Archives of Biochemistry and Biophysics xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Review

Nuclear quantum effects and kinetic isotope effects in enzyme reactions

Alexandra Vardi-Kilshtain, Neta Nitoker, Dan Thomas Major *

Department of Chemistry and the Lise Meitner-Minerva Center of Computational Quantum Chemistry, Bar-Ilan University, Ramat-Gan 52900, Israel

ARTICLE INFO

Article history:
Received 27 January 2015
and in revised form 2 March 2015
Available online xxxx

Keywords:
QM/MM
Kinetic isotope effects
Nuclear quantum effects
Enzyme catalysis

ABSTRACT

Enzymes are extraordinarily effective catalysts evolved to perform well-defined and highly specific chemical transformations. Studying the nature of rate enhancements and the mechanistic strategies in enzymes is very important, both from a basic scientific point of view, as well as in order to improve rational design of biomimetics. Kinetic isotope effect (KIE) is a very important tool in the study of chemical reactions and has been used extensively in the field of enzymology. Theoretically, the prediction of KIEs in condensed phase environments such as enzymes is challenging due to the need to include nuclear quantum effects (NQEs). Herein we describe recent progress in our group in the development of multiscale simulation methods for the calculation of NQEs and accurate computation of KIEs. We also describe their application to several enzyme systems. In particular we describe the use of combined quantum mechanics/molecular mechanics (QM/MM) methods in classical and quantum simulations. The development of various novel path-integral methods is reviewed. These methods are tailor suited to enzyme systems, where only a few degrees of freedom involved in the chemistry need to be quantized. The application of the hybrid QM/MM quantum–classical simulation approach to three case studies is presented. The first case involves the proton transfer in alanine racemase. The second case presented involves orotidine 5′-monophosphate decarboxylase where multidimensional free energy simulations together with kinetic isotope effects are combined in the study of the reaction mechanism. Finally, we discuss the proton transfer in nitroalkane oxidase, where the enzyme employs tunneling as a catalytic fine-tuning tool.

Introduction

Understanding how enzymes catalyze chemical reactions in cells is a contemporary question of great importance. Indeed, enzymes are present in all organisms in nature [1], and in their absence, life as we know it would not be possible. These bio-catalysts enhance the rate of chemical reactions to values approaching the diffusion limit of bimolecular encounters in water [2]. Decades of comparisons between enzymatic reaction rates and their nonenzymatic analogues have revealed astounding rate enhancements of up to 10^{17}-fold, as shown by Wolfenden and co-workers [3]. The ability of enzymes to lower the effective free energy barrier has been ascribed to various effects, including active site preorganization [4], reactant destabilization, desolvation [5], covalent bonding [6], quantum mechanical (QM) tunneling [7,8], and enzyme dynamics [9,10].

A simplified reaction scheme of an enzyme process is:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_{cat}}{\rightarrow} ES^{\ddagger} \rightarrow EP \rightarrow E + P$$

This process is composed of the following distinct steps: (i) initial binding of substrates to form the Michaelis complex with $K_M = (k_{-1} + k_{cat})/k_1 \cong k_{-1}/k_1$ (ii) catalytic step described by k_{cat} (iii) product release. Notably, many enzymes have evolved to optimize k_{cat}/K_M, which spans a very narrow range of values [11]. However, in reality enzyme processes can be far more complex than suggested by the above scheme, making it a daunting task to delineate the intimate details of the workings of enzymes. A crucial tool in studying the intricacies of these biomachines, such as substrate binding, chemical mechanisms, and their dynamic nature is isotope effects.

For each of the steps described above, there might be an associated isotope effect. An isotope effect associated with the initial

* Corresponding author.
E-mail address: majort@biu.ac.il (D.T. Major).

http://dx.doi.org/10.1016/j.abb.2015.03.001
0003-9861/

binding process is termed a binding isotope effect (BIE)[1], whereas an isotope effect on the chemical step is a kinetic isotope effect (KIE). An isotope effect on the reaction equilibrium is denoted as an equilibrium isotope effect (EIE). In particular, KIE is an extremely useful tool in chemistry and biology, and has been used extensively in enzymology [12–16]. In the case of reactions with a single transition state (TS) separating reactants and products, the experimentally observed KIEs provide direct information regarding the change in bonding during the chemical event. Specifically, primary KIEs indicate which atoms are directly involved in bond making or breaking at the TS, while secondary KIEs are indicators of the location of the TS along a reaction coordinate connecting reactants and products.

The KIE is defined as

$$\mathrm{KIE} = k_{\mathrm{L}}/k_{\mathrm{H}} \tag{1}$$

where k_{L} and k_{H} are the rate constants for the reaction involving the light and heavy isotopologues, respectively. Numerous experimental techniques exist for determining KIEs, such as the direct method, internal competition, remote labeling, equilibrium perturbation, and intrinsic isotope effect [1]. However, in many cases there are complicating factors, and the value measured is not the intrinsic isotope effect. Two such confounding matters are substrate binding isotope effect and kinetic complexity. The former effect could arise due to differing binding constants for the two isotopomers, due to induced ligand-strain on binding or interaction with the enzyme active site matrix. Usually, this effect is presumed to be small, although it can often be non-negligible [17,18]. The latter complicating factor is the significant kinetic complexity in enzymatic reactions, which obscures the link between the measured KIE and the chemical nature of the TS. This complexity is a result of the multi-step nature of enzymatic processes, which includes multiple minima separated by TSs. For instance, conformational changes in enzymes are essential for enzyme function, and are often rate limiting in the overall process [19]. Indeed, enzymes have often evolved to a level of chemical perfection so that the chemical transformation is no longer the rate-limiting step [11]. Therefore, complexity arises due to steps that are not related directly to the chemical step of interest and are therefore not isotopically sensitive, but still mask the isotope effect on the chemical step, thus complicating the interpretation of the experimental data. This convolution may be expressed in terms of the reaction's commitment to proceed forward (C_{f}) or in the reverse direction (C_{r}). Thus, the intrinsic KIE may be related to the observed KIE via [20]

$$\mathrm{KIE}_{\mathrm{obs}} = \frac{\mathrm{KIE}_{\mathrm{int}} + C_{\mathrm{f}} + C_{\mathrm{r}} \cdot \mathrm{EIE}}{1 + C_{\mathrm{f}} + C_{\mathrm{r}}} \tag{2}$$

where EIE is the equilibrium isotope effect and C_{f} and C_{r} are the forward and reverse commitments to catalysis, respectively. The commitment to catalysis is defined as the ratio between the isotopically sensitive rate-constant to the isotopically insensitive rate-constants affecting the observed KIE. If the reaction is essentially irreversible the $\mathrm{KIE}_{\mathrm{obs}}$ can vary from $\mathrm{KIE}_{\mathrm{int}}$ to unity (i.e. no KIE). On the other hand, if the reaction is reversible and C_{f} is small, the $\mathrm{KIE}_{\mathrm{obs}}$ may range from $\mathrm{KIE}_{\mathrm{int}}$ to EIE. It is of course of great importance for theoreticians to distinguish between intrinsic and observed KIEs as the computed values typically relate only to the former.

[1] *Abbreviations used:* KIE, kinetic isotope effect; NQEs, nuclear quantum effects; QM/MM, quantum mechanics/molecular mechanics; BIE, binding isotope effect; EIE, equilibrium isotope effect; TS, transition state; TST, transition state theory; PES, potential energy surface; DFT, density functional theory; SRP, specific reaction parameter; EVB, empirical valence bond; US, umbrella sampling; QCP, quantized classical path; EA-VTST/MT, ensemble-averaged variational TST with multi-dimensional tunneling; PI–FEP, path-integral and free-energy perturbation; DHFR, dihydrofolate reductase; ADH, alcohol dehydrogenase; NAO, nitroalkane oxidase; AlaR, alanine racemase.

Theoretical approaches to kinetic isotope effects

In order to compute KIEs for enzymatic reactions from first principles, the theoretical framework of transition state theory (TST) [21] or the more general generalized TST, is usually employed [5,22,23]. Within this framework, the rate constant may be expressed as follows:

$$k = \gamma \cdot k_{\mathrm{CM}}^{\mathrm{TST}} \tag{3}$$

where $k_{\mathrm{CM}}^{\mathrm{TST}}$ is the classical mechanics (CM) TST rate constant, and γ is a prefactor that accounts for deviations from CM TST. $k_{\mathrm{CM}}^{\mathrm{TST}}$ may be obtained from classical simulations that provide the CM potential of mean force (PMF), $W_{\mathrm{CM}}(\zeta)$ [24]:

$$k_{\mathrm{CM}}^{\mathrm{TST}} = 1/2\left\langle|\dot{\zeta}|\right\rangle_{\zeta^{\ddagger}} e^{-\beta W_{\mathrm{CM}}(\zeta^{\ddagger})} / \int_{-\infty}^{\zeta^{\ddagger}} e^{-\beta W_{\mathrm{CM}}(\zeta)} d\zeta \tag{4}$$

where ζ is the reaction coordinate, the prefactor $\left\langle|\dot{\zeta}|\right\rangle_{\zeta^{\ddagger}}$ is the classical dynamical frequency corresponding to the reaction coordinate velocity [25], $\beta = (k_{\mathrm{B}}T)^{-1}$, k_{B} is Boltzmann's constant, and T is the temperature.

The prefactor γ may be defined as

$$\gamma = \Gamma \cdot \kappa \cdot g \tag{5}$$

which accounts for recrossing of the dividing surface (i.e. the TS), nuclear QM effects (NQE), and non-equilibrium distribution in phase-space, respectively. Γ may be computed by activated MD simulations [24], while g is often assumed to be close to unity, which is likely the case for reactions occurring in pre-organized enzyme active sites [26,27]. The QM prefactor κ is defined as

$$\kappa = k_{\mathrm{QM}}^{\mathrm{TST}}/k_{\mathrm{CM}}^{\mathrm{TST}} = e^{-\left(\Delta W_{\mathrm{QM}}^{\ddagger} - \Delta W_{\mathrm{CM}}^{\ddagger}\right)/RT} \tag{6}$$

and may be computed via a variety of methods. We note that the quantum vibrational corrections may be included in $k_{\mathrm{CM}}^{\mathrm{TST}}$ or in the QM prefactor. We will now describe in greater detail multiscale approaches for enzyme simulations followed by a particular approach for computing KIE, based on a path-integral (PI) formulation.

Potential energy surface

The potential energy surface (PES) in enzyme simulations may be treated employing a hybrid quantum mechanics–molecular mechanics (QM/MM) Hamiltonian [28,29]. In the QM/MM approach the region of chemical interest, i.e. the reacting fragment, is treated via QM while the remaining parts of the system are treated by a MM force field. The effect of the solvent and enzyme environment on the QM fragment may be included through a coupling term (i.e. electrostatic embedding). This coupling term is essential, as it accounts for the effect of the enzyme and solvent environment on the reactive fragments. Multi-scale QM/MM methods and their use have been reviewed extensively in the literature [29–34].

Specifically, the QM/MM Hamiltonian of the total system, $\hat{H}_{\mathrm{T}}$, in a typical molecular orbital version of the theory, can be defined by:

$$\hat{H}_{\mathrm{T}} = \hat{H}_{\mathrm{QM}} + \hat{H}_{\mathrm{MM}} + \hat{H}_{\mathrm{QM/MM}} \tag{7}$$

Here, the energy of the full system is described by adding the energy obtained from the QM calculation in the inner layer with a MM calculation in the outer layer. Furthermore, an explicit coupling term is added that describes the interaction between both layers. The coupling term is crucial, and includes the electrostatic and van der Waals interactions between the atoms in both regions.

Please cite this article in press as: A. Vardi-Kilshtain et al., Arch. Biochem. Biophys. (2015), http://dx.doi.org/10.1016/j.abb.2015.03.001