



Editorial

Enzyme transition states from theory and experiment

Mike E. Harris^a, Joseph A. Piccirilli^b, Darrin M. York^c^a Integration of kinetic isotope effect analyses to elucidate ribonuclease mechanism^b Heavy atom labeled nucleotides for measurement of kinetic isotope effects^c Effect of Zn²⁺ binding and enzyme active site on the transition state for RNA 2'-O-transphosphorylation interpreted through kinetic isotope effects

Preface

Understanding how enzymes catalyze specific reactions has yielded important insights into biology, provided the necessary context for the design of novel catalysts, and has facilitated the development of enzyme inhibitors for therapeutic purposes. The contributions of Professor W. Wallace Cleland helped to forge the modern framework for steady state enzyme kinetics, and together with his colleagues he made pioneering contributions to establishing heavy atom isotope effect analyses as a powerful approach for investigating the chemical mechanisms of enzyme-catalyzed reactions. Professor Cleland collaborated with and trained numerous talented enzymologists and generations of scientists. Several facets of W.W. Cleland's contributions to science are illustrated and reflected in this special issue that focuses on using kinetic isotope effects to understand chemical and enzymatic reaction mechanisms.

Along with experimental advances, computation has grown and developed as an essential partner in the interpretation of kinetic isotope effects (KIEs). Transition state theory poses that kinetic isotope effects reveal bonding changes in the transition state and thus the interpretation of KIEs in terms of transition state structure is complicated by kinetic ambiguity KIEs reflect changes in all vibrational modes between the ground state and transition state that involve the atom being analyzed. Therefore, measured KIEs typically reflect contributions from more than one of these modes, or from new modes introduced by interactions with a catalyst. As illustrated in the contributions to this issue, the combination of computational and experiment provides the ability to estimate the magnitude of different contributions to observed KIEs and therefore to evaluate alternative mechanisms and to derive transition state structures corresponding to competing pathways.

One of W. W. Cleland's significant contributions is in the development of steady state enzyme kinetics. His description of internal competition kinetics used to calculate kinetic isotope effects and commitment factors is widely used. Building on this framework, Dr. Vernon Anderson provides a review and derivation of the equations for alternative substrate kinetics. This contribution further includes a detailed description of the expansion of internal competition kinetics to analyze the relative rate constants of large numbers of substrates, which has great promise in high throughput quantitative biochemistry. Importantly, the quantitative framework includes determination of equations for calculation of relative rate constant using both precursor and product ratios, and therefore is a resource adaptable to many enzyme systems.

Although measurement of KIEs using natural abundance isotopes has significant advantages, KIEs are typically measured by internal competition using substrates individually enriched with a stable isotope at atoms undergoing reaction. Therefore, the synthesis of isotopically enriched substrates is an essential requirement. The use of isotopically enriched nucleotides in positional isotope exchange studies of ATPase and phosphodiesterase mechanisms are classic examples of mechanistic enzymology. However, the application of isotopically enriched nucleotides in KIE analyses has been more limited, although there are several successful examples. Piccirilli and colleagues provide a survey of synthetic methods for heavy atom labeled nucleotides for measurement of kinetic isotope effects. The contribution is a valuable resource for researchers interested in taking the measurement of KIEs, however, this information is also extremely valuable for mechanistic applications beyond KIE measurements such as NMR or vibrational spectroscopy.

Along with others, W. W. Cleland made definitive contributions to the interpretation of pH effects on enzyme activity and to the interpretation of multiple isotope effects. The review by Fitzpatrick and colleagues focuses on combining solvent isotope effects with substrate isotope effects in mechanistic studies of alcohol and amine oxidation by flavin- and pyridine nucleotide-dependent enzymes. The review provides an excellent introduction into solvent D₂O effects and the importance of measuring multiple isotope effects for mechanistic analyses. In addition to providing cautionary insights into how selection of alternative substrates for mechanistic studies can influence results, the results from mechanistic studies of alcohol and amine oxidation underscores the utility of combining solvent and kinetic isotope effects to gain insight into the seminal problem of distinguishing between stepwise and concerted reaction mechanisms. Byers and colleagues provide a key illustration of the utility of combining solvent and substrate KIEs for the analysis of β -glucosidase. These studies provide an illustration of using these effects to better understand the relative degree of leaving group bond cleavage and protonation, which is a general question for addition/displacement reactions of esters and alcohols.

Addition/displacement reactions include phosphoryl transfer, and KIE analyses have provided a key pillar in support for our understanding of the mechanism of this class of reactions as well. Classic studies of nitrophenol esters established the mechanistic differences for mono-, di-, and triesters. Cleland and his colleagues helped to define the interpretation of primary and secondary KIEs in terms of mechanism and revealed the mechanistic features governing phosphoryl transfer and as

described by Professor Alvan Hengge, KIEs were a key experimental tool used to analyze the catalytic mechanism and the transition state for phosphoryl transfer by protein tyrosine phosphatases. The ability to probe transition state structure using KIEs has provided a means to investigate the potential role of protein motions in PTP catalysis. Importantly, the KIEs for mutant PTP enzymes reveal perturbations to the transition state due to alteration of residues directly involved in the catalytic mechanism providing evidence for acid/base catalysis.

A further milestone in the development of the framework for interpreting KIEs has been in investigation of the hydrolysis of esters, thioesters and amides, which is expertly reviewed by Robbins et al. Importantly, the course of these studies involved the development of methodology to determine heavy-atom solvent nucleophile KIEs. Their description provides an outstanding perspective on the challenges associated with measuring solvent nucleophile KIEs as well as the importance of the information that can be gained. They also describe the significant challenges associated with unambiguously interpreting nucleophile KIEs in terms of attack by water or hydroxide that is general to solution and enzyme catalyzed hydrolysis reactions. Key considerations for interpreting secondary KIEs are also highlighted, specifically for the carbonyl oxygen of the reacting ester or amide, to account for potential changes in multiple vibrations that include both bonding and bending modes. Similar issues arise in the context of the secondary KIEs for the non-bridging oxygens of phosphoryl transfer reactions as illustrated in the review by Hengge. In both cases computational analyses are useful in providing estimates for the various contributions to the observed KIE.

Using quotes from the 2014 Nobel lecture by Professor Martin Karplus, Wong and colleagues in their review remind us of the integral role of computation in interpretation of KIEs in terms of transition state structure and identification of potential alternative mechanisms. The conventional Bigeleisen equation for isotope effect calculation as well as its refined versions are expertly covered. A series of case studies, ranging from squalene-to-hopene polycyclization and RNA 2'-O-transphosphorylation highlight the author's adaptation of these methods. The potential insights to be gained by combining theory and experiment are further illustrated by Chen et al., who report the use of QM calculations to account for the influence of metal ion and enzyme active site catalytic modes on the transition state for RNA 2'-O-transphosphorylation.

Ribonuclease A has been a classic long-standing system for exploring fundamental aspects of enzyme catalysis such as the role of general acid/base catalysis and the potential for enzyme active sites to alter the transition states of the reactions that they catalyze. Both KIE and LFER data are available for solution and RNase A catalyzed RNA 2'-O-transphosphorylation including key measurements by W. W. Cleland and coworkers for solution and RNase A catalyzed reactions of uridine 3'-m-nitrobenzyl phosphate. Ambiguities regarding nucleophilic activation remain, but the combination of theory and experiment provide a detailed picture for the leaving group, revealing a late TS involving proton transfer to the leaving group as it departs.

As the articles herein demonstrate, KIE analyses of enzyme mechanism continue to provide the best and arguably the only way to experimentally interrogate transition state structure. A recurrent theme in

several contributions is the power of combining solvent and substrate KIE analyses. KIE analyses form a natural partnership with computational chemistry since together they can allow alternative mechanisms and transition state structures to be evaluated and then benchmarked against experiment. The contributions by Anderson and by Piccirilli provide important resources for applications of the conceptual and methodological advances involved in the measurement of KIEs to be extended to ask other questions in mechanistic enzymology. The research described in this issue serves to further illustrate how the advances to applications of KIE analyses made by W. W. Cleland have contributed fundamentally to a foundation and framework that we build from today.



Dr. Harris received his BS from Florida State University and PhD from the University of Alabama at Birmingham. After a postdoctorally fellowship with Norman Pace at Indiana University he joined the faculty at Case Western Reserve University School of Medicine in 1996. Dr. Harris' lab developed methods for measuring kinetic isotope effects on phosphoryl transfer reactions and frameworks for interpreting these effects with Drs. Vernon Anderson, Joseph Piccirilli and Darrin York. His group is currently investigating fundamental aspects of RNA molecular recognition and RNA catalysis.



Dr. Joseph Piccirilli is a professor at the University of Chicago with appointments in the Department of Chemistry and of Biochemistry and Molecular Biology. He received his BS from the University of Scranton and PhD from Harvard working with Steven Benner. He trained as a postdoctoral fellow with Tom Cech at the University of Colorado at Boulder. The Piccirilli laboratory develops new chemical tools for analysis. RNA structure and function is engaged in investigating multiple aspects of RNA biology including ribozymes, riboswitches and mechanisms of splicing.



Dr. Darrin York is Professor at Rutgers University in the Department of Chemistry. He performed his graduate work at the University of North Carolina at Chapel Hill and pursued postdoctoral studies with Martin Karplus at Harvard and Universite Louis Pasteur in Strasbourg. The York group research is involved in the development and application of multi-scale quantum methods for simulations of biological reactions. These methods involve the integration of linear-scaling electronic structure, polarizable force field, generalized solvent boundary and new-generation molecular simulation techniques that work together synchronously to study the detailed mechanisms of RNA catalysis.