

Modeling temperature dependent kinetic isotope effects for hydrogen transfer in a series of soybean lipoxygenase mutants: The effect of anharmonicity upon transfer distance

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Abstract

Soybean lipoxygenase-1 (SLO) catalyzes the oxidation of linoleic acid. The rate-limiting step in this transformation is the net abstraction of the *pro-S* hydrogen atom from the center of the 1,5-pentadienyl moiety in linoleic acid. The large deuterium kinetic isotope effect (KIE) for this step appears in the first order rate constant ($Dk_{\text{cat}} = 81 \pm 5$ at $T = 25^\circ\text{C}$). Furthermore, the KIE and the rate for protium abstraction are weakly temperature dependent ($E_{\text{A,D}} - E_{\text{A,H}} = 0.9 \pm 0.2$ kcal/mol and $E_{\text{A,H}} = 2.1 \pm 0.2$ kcal/mol, respectively). Mutations at a hydrophobic site about 13 Å from the active site Fe(III), Ile⁵⁵³, induce a marked temperature dependence that varies roughly in accordance with the degree to which the residue is changed in bulk from the wild type Ile. While the temperature dependence for these mutants varies from the wild type enzyme, the magnitude of the KIE at 25 °C is on the same order of magnitude. A hydrogen tunneling model [Kuznetsov, A.M., Ulstrup, J. *Can. J. Chem.* 77 (1999) 1085–1096] is utilized to model the KIE temperature profiles for the wild type SLO and each Ile⁵⁵³ mutant. Hydrogenic wavefunctions are modeled using harmonic oscillators and Morse oscillators in order to explore the effects of anharmonicity upon computed kinetic observables used to characterize this hydrogen transfer.

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1. Introduction

1.1. Failures of tunnel corrections

Hydrogen transfer reactions [1] are perhaps the most fundamental chemical reactions. However, many challenges exist concerning a description of these reactions. First, the small mass of the hydrogen atom makes it difficult to accurately treat hydrogen transfer using techniques which have met with success in quantitatively describing reactions in which the movement of more

massive nuclei predominate. Semiclassical transition state theory (TST) in its numerous incarnations [2–5] has proven immensely successful at reproducing or predicting quantitative experimental observables in a host of chemical transformations. In the case of proton, hydride, and hydrogen atom transfers, however, the traditional concept of a transition state loses importance. Because the nuclear wavefunctions for vibrations that consist primarily of hydrogen motion are more diffuse than wavefunctions for vibrations of larger reduced mass, reaction coordinates for hydrogen transfers have significant portions of their length that are appreciably permeable to hydrogen tunneling. This quantum reality is in direct contrast with the establishment of a dividing *surface* which relegates one part of the potential energy

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surface (PES) to reactants and the remaining portion to products under all variants of semiclassical TST. Because of this irresolvable difference between quantum reality and semiclassical TST, quantitative analyses of reactions where observables exhibit a definitive contribution from quantum effects have been treated using a correction factor. In Eq. (1), the rate corrected for tunneling, k , is simply the product of the quantum correction, Q , and the semiclassical transition state theory rate constant, k_{sc} .

$$k = Qk_{sc}. \quad (1)$$

The most utilized quantum mechanical correction to TST was first put forth by Bell [6,7]. This treatment assumes a barrier of parabolic shape with a given energy of activation (see Eq. (2)). In Eq. (2), $u_t = hc\nu^\ddagger/k_B T$ is the reduced imaginary frequency, and $\alpha = E/k_B T$ is the reduced energy of activation; both values are unitless. Northrop has published a version of this correction which is free of typographical errors [8]. The negative curvature of the potential energy barrier at the saddle point can be supplied as a parameter to fit experimental data or originate from the computation of the negative force constant at the transition state using electronic structure calculations. The energy of activation can be provided in a similar fashion, either from empirical rate data or electronic structure calculations.

$$Q = \frac{u_t/2}{\sin(u_t/2)} - \sum_{n=1}^{\infty} (-1)^n \frac{\exp[\alpha(u_t - 2n\pi)/u_t]}{(u_t - 2n\pi)/u_t}. \quad (2)$$

In general, the Bell correction overestimates tunneling amplitudes. This is because the parabolic barrier is, in the case of regularly shaped reaction coordinates, thinner than the barrier along the reaction coordinate. More recent methods of employing tunnel corrections to semiclassical TST utilize a Boltzmann-weighted

JWKB integration of the imaginary action traversed under the potential surface [9,10]. While these calculations are ideally more realistic than the assumption of a barrier of given shape, they suffer from two inadequacies: (1) The computational difficulties inherent in defining a large enough portion of the PES with reliable levels of electronic structure theory lend uncertainty to the imaginary action relevant to the tunneling path. (2) The choice of the tunneling path is difficult, especially when it deviates strongly from both the minimum energy path (small curvature tunneling) and the least action path (extreme limit of large curvature tunneling) between reactant(s) and product(s).

A good illustration of the difficulties outlined above is provided by a recent direct dynamics calculation of the oxidation of 1,4-pentadiene with a truncated model of SLO using the Fe(III) cofactor and its six ligands [11]. Whereas the experimental primary deuterium isotope effect for the hydrogen abstraction step in SLO (Fig. 1) is 81 [12], the value computed by direct dynamics at the PM3/d level of theory is 18.9. Although these calculations cannot quantitatively describe the hydrogen abstraction reaction catalyzed by SLO, there are some salient features of the PES that are captured in this particular calculation. First, the potential energy barrier is calculated to have a frequency of 2913i for the transfer of protium, which is one of the highest magnitude imaginary frequencies ever reported. Second, the reaction coordinate is shown to be composed primarily of hydrogen motion for a significant region in the vicinity of the transition state. These two characteristics suggest that a full tunneling model would be a more reasonable approach to modeling the hydrogen abstraction catalyzed by SLO, since hydrogen motion is, for all practical purposes, uncoupled from heavy atom motion for a significant part of the reaction.

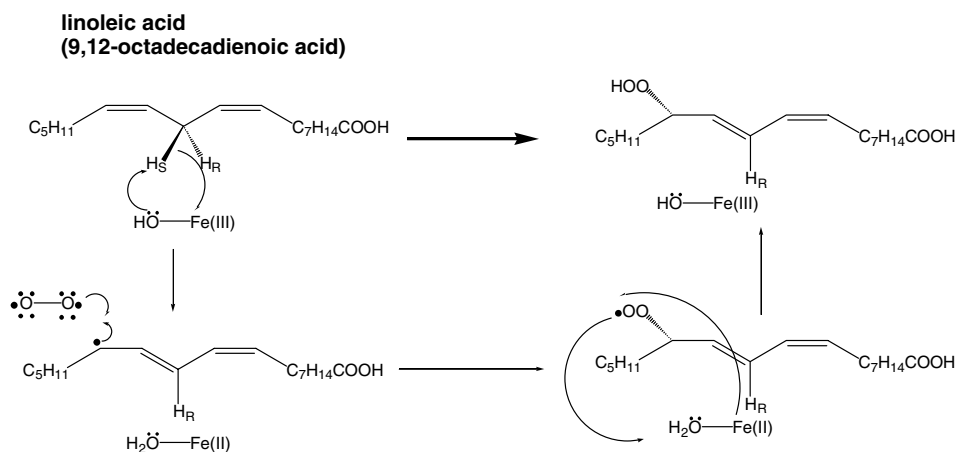


Fig. 1. Consensus mechanism for oxidation of linoleic acid catalyzed by SLO and its mutants. The first step in the mechanism, the abstraction of the 11-*pro-S* hydrogen on linoleic acid is the rate-limiting step, and is the focus of this article. The five amino acid ligands on the Fe(III) cofactor are omitted for clarity.

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