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Review

Review of computer simulations of isotope effects on biochemical reactions: From the Bigeleisen equation to Feynman's path integral☆

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ABSTRACT

Enzymatic reactions are integral components in many biological functions and malfunctions. The iconic structure of each reaction path for elucidating the reaction mechanism in details is the molecular structure of the rate-limiting transition state (RLTS). But RLTS is very hard to get caught or to get visualized by experimentalists. In spite of the lack of explicit molecular structure of the RLTS in experiment, we still can trace out the RLTS unique "fingerprints" by measuring the isotope effects on the reaction rate. This set of "fingerprints" is considered as a most direct probe of RLTS. By contrast, for computer simulations, oftentimes molecular structures of a number of TS can be precisely visualized on computer screen, however, theoreticians are not sure which TS is the actual rate-limiting one. As a result, this is an excellent stage setting for a perfect "marriage" between experiment and theory for determining the structure of RLTS, along with the reaction mechanism, i.e., experimentalists are responsible for "fingerprinting", whereas theoreticians are responsible for providing candidates that match the "fingerprints". In this Review, the origin of isotope effects on a chemical reaction is discussed from the perspectives of classical and quantum worlds, respectively (e.g., the origins of the inverse kinetic isotope effects and all the equilibrium isotope effects are purely from quantum). The conventional Bigeleisen equation for isotope effect calculations, as well as its refined version in the framework of Feynman's path integral and Kleinert's variational perturbation (KP) theory for systematically incorporating anharmonicity and (non-parabolic) quantum tunneling, are also presented. In addition, the outstanding interplay between theory and experiment for successfully deducing the RLTS structures and the reaction mechanisms is demonstrated by applications on biochemical reactions, namely models of bacterial squalene-to-hopene polycyclization and RNA 2′-*O*-transphosphorylation. For all these applications, we used our recently-developed path-integral method based on the KP theory, called automated integration-free path-integral (AIF-PI) method, to perform ab initio path-integral calculations of isotope effects. As opposed to the conventional path-integral molecular dynamics (PIMD) and Monte Carlo (PIMC) simulations, values calculated from our AIF-PI path-integral method can be as *precise* as (not as *accurate* as) the numerical precision of the computing machine. Lastly, comments are made on the general challenges in theoretical modeling of candidates matching the experimental "fingerprints" of RLTS. This article is part of a Special Issue entitled: Enzyme Transition States from Theory and Experiment.

1. Introduction

> "… Isotope labelling and femtosecond spectroscopy can give clues, but rarely produce conclusive evidence for a given mechanism in systems with the complexity characterizing many catalytic chemical processes and almost all biochemical processes. This makes theoretical modelling an important tool as a complement to the experimental techniques. Chemical processes are characterized by a transition state, a configuration with the lowest possible (free) energy that links the product(s) with the reactant(s). This state is normally not experimentally accessible, but there are theoretical methods to search for such structures. Consequently theory is a necessary complement to experiment. …"
> [Advanced Information for the Nobel Prize in Chemistry 2013 [1–5]]

Catalytic chemical reaction steps, e.g., proton transfer, phosphorylation, cleavage of protein polypeptide bonds, etc., play crucial roles in many biological systems [6–24]. Oftentimes in a chemical reaction, it is quite straightforward to find stable reactant and product states in thermal equilibrium. Yet, as stated in the Advanced Information for

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the Nobel Prize in Chemistry 2013 [1–5], every possible reaction path connecting the reactant and product states is actually characterized by its own rate-limiting transition state, which, by its intrinsic nature, is unsteady, and thus is very difficult to be accessible in experiment. Thereby, deducing the molecular structure (as well as the bonding nature) of the rate-limiting transition state is a key step to elucidate the enzymatic mechanism in biocatalysis [1–24].

To better understand the properties of transition states, one common approach is investigating equilibrium and kinetic isotope effects (EIE and KIE) on a biochemical reaction, i.e., the so-called isotope labeling [1,6–20]. The EIE is defined as the ratio of equilibrium constant of light isotope to that of heavy isotope:

$$\mathrm{EIE} \equiv \frac{\text{Equilibrium constant (light isotope)}}{\text{Equilibrium constant (heavy isotope)}}. \tag{1}$$

And the KIE is defined as the ratio of reaction rate of light isotope to that of heavy isotope:

$$\mathrm{KIE} \equiv \frac{\text{Reaction Rate (light isotope)}}{\text{Reaction Rate (heavy isotope)}}. \tag{2}$$

If a value of KIE is larger than unity, we then have a "normal KIE" because the reaction with light isotope is *faster* than that with heavy isotope (it is called "normal" because intuitively an object with lighter mass should "react" and move faster). Conversely, an "inverse KIE" means a KIE value is smaller than unity, i.e., the reaction with light isotope is *slower* than that with heavy isotope. This reaction rate ratio, i.e., KIE, is very sensitive to the structure and bonding nature of the rate-limiting transition state. Hence, measuring KIE values has been considered as a (most) direct and robust probe of transition state (e.g., for its structure and bonding nature) in experiment [6–20].

Nonetheless, merely having all these experimental KIE numerical values are still not enough for us to quantitatively determine the molecular structure of the rate-limiting transition state. On the other hand, the irreplaceable role of theory, computer simulations and visualization in Chemistry and Chemical Physics, which complements the shortcomings of experiment, has been at least recognized by the 1998 and 2013 Nobel Prizes in Chemistry [1–5,25,26]. Indeed, this complementary interplay can also be well demonstrated in the computer simulations of isotope effects [15–20].

> "… I would like to remind the audience that a very difficult problem in the field of molecular dynamics simulations of biomolecules is to have a way of checking that the results are correct. Experimental data (e.g., NMR measurements) that can be used for validation of the results are important but limited; i.e., they do not provide enough information for a quantitative test. Despite what the Nobel Prize press citation implies ("The computer is just as important as the test tube."), experiments are essential to verify that what we are doing is meaningful. …"
>
> [Nobel Lecture by Martin Karplus for the Nobel Prize in Chemistry 2013 [2]]

For simulations, in contrast to experiment, in fact first we hypothesize a possible reaction path, for which an explicit molecular structure of the rate-limiting transition state needs to be already attained [6–20]. Afterwards, as the reminder underscored in the 2013 Nobel Lecture by Martin Karplus about the importance of verification for simulations [2], we compute a set of isotope effect values and test whether or not our computed values associated with the inferred transition-state structure match with experimental results. If so, we then can declare that the reaction mechanism, along with the molecular structure and other properties of the rate-limiting transition state, are successfully and quantitatively identified and concluded in silico [6–20].

Indeed, this kind of justifications for the rate-limiting transition state by using a set of experimental isotope effects values is more convincing than using an experimental reaction rate constant for validations. This is largely because contrary to a set of unique isotope effect values, practically-identical reaction rate constants can be shared with a wide variety of biochemical reactions that share *neither* the same reaction mechanisms *nor* the same transition states.

In this Review, first we will describe the underlying theories for isotope effects, including the classical and quantum origins of the isotope effects. The formulation and limitations of the well-known Bigeleisen equation [27–32] for computing isotope effects will then be briefly discussed. Next, we will talk about our recently-developed ab initio path-integral method, called automated integration-free path-integral (AIF-PI) method, which is in the framework of Feynman's path integral and based on Kleinert's variational perturbation (KP) theory, for having accurate EIE and KIE computations [15–18,33–36]. Our ab initio path-integral method can be *exactly* reduced to the Bigeleisen equation in EIE and KIE calculations, and can also methodically go beyond this equation, e.g., by systematically including (non-parabolic) quantum tunneling effects, as well as anharmonic corrections to harmonic zero-point and vibrational energies. Further, in contrast to the commonly-used path-integral Monte Carlo (PIMC) [37–42] and molecular dynamics (PIMD) [43–45] simulations, calculated values using our ab initio path-integral method can be as precise as (though *not* as accurate as) the numerical precision of the computing machine. Illustrations of our ab initio path-integral method by some applications on reactions in solution and in protein enzymes, together with some other biologically relevant reactions, will follow [15–20,33–36]. At the end, we will conclude this Review with some remarks on the difficulties in simulating the isotope effects on the RNA 2′-*O*-transphosphorylation reaction in various environments, ranging from the cases in acidic and alkaline solution to the case catalyzed by ribonuclease A (RNase A).

2. Theory and method

The pioneers of studying isotope effects in Chemistry and Chemical Physics are at least represented by Urey, Eyring, Polanyi, de Hevesy, Van Vleck, Bigeleisen, etc. [27–32,46–61]. For example, according to Ref. [46], Cremer and Polanyi, as well as Eyring and Sherman, independently predicted that protium and deuterium isotopes should *not* result in the same reaction rates owing to their difference (at least) in zero point energy.

The origin of isotope effects on a chemical reaction can be divided into two parts. One origin is from the *classical* world, whereas another origin is from the *quantum* world.

2.1. The classical origin of isotope effects

In order to show the *classical* origin of isotope effects, let us consider a simplest single-molecule (i.e., unimolecular) one-dimensional system. For this simplest system, the molecule would like to overcome an asymmetric double-well potential-energy barrier (Fig. 1) for forming a product from a reactant. According to the conventional *classical* transition state theory (TST) [62–74], the reaction rate constant k_{TST} can be expressed in terms of the free energies or partition functions at the reactant and transition states:

$$k_{\mathrm{TST}} = \frac{k_B T}{h}\left(\frac{Q^{\ddagger}}{Q_R}\right) = \frac{k_B T}{h}\left[\frac{\exp\left(-\beta \Delta G^{\ddagger}\right)}{Q_R}\right] \tag{3}$$

where k_B is Boltzmann's constant, T is temperature, $\beta = 1/k_B T$, h is Planck's constant, Q is the partition function, $\Delta G^{\ddagger}$ is the *free energy* barrier to overcome, and the superscript ‡ and the subscript R denote the transition state (a first-order saddle point in the free-energy surface)