squalene-hopene, aristolochene and bornyl diphosphate synthases, the authors reveal the diverse array of physicochemical tools utilized by these enzymes to achieve chemical control in the specific synthesis of a target terpene. This includes appropriate active site architecture to permit the correct substrate folding and subsequent sequestering of the relevant carbocation intermediate, extremely precise positioning of active site acid and bases and a bifacial active site that allows for both strong electrostatic interactions in addition to weaker $\pi$-cation dispersion interactions.

A fundamental mechanistic understanding of the origin of enzymatic product specificities is a recognized challenge in the biocatalysis community, in particular for enabling the application of enzymes towards the synthesis of a more diverse array of products. The most studied enzymes in this respect are those that facilitate acyl transfer and hydrolysis, such as lipases and epoxide hydrolases. Mikael Widersten reviews recent progress in this area and highlights how the combination of protein engineering and kinetic resolution tools, founded on mechanistic understanding, have permitted the development of new, useful biocatalysts. The harnessing of promiscuous enzyme functions to increase the diversity of products accessible by biocatalysis is also discussed. As an example, a single point mutation of Candida antartica lipase B (CALB) resulted in an enzyme that could catalyse a novel Michael addition reaction when the catalytic nucleophile for the normal enzymatic reaction was removed (S105A).

Enzymatic catalysis of phosphoryl transfer presents examples of Nature’s most efficient biocatalysts. David Hodgson, Heidi Korhonen and Louis Conway discuss the application of small molecule phosphate analogues as probes of biological mechanism in this area. The understanding of phosphoryl transfer in phosphate monoester, diester and phosphoanhydride systems is the main focus of their review. Both reactive and stable (inhibitory) substrate analogues are covered, which offer insight into the nature and sites of transfer in addition to the dynamic processes that occur at phosphorus during bond making and cleavage events. The authors provide an overview of a range of key enzyme systems in this regard, including RNA ligase...
RtcB and DNA polymerase β, allowing for a comparative picture of a broad range of phosphoryl transfer strategies.

Michael Harris, Daniel Kellerman and Darrin York discuss how recent experimental and theoretical advances are addressing long standing mechanistic questions on RNA 2'-O-transphosphorylation. These studies probe the differential contributions of proton transfer, metal ion interactions and reaction co-ordinate motion at the transition states for the solution and enzyme reactions of this important phosphoryl transfer process. Through Linear Free Energy Relationship (LFER) and Kinetic Isotope Effect (KIE) experimental analyses made possible by recent synthetic access to substrate analogues, in tandem with multi-scale quantum simulations, a more consistent mechanistic picture is emerging. The authors highlight how specific substrate interactions with active site residues are intricately related with transition state charge distribution resulting in defined catalytic modes. The detailed understanding of the interplay of catalytic modes and the environment allows for the more precise design of new classes of transition state analogues as inhibitors.

Although the mechanisms and biochemical consequences of DNA damage have been studied for many years, recent experimental evidence highlights that certain lesions are more significant and less repairable than previously thought. Marc Greenberg discusses how apurinic/apyrimidinic (AP) and oxidized abasic sites (which are frequently occurring DNA lesions) can lead to the formation of more deleterious interstrand cross-links and double strand breaks as well as result in the irreversible inactivation of DNA polymerase β, a vital component of base excision repair. The study of the reactivity of these lesions reveals the formation of intermediate DNA-protein cross-links, which is dependent on Schiff-base formation involving lysines present in nucleosomes. These newly identified reactivity modes have enabled advancement to a more detailed mechanistic picture of the initial formation of these lesions by DNA damaging agents.

The role of protein motions in enzymatic catalysis continues to be a subject of much debate. Amnon Kohen and Kevin Francis present their opinions on the role of these effects in catalysis by dihydrofolate reductase (DHFR), via an analysis of the temperature dependence of intrinsic KIEs analyzed using Marcus-like models. According to these authors, the alterations of KIEs between wild-type and site-directed mutant forms of DHFR may be interpreted as a dynamical link between barrier crossing and protein motions. They propose a structural and dynamic role of residues across the protein in enzymatic catalysis of C-H→C transfer including both active site and remote amino acid residues. It is further suggested, based on phylogenetic analyses of DHFR sequences, that this dynamic network of functionally correlated motions is evolutionarily conserved. Iñaki Tuñón, Katarzyna Świderek, J Javier Ruiz-Pernía and Vicent Moliner review recent experimental and theoretical studies on the use of isotopically substituted enzymes, or ‘heavy’ enzymes, to provide insight into the effects of protein motions on the chemical steps. These heavy enzyme KIEs are frequently interpreted as providing a link between mass-dependent protein vibrations and barrier crossing. Studies of ‘heavy’ DHFR in conjunction with computer simulations are suggested to provide evidence against
the coupling of large-scale millisecond protein motions to the reaction co-ordinate. The barrier and contribution of quantum tunneling were not significantly affected in the heavy enzyme suggesting a less important role of protein motions in tunneling and alteration of the barrier. Clearly, despite extensive research effort, this is still an area where both experiment and theory can continue to make valuable contributions to resolve open questions.

Finally, the ability to design an efficient artificial enzyme remains one of the “Holy Grails” of theoretical biochemistry. The successful design of a ‘Theozyme’ would provide strong evidence that the elusive origin of the tremendous catalytic proficiency of naturally occurring enzymes has been finally unmasked. Recent years have seen tremendous progress in this area, however, there still remains a significant (but decreasing) gap between the efficiencies of natural and rationally-designed systems. Warshel and coworkers provide their opinions of the relative importance of current catalytic concepts and go on to discuss how the molecular rationale devised by theoretical analysis can be applied to artificial enzyme design. They highlight the power of the empirical valence bond approach as a tool to achieve this, as well as the importance of electrostatic preorganized active sites in efficient enzyme catalysis. This is further emphasized by Fuxreiter and Mones who present their opinions on the role of reorganization energy in the rational design of computational enzymes, and how this may be exploited to potentially fill the gap with natural highly-efficient enzymes. They propose the utilization of the enzyme’s “reorganization energy” along the chemical coordinate as a tool for the refinement and final evaluation of top ranked computational designed constructs, suggesting that this will provide a powerful starting point for highly evolvable initial designs.

This series of reviews demonstrates that combinations of traditional and contemporary chemical and computational methods can give detailed fundamental understanding of molecular mechanism at the chemistry-biology interface. Substantial recent progress in both experimental and computational techniques suggests that the next decade will likely witness several exciting developments in our understanding of the mechanisms of biological reactivity.