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Enzyme mechanisms from molecular modeling and isotope effects

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Abstract

The application of kinetic isotope effects and molecular modeling to characterize three enzyme-catalyzed reactions is presented; the mechanism of the chloroacid dehalogenase catalyzed reaction is approached using chlorine kinetic isotope effects and solvent kinetic isotope effects. The pre-steady-state phase of the reaction catalyzed by methylmalonyl-CoA mutase is approached by different QM/MM schemes and the results are validated by comparison with the experimental value of the deuterium kinetic isotope effect. Finally, a procedure for improving QM/MM calculations is illustrated by analysis of the trihydroxynaphthalene reductase-catalyzed reaction.

Keywords: Isotope effects; Molecular modeling; Chloroacid dehalogenase; Methylmalonyl-CoA mutase; Trihydroxynaphthalene reductase

Advances in new theory levels, development of improved algorithms, and the enormous increase in computational power of modern computers has resulted in the reliability of theoretical calculations becoming comparable with the experimental error. The calculations are now recognized as valid tools in studies of organic reaction mechanisms. Biological systems, on the other hand, because of their size and complexity, still present challenges that we are only recently becoming able to tackle. The most fruitful approaches seem to be so called QM/MM methods, in which the reactants and most important elements of the biological system (e.g., cofactor(s) and active site aminoacids in the case of enzymes) are treated quantum-mechanically while the remaining part of the system is treated at lower level of theory, usually at the molecular mechanics level. This approach delivers quantum chemical insight while maintaining manageable computational costs. These QM/MM calculations can be combined with a dynamic method that allows sampling of different pathways, enabling a greater amount of the enormous conformational space available to biological systems with potentially different transition states to be accessed. While QM/MM methods are attractive and economic they have not matured to the point that they can be trusted in the same way results from theoretical calculations are trusted in studies of organic reactions. For example the boundary conditions between QM and MM domains still pose several theoretical and practical problems that await solution. It is, therefore, important to calibrate and validate these methods against experimental results.

In our laboratory we approach mechanisms of enzymatic catalysis using theoretical calculations combined with both theoretical and experimental determinations of isotope effects. These two approaches nicely complement each other, allowing in many cases to highlight the details of reactions occurring in enzyme active sites. Herein we will review our recent studies on three enzymatic systems: haloacid dehalogenase (DL-DEX113), methylmalonyl-CoA mutase (MCM)[1], and trihydroxynaphthalene reductase (3HNR). These examples were chosen to cover a few aspects of such studies. From the point of view of isotope effects studies we will present heavy-atom (chlorine), hydrogen (deuterium), and solvent (deuterium) kinetic isotope effects. From the point of view of theoretical calcula-

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[1] *Abbreviations used:* MCM, methylmalonyl-CoA mutase; PES, potential energy surface.

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tions two aspects will be introduced. Firstly, two different methodologies will be presented; QM/MM and ONIOM. Secondly, for MCM inclusion of tunneling will be discussed. Finally, from the kinetic point of view, the use of isotope effects only in the lieu of crystal structure of the enzyme, as well as studies that combine theoretical calculations with the experimental measurements of kinetic isotope effects, will illustrate approaches used in our laboratory to study the mechanisms of enzyme-catalyzed reactions.

Our discussion requires an introduction to the influence of the complexity of enzyme-catalyzed reactions on the interpretation of the kinetic isotope effects. The simple mechanism of an enzymatic reaction, presented in Eq. (1) together with individual rate constants, includes a binding step, in which substrate, S, forms the Michaelis complex, ES, with the enzyme, followed by the "chemical step" that interconverts substrate and product, P, which is subsequently released.

$$E + S \underset{k_{offS}}{\overset{k_{onS}}{\rightleftharpoons}} ES \underset{k_{rev}}{\overset{k_{cat}}{\rightleftharpoons}} EP \underset{k_{onP}}{\overset{k_{offP}}{\rightleftharpoons}} E + P \tag{1}$$

Assumptions that binding does not exhibit noticeable isotopic fractionation and the overall equilibrium isotope effect is unity lead to Eq. (2) that relates the apparent kinetic isotope effect k^L_{app}/k^H_{app} to the "intrinsic" kinetic isotope effect k^L_{cat}/k^H_{cat}, i.e. the isotope effect of the chemical step (k_{cat} in the above example). Subscripts L and H denote light and heavy isotopomers, respectively. The ratio k_{cat}/k_{offS} is called forward commitment to catalysis (or commitment for short) while the ratio k_{rev}/k_{offP} is called reverse commitment to catalysis.

$$k^L_{app}/k^H_{app} = \frac{k^L_{cat}/k^H_{cat} + k_{cat}/k_{offS} + k_{rev}/k_{offP}}{1 + k_{cat}/k_{offS} + k_{rev}/k_{offP}} \tag{2}$$

Eq. (2) signifies that the apparent isotope effect that can be directly compared with the experimentally determined value may differ from the isotope effect of the chemical step. Only in the limiting case, when the commitments approach zero, does the observed KIE approach the value of the intrinsic KIE. If a commitment is larger than zero, part of the intrinsic KIE is masked and the observed KIE becomes smaller than the intrinsic KIE. In order to be able to draw conclusions about the mechanism of an enzymatic reaction it is necessary to find out the relation between the values. We have summarized elsewhere [1] some of the experimental methods that can be used to unmask the intrinsic KIEs. Alternatively, they can be obtained from QM models of the reaction.

Haloacid dehalogenase (DL-DEX 113)

Bacterial dehalogenases are very important enzymes that are essential for biodegradation of chlorinated pollutants in the ecosystem. The typical mechanism of reactions catalyzed by hydrolytic dehalogenases consists of nucleophilic substitution of chloride by a carboxylate group of an enzyme aminoacid with formation of an enzyme-bound ester intermediate and its subsequent hydrolysis to the product alcohol [2]. DL-DEX 113, is atypical as it uses an enzyme-activated water molecule as the nucleophile (Fig. 1) and the reaction proceeds without formation of an intermediate [3]. It was thus of great interest to learn details of this direct S_N2 substitution mechanism. We have approached the problem of the characterization of DL-DEX 113 mechanism by determining the primary chlorine kinetic isotope effects for both stereoisomers of the substrate, R(+)- and S(−)-2-chloropropionate to be 1.0105 ± 0.0001 and 1.0082 ± 0.0005, respectively [4].

Unfortunately, when we measured these isotope effects the enzyme crystal structure was not available, and in fact only recently a communication reporting preliminary results of the crystal structure studies on a homologous enzyme appeared in the literature [5]. Thus, it was necessary to resort to other approaches in order to interpret the magnitude of measured values.

The major problem that had to be addressed was the difference in the KIE values obtained for both stereoisomers. In the framework of Eq. (2) the results could be interpreted in two different ways. Since the measured KIEs are quite large for Cl^- elimination, one could assume that in both cases they correspond to intrinsic KIEs. This line of argument implies that the intrinsic KIEs are different for both substrates. Alternatively, it can be argued that only the larger value corresponds to the intrinsic KIE and the other is smaller because the forward commitment is larger than zero (since the dehalogenation is irreversible the reverse commitment in Eq. (2) is equal to zero as well).

It is impossible to explore these hypotheses theoretically due to the lack of the crystal structure of the enzyme. Thus we have resorted to measuring additional isotope effects. In particular, we have measured solvent isotope effects on V_{max} and V_{max}/K_M for both substrates. The information embedded in these isotope effects is different; the isotope effect on V_{max} reports on the fate of the enzyme bound substrate up to the overall rate-determining step. The isotope effect on V_{max}/K_M, on the other hand, provides information connected with the conversion of the free substrate in the solution up to the first irreversible step. The values

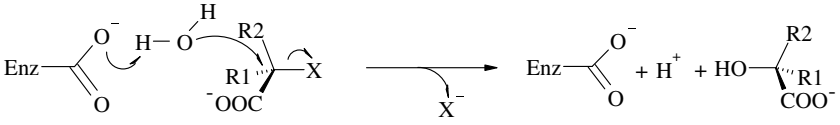

Fig. 1. Mechanisms of the DL-DEX catalyzed dehalogenation.