Archives of Biochemistry and Biophysics 544 (2014) 96–104

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

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

Review

Kinetic isotope effects as a probe of hydrogen transfers to and from common enzymatic cofactors

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ARTICLE INFO

Article history:
Available online 22 October 2013

Keywords:
Kinetic isotope effects
Marcus-like models
Nicotinamide
Folate
Hydrogen tunneling

ABSTRACT

Enzymes use a number of common cofactors as sources of hydrogen to drive biological processes, but the physics of the hydrogen transfers to and from these cofactors is not fully understood. Researchers study the mechanistically important contributions from quantum tunneling and enzyme dynamics and connect those processes to the catalytic power of enzymes that use these cofactors. Here we describe some progress that has been made in studying these reactions, particularly through the use of kinetic isotope effects (KIEs). We first discuss the general theoretical framework necessary to interpret experimental KIEs, and then describe practical uses for KIEs in the context of two case studies. The first example is alcohol dehydrogenase, which uses a nicotinamide cofactor to catalyze a hydride transfer, and the second example is thymidylate synthase, which uses a folate cofactor to catalyze both a hydride and a proton transfer.

Introduction

The chemical and physical mechanisms by which enzymes catalyze reactions receives considerable interest from chemists, owing to the many important intellectual and practical problems associated with enzymes. Enzymes catalyze a very diverse range of reactions, increasing rates by many orders of magnitude, but as of yet, there exists no thorough understanding of why they are so successful. Determining the mechanisms and sources of the catalytic power of enzymes would enable the development of useful biomimetic catalysts [1] and would facilitate the development of specific and potent drugs that affect enzymatic activity [2]. Though much mystery remains, some common themes have begun to emerge in recent years, especially among reactions involving the same (or similar) cofactors. Certain ubiquitous cofactors (e.g. nicotinamides, folates, flavins, etc.) play roles in very diverse reactions, but the physical mechanisms involved in these reactions are often strikingly similar. Here we seek to describe our current understanding of the physical mechanism of hydrogen transfer to and from some very well studied cofactors. One of the most useful experimental techniques for understanding H-transfers is the measurement of kinetic isotope effects (KIEs)[2] and that will be the primary focus of this review. We note that from the perspective of the physical enzymologist, there is no distinction between a "cofactor" and a "substrate": both molecules react during the reaction and the molecules' overall roles in metabolic pathways are inconsequential to the physics of any given reaction. Thus, our discussion of the theory and interpretation of KIEs will be fairly general, but then we will highlight the use of KIEs in examples that involve H-transfers to and from ubiquitous cofactors: nicotinamide in alcohol dehydrogenase (ADH) and folate in thymidylate synthase (TSase).

Theory of KIEs

A KIE is the ratio of rates between two reactions that differ only in the isotopic composition of reactants (isotopologues):

$$\mathrm{KIE} = \frac{k_{\mathrm{Light}}}{k_{\mathrm{Heavy}}} \tag{1}$$

Here, k_{Light} is the rate with the light isotope and k_{Heavy} is the rate with the heavy isotope. Isotopic substitution serves as a minimal

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[2] *Abbreviations used:* KIEs, kinetic isotope effects; TRS, tunneling ready state; ADH, alcohol dehydrogenase; TSase, thymidylate synthase; PES, potential energy surface; TS, transition state; ZPE, zero point energy; DAD, donor–acceptor distance; SSE, Swain–Schaad Exponent; NADH, nicotinamide adenine dinucleotide; yADH, yeast enzyme; RGM, geometric mean; mSSE, mixed-labeling SSE; dTMP, 2′-deoxythymidine-5′-mono phosphate.

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http://dx.doi.org/10.1016/j.abb.2013.10.010

perturbation to the reaction allowing an experiment to probe the nature of a reaction's traversal across a potential energy surface (PES). To be clear, according to the Born–Oppenheimer approximation, the isotopic substitution does not affect a reaction's electronic PES, so the kinetic changes that occur upon substitution reveal the nature of how a reaction proceeds from a reactant state to a transition state (TS). These kinetic changes result primarily from nuclear quantum effects including vibrational zero point energy (ZPE) and quantum mechanical tunneling. We have recently reviewed how both of these effects appear in measured KIEs, with an emphasis on why interpretations that ignore tunneling fail to explain H-transfers [3]. Here we will take it for granted that H-transfers involve a large degree of tunneling, which is the prevailing view among enzymologists [4–9], and focus on the interpretation of experiments in this context.

KIEs can be interpreted using Marcus-like models (Fig. 1), which have also been referred to as environmentally coupled tunneling [10], vibrationally enhanced tunneling [11], and other names. Following the footsteps of Marcus theory of electron transfer [12], the key to this kind of model is that it makes a Born–Oppenheimer-like separation between the fast motion of the transferred H and the slow motion of the surrounding atoms, which includes the remainder of the substrates and the enzyme. Marcus-like models assume a mechanism of H-transfer where the surrounding atoms (the heavy atoms) rearrange from the ground state to a tunneling ready state (TRS), where the energy levels of the reactant well and the product well are degenerate (vibrational ground state or excited state), so efficient tunneling can occur. The TRS is the heavy atom configuration where the transferred particle is delocalized (i.e., in the process of tunneling) between donor and acceptor wells. This state is simply a delocalized transition state, which is typical to small particles transfer (e.g., electrons and protons), and thus involves longer DAD than the DAD at the peak of the energy barrier (i.e., the localized transition state). In analogy to the dividing surface between reactants and products in traditional transition state theory, a system has a whole ensemble of TRSs. At each TRS, the efficiency of tunneling depends on the mass of the tunneling particle and the donor–acceptor distance (DAD). The rate (k) in this kind of model takes the functional form [6,10,13–17].

$$K = \frac{|V|^2}{\hbar}\sqrt{\frac{\pi}{\lambda k_B T}}e^{-\frac{(\Delta G^\circ + \lambda)^2}{4k_B T\lambda}}\int_0^\infty F(m,\mathrm{DAD})e^{-E(\mathrm{DAD})/k_B T}d\mathrm{DAD} \quad (2)$$

The leading factors of this equation compute the rate of heavy atom rearrangement to reach a TRS based on the electronic coupling between reactants and products (V, the adiabaticity), the reorganization energy (λ), and the reaction driving force (ΔG°). The mass-sensitivity of these leading factors is generally negligible for 1° KIEs, so they cancel out when using this equation to model experimental KIEs [13]. The integral yields the probability of tunneling to products once the system reaches the TRS and depends on the transmission probability, $F(m,\mathrm{DAD})$, as a function of mass (m) and DAD, and a Boltzmann factor giving the probability of being at any given DAD. The transmission probability can be calculated assuming vibrationally diabatic transfer using either harmonic [14,17] or Morse potentials[18] to describe the H-wavefunctions, or, where a vibrationally adiabatic approach is necessary model calculations of relevant systems can be used to calculate the transmission probability [13]. While the Boltzmann factor assumes a statistical distribution of states, the models would have very similar mathematical form if non-equilibrium dynamics were introduced, though currently, we are not aware of experimental findings that cannot be rationalized by a Boltzmann factor. Integrating the tunneling probability (weighted by the probability of being at each DAD) over all DADs gives the total tunneling probability. Since the thermal activation leading to the TRS in this model is insensitive to the mass of the 1° isotope (see below regarding 2°

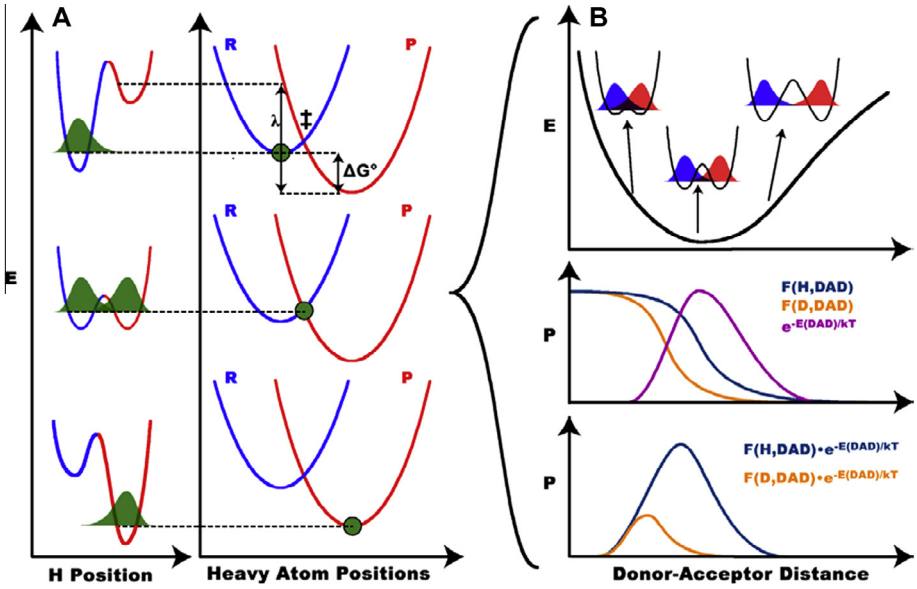

Fig. 1. Marcus-like model of H-tunneling. (A) The three panels (top to bottom) represent three positions during the course of the reaction: reactant state, TRS, and product state. The reaction coordinate consists of heavy atom motion which is separated from the motion of the transferred particle via a Born–Oppenheimer-like approximation. In the reactant state (top), the ZPE of the transferred H is lower in the reactant well (blue) than the product well (red), so its wavefunction (green) is localized in the reactant well. When heavy atoms rearrange to a TRS (middle), the reactant well and product well are degenerate, so the H wavefunction is delocalized between the two and tunneling occurs. Upon further heavy atom rearrangement (bottom), the transferred H can be trapped in the product well. (B) At the TRS, fluctuations of the DAD affect the probability of tunneling. The top panel shows the PES along the DAD coordinate, highlighting the different levels of reactant–product wavefunction overlap at different DADs, which is proportional to the tunneling probability as a function of DAD. The middle panel shows the population distribution (magenta) corresponding to the PES in the top, along with the tunneling probability of H (purple) and D (orange) as a function of DAD. The bottom panel shows the product of the tunneling probability and population distribution shown in the middle panel, which gives the overall flux of reactive trajectories for each isotope as a function of DAD (the integrand of Eq. (2). Note that this model predicts that H-transfer occurs from a longer average DAD than D-transfer. Figure reproduced from Ref. [19] with permission from ACS.