IMPACT OF MUTATION ON THE PROTON-COUPLLED ELECTRON TRANSFER REACTION CATALYZED BY SOYBEAN LIPROXYGENASE

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by
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ABSTRACT

The hydrogen atom abstraction catalyzed by soybean lipoxygenase occurs by way of a proton-coupled electron transfer mechanism. Experimental kinetic studies indicate that the deuterium kinetic isotope effect for the reaction is ~80 at room temperature and exhibits weak temperature dependence. Kinetic studies also show that mutation of the I553 residue in lipoxygenase, which is ~15 Å from the active site Fe but borders the substrate, can significantly alter the magnitude and temperature dependence of the kinetic isotope effect for this reaction. We have studied the wild-type and mutant lipoxygenase with a vibronically nonadiabatic theory of proton coupled-electron transfer that includes the quantum effects of the electrons and transferring proton. This theory indicates that the magnitude and temperature dependence of the kinetic isotope effect depend on the proton donor-acceptor equilibrium distance and vibrational frequency, as well as the overlap between the reactant and product proton vibrational wavefunctions. We used this theoretical treatment to analyze the experimental data for the wild-type and four I553 mutant lipoxygenases. We found that the proton donor-acceptor equilibrium distance increases and the vibrational frequency decreases as the residue I553 becomes less bulky. These changes at the proton transfer interface are correlated to the experimentally observed increase in both the magnitude and temperature dependence of the KIE as the residue I553 becomes less bulky. We performed all-atom molecular dynamics simulations for the entire solvated lipoxygenase enzyme, as well as two I553 mutants, in order to understand how a mutation at residue I553 impacts the proton transfer interface. These simulations provided insight into the impact of mutation on the substrate mobility, conformation and orientation.
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Chapter 1

Introduction

The reaction in which a proton and an electron are simultaneously transferred from donor to acceptor is called proton-coupled electron transfer (PCET). The conditions and mechanisms of these reactions have been studied extensively both experimentally\textsuperscript{1-20} and theoretically\textsuperscript{21-30} in a wide variety of systems. PCET occurs in a broad range of biological and chemical reactions. In this dissertation we present a model of the reaction within the enzyme soybean lipoxygenase with a particular focus on the impact of mutation on the PCET reaction this enzyme catalyzes. Using model systems and a mathematical approach, we have been able to develop a deeper understanding of experimental observations and to predict the physical trends in proton donor-acceptor distance and vibrational frequency in a series of soybean lipoxygenase mutants. Performing molecular dynamics simulations, we have studied the impact of mutation on substrate mobility, conformation, and orientation.

To study PCET, we used a theoretical formulation that includes the quantum effects of the transferring electron and proton, and the motion of both the protein/solvent environment and the proton donor-acceptor.\textsuperscript{25,27} This formulation is based on linear response theory for the solvent/protein environment and can be viewed as Marcus theory for electron transfer extended to PCET. Instead of pure electronic states, we use mixed electron-proton vibronic states. Since there are two collective solvent coordinates, one for electron transfer and one for proton transfer (PT), we have two-dimensional paraboloid free energy surfaces rather than one-dimensional parabola free energy curves. The general form of the rate constant expression we use is:

\[
k = \sum_{\mu,\nu} P_{\mu} k_{\mu\nu}
\]  

(1-1)
where $P_\mu$ is the Boltzmann probability of reactant state $\mu$, and $k_{\mu \nu}$ is the partial nonadiabatic rate constant for transfer from reactant state $\mu$ to product state $\nu$. Input quantities for these rate constant expressions are calculated from classical molecular dynamics simulations, quantum mechanical models, and experiment.

We employ this theoretical formulation to further elucidate the physics underlying three experimental observations relating to the deuterium kinetic isotope effect ($k_H/k_D$, KIE). One, the KIE typically increases as the proton donor-acceptor distance increases if all other parameters are held constant. The opposite trend could be observed if the proton donor-acceptor vibrational frequency decreases as the proton donor-acceptor distance increases. These two effects impact the KIE in opposite directions, so either trend could be observed. Two, the KIE has been shown to both increase and decrease with increasing temperature. The complex interplay between distance, frequency, driving force, and solvent reorganization energy allows either trend to be observed. Three, we have gained insight into the driving force dependence of both the rate and KIE. The rate increases as the driving force becomes more negative due to the increased accessibility of excited product vibronic states with low free energy barriers and large vibronic couplings. The KIE decreases as the reaction becomes more asymmetric because the increased contributions from excited states decrease the KIE.

This study of driving force dependence led to a more in depth study of the Marcus inverted region for PT and PCET. In the normal Marcus regime, the rate increases as the driving force becomes more negative. When the driving force passes a certain threshold, the rate begins to decrease with more negative driving force. This is a well understood phenomenon for electron transfer, but is misunderstood for PT and PCET. According to our theoretical framework, the Marcus inverted region is not experimentally accessible for PT and PCET. However, inverted region behavior for PT has been observed experimentally for a variety of systems. We
explain this apparent discrepancy in two possible ways.\textsuperscript{34} First, for low values of the solvent reorganization energy (i.e. in a non-polar solvent), there are oscillations in the driving force dependence. If the experimental driving force range is sufficiently small, this would lead to the observation of an apparent inverted region. Second, inverted region behavior may also be observed if the proton transfer barrier or proton donor-acceptor distance increases as the driving force becomes more negative.

With a firm understanding of how system parameters affect the rate – and therefore the KIE – we are well equipped to study the impact of mutation on the KIE of soybean lipoxygenase. Soybean lipoxygenase is a non-heme metalloenzyme, studied for its pharmaceutical implications,\textsuperscript{35-40} that catalyzes the oxidation of linoleic acid to a fatty acid hydroperoxide.\textsuperscript{19} The first step of this reaction is PCET from the substrate linoleic acid to the Fe(III)-OH cofactor. Klinman \textit{et al.} have studied this system experimentally\textsuperscript{19,20,42} and found an unusually high KIE of 81 at room temperature as well as a weak temperature dependence, indicating that hydrogen tunneling plays an important role in this step of the reaction. A series of mutations were made to residue 553,\textsuperscript{42-44} an isoleucine that borders the substrate but is \textasciitilde{}15 Å from the active site Fe, causing this residue to become progressively less bulky. Both the magnitude and temperature dependence of the KIE were found to increase as the residue bulk decreases. Our calculations\textsuperscript{43} on model systems predict that as residue 553 becomes less bulky, the proton donor-acceptor distance will increase and the proton donor-acceptor vibrational frequency will decrease. These changes in distance and frequency are also correlated to changes in substrate mobility, conformation, and orientation.\textsuperscript{43}

The structure of my dissertation is twofold: first, I present our studies of PCET reactions in model systems, second I present our results of simulations of soybean lipoxygenase. In Chapter 2, I present an analysis of the impact of various system properties on the rate and KIE of PCET reactions. In Chapter 3, I take a deeper look at the driving force dependence of the rate
and KIE of PT and PCET reactions. In Chapter 4, I apply our theoretical formulation to the KIE of soybean lipoxygenase. I find that the changes in the magnitude and temperature dependence of the KIE are related to changes in the proton donor-acceptor distance and vibrational frequency and changes in the substrate mobility, conformation and orientation. In Chapter 5, I conclude with a summary of the important findings of my dissertation.

References

Chapter 2

Analysis of kinetic isotope effects for proton-coupled electron transfer reactions


A series of rate constant expressions for nonadiabatic proton-coupled electron transfer (PCET) reactions are analyzed and compared. The approximations underlying each expression are enumerated, and the regimes of validity for each expression are illustrated by calculations on model systems. In addition, the kinetic isotope effects (KIEs) for a series of model PCET reactions are analyzed to elucidate the fundamental physical principles dictating the magnitude of the KIE and the dependence of the KIE on the physical properties of the system, including temperature, reorganization energy, driving force, equilibrium proton donor-acceptor distance, and effective frequency of the proton donor-acceptor mode. These calculations lead to three physical insights that are directly relevant to experimental data. First, these calculations provide an explanation for a decrease in the KIE as the proton donor-acceptor distance increases, even though typically the KIE will increase with increasing equilibrium proton donor-acceptor distance if all other parameters remain fixed. Often the proton donor-acceptor frequency decreases as the proton donor-acceptor distance increases, and these two effects impact the KIE in opposite directions, so either trend could be observed. Second, these calculations provide an explanation for an increase in the KIE as the temperature increases, even though typically the KIE will decrease with increasing temperature if all other parameters remain fixed. The combination of a rigid hydrogen bond, which corresponds to a high proton donor-acceptor frequency, and low
solvent polarity, which corresponds to small solvent reorganization energy, allows the KIE to either increase or decrease with temperature, depending on the other properties of the system. Third, these calculations provide insight into the dependence of the rate constant and KIE on the driving force, which has been studied experimentally for a wide range of PCET systems. The rate constant increases as the driving force becomes more negative because excited vibronic product states associated with low free energy barriers and relatively large vibronic couplings become accessible. The $\ln[KIE]$ has a maximum near zero driving force and decreases significantly as the driving force becomes more positive or negative because the contributions from excited vibronic states increase as the reaction becomes more asymmetric, and contributions from excited vibronic states decrease the KIE. These calculations and analyses lead to experimentally testable predictions of trends in the KIEs for PCET systems.

**Introduction**

Proton-coupled electron transfer (PCET) plays an important role in a wide range of chemical and biological processes. PCET reactions involve the coupled transfer of both an electron and a proton. According to the general definition of PCET, the mechanism can be either sequential or concerted, and the electron and proton can transfer in different directions or in the same direction, either between the same sites or between different sites. This chapter focuses on the broad class of PCET reactions that involve the transfer of an electron and a proton with no stable intermediate. These types of PCET reactions have been extensively studied both experimentally\textsuperscript{1-20} and theoretically.\textsuperscript{21-30}

The fundamental properties of PCET systems can be characterized by measuring the kinetic isotope effect (KIE), which is the ratio of the rate for hydrogen to the rate for deuterium. The experimentally observed magnitudes of the KIE, as well as the dependence of the KIE on
properties such as temperature and driving force, vary widely for different PCET systems. Numerous theoretical models have been developed to describe these experimental data.\textsuperscript{17-30} Many of these models build upon previous theoretical studies of electron transfer\textsuperscript{31,32} and vibrationally nonadiabatic proton transfer reactions.\textsuperscript{33-36}

In this chapter, we analyze and compare a series of rate constant expressions for nonadiabatic PCET reactions. We enumerate the approximations underlying each expression and illustrate the regimes of validity for each expression through calculations on model systems. In addition, we analyze the KIEs for a series of model systems to elucidate the fundamental physical principles dictating both the magnitude of the KIE and the dependence of the KIE on the properties of the system. Specifically, we examine the impact of the proton transfer interface properties (i.e., the equilibrium proton donor-acceptor distance and the effective frequency of the proton donor-acceptor mode) on the KIE. We also study the dependence of the KIE on the solvent reorganization energy, the temperature, and the driving force. These calculations and analyses lead to physical insights that assist in the interpretation of existing experimental data and provide experimentally testable predictions of trends in the KIEs.

An outline of this chapter is as follows. In “Theory,” we present a series of rate constant expressions and discuss the approximations underlying each expression. In “Model calculations and discussion,” we analyze these rate constant expressions by applying them to model PCET systems. This analysis illustrates the regimes of validity for each rate constant expression and elucidates the dependence of the KIE on the physical properties of the system, including temperature, reorganization energy, driving force, equilibrium proton donor-acceptor distance, and effective frequency of the proton donor-acceptor mode. “Conclusions” summarizes the new physical insights that are directly relevant to experimental data and the experimentally testable predictions provided by these calculations.
Theory

Rate constant expressions

In this chapter, we use a series of vibronically nonadiabatic rate constant expressions that have been derived previously. In this formulation, the PCET reaction is described in terms of nonadiabatic transitions between pairs of reactant and product mixed electron-proton vibronic states. The rate constant expressions are based on Fermi’s golden rule formalism in conjunction with linear response theory for the solvent environment. For simplicity, we use the term solvent to denote both solvent and protein. The detailed derivations of these rate constant expressions are presented elsewhere. Here we simply present the final expressions and discuss the approximations underlying each expression.

In the derivations of the rate constant expressions, the nonadiabatic coupling between the reactant and product vibronic states is approximated to be of the form:

$$V_{\mu \nu}(R) = V_{\mu \nu}^{(0)} \exp \left[ -\alpha_{\mu \nu} (R - \bar{R}_{\mu}) \right]$$

(2-1)

where $\bar{R}_{\mu}$ is the equilibrium value of $R$ for the reactant state $\mu$, $V_{\mu \nu}^{(0)}$ is the vibronic coupling between reactant state $\mu$ and product state $\nu$ at distance $R = \bar{R}_{\mu}$, and $\alpha_{\mu \nu}$ is the exponential decay parameter. This form of the coupling is a reasonable approximation in the region of $R$ near its equilibrium value. In the electronically nonadiabatic limit for proton transfer, the vibronic coupling is of the form $V_{\mu \nu}(R) = V_{\mu \nu}^{el} S_{\mu \nu}(R)$, where $V_{\mu \nu}^{el}$ is the electronic coupling and $S_{\mu \nu}(R)$ is the overlap between the reactant and product proton vibrational wavefunctions for states $\mu$ and $\nu$ at a distance $R$. In this limit, $V_{\mu \nu}^{(0)} = V_{\mu \nu}^{el} S_{\mu \nu}^{(0)}$ in Eq. (2-1), where $S_{\mu \nu}^{(0)}$ is the overlap at the
distance $\overline{R}_\mu$ and the parameter $\alpha_{\mu\nu}$ describes the approximately exponential decay of the overlap with $R$ near $\overline{R}_\mu$.

Using the short-time, high-temperature approximation for the solvent modes and representing the $R$-mode time correlation function by that of a quantum mechanical harmonic oscillator, the rate constant can be expressed as:

$$k_{\text{quant}} = \sum_\mu \sum_\nu P_\mu \sum_\nu \left| \frac{V_{\mu\nu}(0)}{h^2 \Omega} \right|^2 \exp \left[ -\frac{2 \lambda_{\mu\nu}(a)}{h \Omega} \right] \int d\tau \exp \left[ -\frac{1}{2} \chi \tau^2 + p (\cos \tau - 1) + i (q \sin \tau + \theta \tau) \right]$$

(2-2)

with the dimensionless parameters defined as:

$$\zeta = \coth \left( \frac{1}{2} \beta \hbar \Omega \right) ; \chi = \frac{2 \lambda}{\beta \hbar^2 \Omega} ; \theta = \frac{\Delta G_{\mu\nu}^0 + \lambda}{h \Omega} ; p = \frac{\lambda_{\mu\nu}^{(a)}}{h \Omega} ; q = \frac{\lambda_{\mu\nu}^{(a)}}{h \Omega}$$

(2-3)

Here the summations are over reactant and product vibronic states, $\beta = 1/k_B T$, $P_\mu$ is the Boltzmann probability for the reactant state $\mu$, $\lambda$ is the solvent reorganization energy, $\Delta G_{\mu\nu}^0$ is the free energy of reaction for states $\mu$ and $\nu$, $\lambda_{\mu\nu}^{(a)}$ is the coupling reorganization energy defined as $\lambda_{\mu\nu}^{(a)} = \hbar^2 \alpha_{\mu\nu}^2 / 2 M$, and $M$ and $\Omega$ are the $R$-mode effective mass and frequency, respectively. The free energy of reaction is often expressed as $\Delta G_{\mu\nu}^0 = \Delta G^0 + \Delta \epsilon_{\mu\nu}$, where $\Delta G^0 \equiv \Delta G_{\mu0}^0$ and $\Delta \epsilon_{\mu\nu}$ is the difference between the product and reactant vibronic energy levels $\nu$ and $\mu$ relative to their respective ground states. The short-time, high-temperature approximation for the solvent is valid when the solvent reorganization energy $\lambda$ is large enough to ensure that the dynamics of the solvent fluctuations are fast on the time scale of the coherent nonadiabatic transitions. These expressions also assume that the solvent reorganization energy is the same for all pairs of reactant/product vibronic states and that the equilibrium proton donor-acceptor distance is the same for all reactant and product states. Furthermore, the inner-sphere reorganization energy for
the intramolecular solute modes can be added to the solvent reorganization energy in the high-temperature (low-frequency) limit for these modes.\textsuperscript{25,39-41} These approximations are valid for the majority of homogeneous PCET systems of interest, and Eq. (2-2) will be used as a benchmark for the more approximate methods discussed below.

This rate constant expression can be simplified in certain limiting regimes pertaining to the $R$-mode frequency. In the high-temperature (low-frequency) limit for the $R$-mode ($\hbar \Omega < k_B T$), the rate constant has the form:\textsuperscript{27,42}

$$k_{\text{high}} = \sum_{\mu} P_{\mu} \sum_{\nu} \frac{|V_{\mu \nu}^{(0)}|^2}{\hbar} \exp \left[ \frac{2k_B T \alpha_{\mu \nu}^0}{\hbar} \right] \frac{\pi}{\sqrt{\lambda + \lambda_{\mu \nu}^{(0)}}} \exp \left[ -\frac{(\Delta G_{\mu \nu}^0 + \lambda + \lambda_{\mu \nu}^{(0)})^2}{4(\lambda + \lambda_{\mu \nu}^{(0)}) k_B T} \right]$$  \hspace{1cm} (2-4)

This high-temperature rate constant expression is derived from Eq. (2-2) by performing a short-time expansion of the trigonometric functions up to second order and evaluating the time integral analytically.

In the low-temperature (high-frequency) limit for the $R$-mode ($\hbar \Omega > k_B T$), the rate constant has the form:\textsuperscript{27}

$$k_{\text{low}} = \sum_{\mu} P_{\mu} \sum_{\nu} \frac{|V_{\mu \nu}^{(0)}|^2}{\hbar} \sqrt{\lambda_{\mu \nu}} \exp \left[ \frac{\hbar \alpha_{\mu \nu}^2}{2 M \Omega} \right] \exp \left[ -\frac{(\Delta G_{\mu \nu}^0 + \lambda)^2}{4 \lambda k_B T} \right]$$  \hspace{1cm} (2-5)

This low-temperature rate constant expression is derived from Eq. (2-2) using the stationary phase method and is valid only in the strong solvation regime (i.e., $\lambda > |\Delta G_{\mu \nu}^0|$ for all relevant pairs of states). An equivalent expression can be obtained by averaging the squared vibronic coupling over the ground state vibrational wavefunction of the $R$-mode earlier in the derivation.

An alternative rate constant expression attributed to Kutznetsov and Ulstrup\textsuperscript{33} has been implemented by Klinman and others to study PCET reactions in enzymes.\textsuperscript{19,20} This expression is based on the Marcus theory rate constant for nonadiabatic electron transfer modified by the
inclusion of $R$-dependent Franck-Condon overlap terms for the transferring hydrogen, thermally averaged over a Boltzmann distribution for $R$. This expression is of the form:

$$k_{UK} = \sum_{\mu} P_{\mu} \sum_{\nu} |V^{d\nu}_{\mu}|^2 \sqrt{\frac{\pi}{\lambda k_n T}} \exp \left[ -\frac{(\Delta G^0_{\mu\nu} + \lambda)^2}{4\lambda k_n T} \right] \int P(R) [S_{\mu\nu}(R)]^2 dR \quad (2-6)$$

where $P(R) = \sqrt{\frac{M\Omega^2}{2\pi k_n T}} \exp \left[ -\frac{M\Omega^2}{2k_n T} \right]$ is the Boltzmann probability for a classical harmonic oscillator representation of the $R$-mode. Note that the normalization constant for the Boltzmann probability assumes integration over all values of $R$; this assumption is reasonable because $P(R)$ is negligible for negative values of $R$. The Marcus theory rate constant for nonadiabatic electron transfer with the Franck-Condon overlap terms is based on the treatment of an intramolecular solute mode quantum mechanically in the low-temperature (high-frequency) limit, and this solute mode is assumed to be uncoupled from the solvent.\textsuperscript{39-41} Thus, Eq. (2-6) is based on the reasonable assumption that the proton vibrational frequency is in the high-frequency regime relative to the thermal energy, as well as the additional assumption that the proton motion is not coupled to the solvent. Furthermore, the thermal averaging procedure with the classical Boltzmann probability used in Eq. (2-6) is valid only in the high-temperature (low-frequency) limit for the $R$-mode.

The rate constants $k_{UK}$ and $k_{\text{high}T}$ become identical when the vibronic coupling is assumed to be the product of an electronic coupling and the proton vibrational wavefunction overlap (i.e., $V_{\mu\nu} = V^d S_{\mu\nu}$), the overlap is assumed to decrease exponentially with $R$ near its equilibrium value (i.e., $S_{\mu\nu} = S_{\mu\nu}^{(0)} \exp \left[ -\alpha_{\mu\nu} \left( R - \bar{R}_\mu \right) \right]$), and $\lambda^{(d)}_{\mu\nu} \ll \lambda$.\textsuperscript{43} Since $\lambda^{(d)}_{\mu\nu}$ is inversely proportional to the mass $M$ corresponding to the $R$-mode, the difference between $k_{UK}$ and $k_{\text{high}T}$ is smaller for larger
Note that both of these rate constant expressions are only strictly valid only in the low-frequency regime for the $R$-mode, where $\hbar \Omega \ll k_B T$.

**Kinetic isotope effects**

In the high-temperature (low-frequency) regime for the $R$-mode, the KIE has a simple form if the reorganization energy and driving force are independent of isotope, only the ground reactant and product vibronic states contribute to the rates, and $\lambda_{\mu \nu}^{(e)} \ll \lambda$. In this case, the KIE is of the form:

$$\text{KIE} = \frac{S_H}{S_D} \exp \left[ -\frac{2k_B T}{M \Omega^2} (\alpha_D^2 - \alpha_H^2) \right]$$  \hspace{1cm} (2-7)

where $S_H$ and $S_D$ represent the overlaps of the hydrogen and deuterium ground state wavefunctions, and $\alpha_H$ and $\alpha_D$ represent the exponential dependence of these overlaps on $R$.

The temperature dependence of the KIE can be analyzed from the derivative of the $\ln[$KIE$]$ with respect to temperature:

$$\frac{d}{dT} \ln[\text{KIE}] = -\frac{2k_B}{M \Omega^2} (\alpha_D^2 - \alpha_H^2)$$  \hspace{1cm} (2-8)

The simple expressions in Eqs. (2-7) and (2-8) enable the analysis of qualitative trends pertaining to the magnitude and temperature dependence of the KIE, but we emphasize that they are valid only when excited vibronic states do not contribute to the rates and in the low-frequency regime for the $R$-mode.

For all of the rate constant expressions given above, the KIE depends strongly on the equilibrium $R$ value and the frequency $\Omega$ of the $R$-mode. Equations (2-7) and (2-8) can be used to examine qualitative trends when only the ground reactant and product vibronic states contribute.
to the rates in the low-frequency regime for the \( R \)-mode. In this case, Eq. (2-7) indicates that the KIE is proportional to the ratio of the overlaps for hydrogen and deuterium. These overlaps are depicted in Figure 2-1a for Morse potentials representing the reactant and product proton potential energy curves. This figure illustrates that the hydrogen overlap is greater than the deuterium overlap for the same value of \( R \). In general, the overlap between the reactant and product vibrational wavefunctions decreases as \( R \) increases for both hydrogen and deuterium. However, the overlap decreases much faster with \( R \) for deuterium than for hydrogen due to the larger mass of deuterium, so this ratio becomes larger as \( R \) increases. Figure 2-1b illustrates these effects for the ground reactant and product vibrational wavefunctions in Figure 2-1a. Thus, if all other quantities remain the same, the KIE increases as the equilibrium \( R \) value increases. Moreover, since the overlap decreases faster with \( R \) for deuterium than for hydrogen, \( \alpha_d > \alpha_H \), and the overall quantity in the exponential of Eq. (2-7) is negative. Therefore, if all other quantities remain the same, the magnitude of the KIE will increase as the frequency \( \Omega \) increases. The physical basis for this trend is that a higher frequency typically does not enable effective sampling of smaller distances.

**Figure 2-1:** (a) Proton potential energy curves and the associated hydrogen (solid) and deuterium (dashed) vibrational wavefunctions for the ground reactant (blue) and product (red) states. The proton potential energy curves are Morse potentials with the parameters given in the text. The proton donor-acceptor distance is 2.7 Å. (b) Square of the ratio of the hydrogen and deuterium overlaps for the ground reactant and product vibrational states 0/0 (black) and the ground reactant and first excited product states 0/1 (red) as functions of the proton donor-acceptor distance \( R \). Note that \( \left( \frac{S_H}{S_D} \right)^2 \) increases dramatically as \( R \) increases and is significantly smaller for the 0/1 pair of states.
The inclusion of excited vibronic states will influence these general trends. Figure 2-1b also depicts the ratio of overlaps for the ground reactant and first excited product vibrational states. For excited vibrational states, the overlap is greater, and therefore the ratio of hydrogen to deuterium wavefunction overlaps is smaller, than for the ground reactant and product vibrational states. In addition, the excited states often contribute more for deuterium than for hydrogen because the splittings between the energy levels are smaller for deuterium. Thus, contributions from excited vibronic states tend to decrease the magnitude of the KIE.

To analyze the temperature dependence of the KIE, we focus on the ln[KIE] because this quantity is directly related to the difference in the apparent activation energies for deuterium and hydrogen transfer. Specifically, the derivative of ln[KIE] with respect to the inverse temperature is proportional to $E_D - E_H$, where $E_D$ and $E_H$ are the apparent activation energies for deuterium and hydrogen transfer, respectively (i.e., $E_H$ is proportional to the derivative of ln[$k_H$] with respect to inverse temperature). Eq. (2-8) indicates that the ln[KIE] will decrease with temperature in the high-temperature (low-frequency) limit for the $R$-mode with only the ground vibronic states contributing. Moreover, the temperature dependence of ln[KIE] will increase as the frequency $\Omega$ decreases in this regime. A more detailed analysis of these trends will be presented below by calculations on model systems.

We will also analyze the dependence of the rate constant and KIE on the driving force $\Delta G^0$. For this purpose, we derive an approximate expression for the KIE in the vicinity of $\Delta G^0 = 0$ in the high-temperature (low-frequency) limit for the $R$-mode, assuming again that the reorganization energy and driving force are independent of isotope and that only the ground reactant and product vibronic states contribute to the rates. Performing a Taylor series of $\ln[k_{H}^{\text{highT}}]$ and $\ln[k_{D}^{\text{highT}}]$ about $\Delta G^0 = 0$ and retaining terms up to second order in $\Delta G^0$ leads to:
where $k^0_L$ with $L = H$ or D is the high-temperature rate constant for hydrogen or deuterium, respectively, at $\Delta G^0 = 0$ and $\lambda^L_w$ with $L = H$ or D denotes $\lambda^{(c)}_{w0}$ for hydrogen or deuterium, respectively. Eq. (2-9) leads to the following approximate forms of $\ln[KIE]$ and KIE:

$$\ln\left( \frac{k^{\text{high}T}_L}{k^0_L} \right) = -\frac{\Delta G^0}{2k_BT} - \frac{\left( \Delta G^0 \right)^2}{4(\lambda + \lambda^L_w)k_BT}$$

where $k^0_L$ is the KIE evaluated at $\Delta G^0 = 0$:

$$KIE^0 = \frac{S_{\text{HH}}}{S_{\text{DD}}} \exp \left[ -\frac{2k_BT}{M\Omega^2} \left( \alpha^D_{\text{HH}} - \alpha^H_{\text{DD}} \right) \right] \frac{\lambda + \lambda^D_w}{\lambda + \lambda^H_w} \left[ \frac{\lambda^D_w - \lambda^H_w}{4k_BT} \right]$$

Eq. (2-11) differs from Eq. (2-7) because the derivation of Eq. (2-11) is not based on the assumption that $\lambda^{(c)}_{w0} = 0$, which was assumed in the derivation of Eq. (2-7). As will be shown below, the contributions from excited vibronic states can lead to qualitative deviations from the quadratic behavior in Eqs. (2-9) and (2-10), particularly for the rate constant. A similar analysis was performed previously for vibrationally nonadiabatic proton transfer reactions.\textsuperscript{45,46} Note that this driving force dependence of the KIE will not be evident using the $k^{\text{UK}}$ rate constant expression given in Eq. (2-6).

The preceding analyses of the dependence of the KIE on temperature and driving force are based on the rate constant expression in the high-temperature (low-frequency) regime for the $R$-mode. In the low-temperature (high-frequency) regime for the $R$-mode, the KIE obtained from Eq. (2-5) including contributions from only the ground reactant and product vibronic states is:
As in the high-temperature regime, the magnitude of the KIE will increase as the equilibrium $R$ value and the frequency $\Omega$ increase. In this low-temperature regime, however, the KIE is independent of both temperature and driving force when only ground states contribute. As shown below, contributions from excited vibronic states can lead to temperature dependence of the KIE in the low-temperature regime for the $R$-mode.

### Model calculations and discussion

#### Model system

In this section, we analyze the rate constant expressions given above by calculating the rates and KIEs for model PCET systems. For simplicity, the reactant and product proton potential energy curves are described by Morse potentials of the form:

$$ E_{XY} = D_{XY} \left(1 - e^{-\beta_{XY} \left(R_{XY} - R_{XY}^0\right)}\right)^2 $$  \hspace{1cm} (2-14)

In all model systems studied in the present chapter, the proton is assumed to transfer from a carbon atom to an oxygen atom. Thus, the Morse potential for the reactant corresponds to a C–H vibrational mode, and the Morse potential for the product corresponds to an O–H vibrational mode. We chose parameters that are typical for these types of vibrational modes.\footnote{The values for the dissociation energies $D_{CH}$ and $D_{OH}$ were chosen to be 77 and 82 kcal/mol, and the values of $R_{CH}^0$ and $R_{OH}^0$ were chosen to be 1.09 and 0.96 Å. The values for parameters $\beta_{CH}$ and $\beta_{OH}$ were chosen to be 2.068 and 2.442 Å$^{-1}$ to reproduce the typical C–H and O–H frequencies of 2885 and 3609 cm$^{-1}$, respectively.}
2900 and 3500 cm$^{-1}$. These Morse parameters may be varied to describe other vibrational modes, but the qualitative trends discussed below will not be significantly altered. The other parameters in the rate constant expressions are varied in these model systems. Unless otherwise stated, $M = 100$ amu, $\lambda = 30$ kcal/mol, $\Delta G^0 = -5$ kcal/mol, $T = 303$ K, $\Omega = 150$ cm$^{-1}$, and $\tilde{R} = 2.7$ Å.

The hydrogen and deuterium vibrational wavefunctions are calculated by solving a one-dimensional Schrödinger equation for the hydrogen or deuterium moving in the reactant and product Morse potentials. Analytical solutions are available for the eigenfunctions of Morse potentials. The Morse potentials and corresponding hydrogen and deuterium wavefunctions are depicted in Figure 2-1a. In this model, the Morse potentials are positioned so that the minima are separated by a distance of $\tilde{R} - R^o_{\text{CH}} - R^o_{\text{OH}}$. The overlaps $S^{(0)}_{\mu \nu}$ between the reactant and product vibrational wavefunctions at $\tilde{R}$ are calculated numerically. The parameters $\alpha_{\mu \nu}$ are determined for each pair of vibronic states by calculating the numerical derivatives of the natural logarithm of the corresponding overlap integrals with respect to $R$ at $\tilde{R}$. For $\tilde{R} = 2.7$ Å, $\alpha_{00} = 17.80$ Å$^{-1}$ and 24.79 Å$^{-1}$ for hydrogen and deuterium, respectively. These values vary slightly with $\tilde{R}$: at $\tilde{R} = 2.8$ Å, $\alpha_{00} = 19.77$ Å$^{-1}$ and 27.47 Å$^{-1}$ for hydrogen and deuterium, respectively. These values also vary slightly for different pairs of states: at $\tilde{R} = 2.7$ Å, $\alpha_{01} = 15.55$ Å$^{-1}$ and 22.58 Å$^{-1}$ for hydrogen and deuterium, respectively.

**Comparison of rate constant expressions**

As discussed above, the rate constant $k^{\text{quant}}_{\text{highT}}$ in Eq. (2-2) is valid for all frequency regimes of the $R$-mode, whereas $k^{\text{highT}}$ in Eq. (2-4) is valid only in the high-temperature (low-frequency) limit for the $R$-mode, and $k^{\text{lowT}}$ in Eq. (2-5) is valid only in the low-temperature (high-frequency)
limit for the $R$-mode. Figure 2-2a depicts the dependence of the KIE on the frequency $\Omega$ of the $R$-mode for all three of these rate constant expressions. This figure illustrates that $k_{\text{highT}}$ agrees well with $k_{\text{quant}}$ for low $R$-mode frequencies, and $k_{\text{lowT}}$ agrees well with $k_{\text{quant}}$ for high $R$-mode frequencies. As discussed above, the simple KIE in Eq. (2-7) predicts the increase of the KIE as the frequency increases for the high-temperature limit of the $R$-mode. From a physical perspective, the KIE increases as the frequency increases because a higher frequency limits the ability of the system to sample smaller $R$ values, and larger $R$ values are associated with greater KIEs.

Figure 2-2b depicts the dependence of the KIE on the temperature for two different $R$-mode frequencies, $\Omega = 150 \text{ cm}^{-1}$ and $\Omega = 850 \text{ cm}^{-1}$, with equilibrium $R$ values of $\bar{R} = 2.7$ and 2.6 Å, respectively. For the lower $R$-mode frequency, the temperature dependence of the KIE is similar using $k_{\text{quant}}$ and $k_{\text{highT}}$. For the higher $R$-mode frequency, the temperature dependence of the KIE is similar using $k_{\text{quant}}$ and $k_{\text{lowT}}$. A comparison of Figures 2-2a and 2-2b illustrates that decreasing $\bar{R}$ from 2.7 to 2.6 Å for $\Omega = 850 \text{ cm}^{-1}$ significantly decreases the KIE, mainly due to the decrease in the ratio of the hydrogen and deuterium overlaps. Typically a higher-frequency
R-mode is associated with a stronger hydrogen bond, which would have a smaller equilibrium $R$ value and therefore a lower KIE.

Figure 2-3 compares the KIE calculated using the rate constant $k^{UK}$ to the KIE calculated using the rate constants $k^{quant}$, $k^{highT}$, and $k^{lowT}$. As discussed above, $k^{UK}$ and $k^{highT}$ become identical when $\lambda_{\mu\nu}^{(a)} = 0$ and the overlap decays exponentially with $R$ near its equilibrium value.

Figure 2-3a depicts the frequency dependence of the KIE for all of these rate constant expressions with $M = 100$ amu. The KIEs obtained from $k^{quant}$ and $k^{highT}$ converge to the same value at low frequencies, but the KIE obtained from $k^{UK}$ approaches a different value at low frequencies. This discrepancy arises because $k^{quant}$ and $k^{highT}$ are based on the assumption that the vibronic coupling decreases exponentially with $R$, which is valid only near the equilibrium $R$ value, and a smaller frequency enables sampling of a wider range of $R$ values. Although not shown in this figure, the KIEs obtained from $k^{UK}$ and $k^{highT}$ with $\lambda_{\mu\nu}^{(a)} = 0$ are virtually identical at high frequencies, where $R$ is nearly fixed to its equilibrium value. Furthermore, the KIEs obtained from $k^{highT}$ with $\lambda_{\mu\nu}^{(a)} = 0$ agree with those calculated using $k^{quant}$ in the high-frequency $R$-mode limit because the expression for $k^{highT}$ given in Eq. (2-4) becomes similar to the expression for $k^{lowT}$ given by Eq. (2-5) in this limit. Figure 2-3b depicts the same curves as Figure 2-3a with $M = 20$ amu. Similar
Dependence of KIE on system properties

In this subsection, we examine the dependence of the KIE on the physical properties of the system. First we investigate the impact of the reorganization energy $\lambda$ on the magnitude and temperature dependence of the KIE. These results were calculated using the rate constant expression given in Eq. (2.2). Figure 2-4 indicates that the KIEs are virtually identical for reorganization energies in the range of 10 to 40 kcal/mol. The magnitude of the KIE does not change monotonically as $\lambda$ is increased because of changes in the relative contributions from excited states.

Excited vibronic states often contribute significantly to the overall rate of PCET reactions. Given the reorganization energy and the driving force, we can generate the standard parabolic Marcus free energy curves as functions of a collective solvent coordinate. Figure 2-5 depicts these free energy curves, as well as the reactant and product proton potential energy.
curves and associated proton vibrational wavefunctions, for the lowest three reactant and product vibronic states. Note that the energy splittings between the ground and excited vibronic states are determined by the energy splittings of the proton vibrational states in the Morse potentials for the reactant and product states. For reasons discussed above, the contributions of excited vibronic states tend to decrease the KIE because the overlaps between excited vibrational wavefunctions are larger than the overlap between the ground reactant and product proton vibrational wavefunctions.

The qualitative analysis of the contributions from excited vibronic states is simplified by using the high-temperature rate constant expression given in Eq. (2-4). The relative contribution of each pair of reactant/product vibronic states is determined by a balance among the Boltzmann probability $P_\mu$ for the reactant state, the vibronic coupling $V^{(0)}_{\mu\nu} = V^{el}S^{(0)}_{\mu\nu}$, and the free energy barrier $\Delta G^*_{\mu\nu} = \left(\Delta G^0_{\mu\nu} + \lambda + \lambda^{(0)}_{\mu\nu}\right)^2 / 4\left(\lambda + \lambda^{(0)}_{\mu\nu}\right)$. The reorganization energy $\lambda$, driving force $\Delta G^0$, and free energy barrier $\Delta G^*_{\mu\nu}$ for the ground reactant and product vibronic states are depicted in Figure 2-5. The free energy barrier contributes a factor of $\exp\left[-\Delta G^*_{\mu\nu}/k_BT\right]$ to the rate constant.
for each pair of reactant/product vibronic states. The Boltzmann probability strongly favors the ground reactant state. The vibronic coupling favors excited vibronic states because the overlaps are greater for excited state proton vibrational wavefunctions. In the normal Marcus regime, the free energy barrier favors the ground product state when the reaction initiates from the ground reactant state. The relative contributions of the pairs of reactant/product vibronic states are determined from a complex interplay among these various factors.

We examined the contributions from the excited vibronic states for three model systems with $\Delta G^0 = -5, 0, \text{ and } +5 \text{ kcal/mol}$. The detailed analysis of the various terms for both hydrogen and deuterium is provided in Table 2-1. For all three model systems, the ground vibronic states dominate for hydrogen, and excited vibronic states contribute much more for deuterium. This difference can be understood in the context of Figure 2-6, which depicts the free energy curves for hydrogen and deuterium. In this figure, the ground vibronic states are chosen to be at the same energies for hydrogen and deuterium, but the first excited vibronic states are lower for

Table 2-1: Analysis of the contributions of pairs of reactant/product vibronic states $\mu/\nu$ for a model system with $\lambda = 30 \text{ kcal/mol}$, $\bar{R} = 2.7 \text{ Å}$, $M = 100 \text{ amu}$, $\Omega = 150 \text{ cm}^{-1}$, and $T = 303 \text{ K}$. Free energies are given in units of kcal/mol.
Figure 2-6: Free energy curves as functions of a collective solvent coordinate for hydrogen (solid) and deuterium (dashed) for a model system with $\lambda = 30$ kcal/mol and $\Delta G^0 = 5$ kcal/mol. The lowest two reactant and the lowest two product states are shown for both hydrogen and deuterium. Since the ground reactant state is chosen to have the same absolute energy for hydrogen and deuterium, the ground reactant and product states for deuterium exactly overlay those for hydrogen and therefore are not distinguishable.

deuterium than for hydrogen. As a result, the free energy barrier for the (0/1) pair of vibronic states (i.e., the pair corresponding to the ground reactant and first excited product state) is lower for deuterium than for hydrogen, leading to a greater contribution from this pair of states for deuterium. In addition, the Boltzmann probability for the first excited reactant state is significantly greater for deuterium than for hydrogen, leading to a greater contribution from the (1/0) pair of vibronic states for deuterium. The contributions from excited vibronic states are greater for the model system with $\Delta G^0 = 5$ kcal/mol, leading to a lower KIE.

We emphasize that the excited reactant states may contribute significantly to the overall PCET rate. For these model systems, the excited reactant state contributes significantly for deuterium. Although the Boltzmann probability is small for this excited state, the free energy barrier is significantly lower for the (1/0) pair of reactant/product vibronic states than for the (0/0) pair of reactant/product vibronic states. This difference in free energy barriers for the (0/0) and (1/0) pairs of reactant/product vibronic states is evident in Figure 2-6. In addition, the vibronic coupling is larger for the excited states. For the model system with $\Delta G^0 = 5$ kcal/mol, the first excited reactant state contributes 28% to the overall rate for deuterium. Thus, in practice, all reactant and product vibronic states with significant contributions to the overall rate should be included when calculating PCET rates.
As discussed above, the magnitude and temperature dependence of the KIE are primarily dictated by the equilibrium proton donor-acceptor distance $\bar{R}$ and the $R$-mode vibrational frequency $\Omega$. This dependence can be analyzed in the context of Eqs. (2-7) and (2-8) corresponding to the KIE and the derivative of the $\ln[KIE]$ with respect to the temperature. The curves in Figure 2-7 are calculated using the full form of the rate constant given in Eq. (2-2), and the approximate forms in Eqs. (2-7) and (2-8) are used only for the purposes of qualitative analysis. Figure 2-7a depicts the temperature dependence of the $\ln[KIE]$ for $\bar{R} = 2.7$ Å and $\Omega = 140$ cm$^{-1}$ (black), $\bar{R} = 2.7$ Å and $\Omega = 180$ cm$^{-1}$ (red), and $\bar{R} = 2.8$ Å and $\Omega = 140$ cm$^{-1}$ (blue). (b) $\bar{R} = 2.7$ Å and $\Omega = 150$ cm$^{-1}$ (black), $\bar{R} = 2.7$ Å and $\Omega = 300$ cm$^{-1}$ (red). The curves are labeled according to the $\bar{R}/\Omega$ values in units of Å and cm$^{-1}$.

As discussed above, the magnitude and temperature dependence of the KIE are primarily dictated by the equilibrium proton donor-acceptor distance $\bar{R}$ and the $R$-mode vibrational frequency $\Omega$. This dependence can be analyzed in the context of Eqs. (2-7) and (2-8) corresponding to the KIE and the derivative of the $\ln[KIE]$ with respect to the temperature. The curves in Figure 2-7 are calculated using the full form of the rate constant given in Eq. (2-2), and the approximate forms in Eqs. (2-7) and (2-8) are used only for the purposes of qualitative analysis. Figure 2-7a depicts the temperature dependence of the $\ln[KIE]$ for $\bar{R} = 2.7$ and 2.8 Å and $\Omega = 140$ and 180 cm$^{-1}$. For a fixed frequency of $\Omega = 140$ cm$^{-1}$, the magnitude of the KIE increases as $\bar{R}$ increases from 2.7 to 2.8 Å because the ratio of the hydrogen and deuterium overlaps in Eq. (2-7) increases. For a fixed $\bar{R}$ value of 2.7 Å, the magnitude of the KIE increases as $\Omega$ increases, as predicted by Eq. (2-7). This trend is physically reasonable because the higher frequency restricts the value of $R$ to larger values corresponding to a greater ratio of the hydrogen and deuterium overlaps. In addition, the temperature dependence of the $\ln[KIE]$ decreases as $\Omega$ increases for fixed $\bar{R}$, as predicted by Eq. (2-8). This trend is more evident in Figure 2-7b,
which depicts the temperature dependence of the ln[KIE] for $\bar{R} = 2.7$ and $\Omega = 150$ and 300 cm$^{-1}$, because the difference in frequencies is greater than Figure 2-7a.

Furthermore, the equilibrium $R$ value and the proton donor-acceptor frequency cannot usually be varied independently when designing experimental systems. Typically the frequency will increase as the equilibrium $R$ value decreases because the hydrogen bond is stronger for shorter distances. When the frequency varies even slightly, the KIE could be observed to either increase or decrease as $\bar{R}$ increases. For example, Figure 2-7a depicts a comparison of two systems in which $\bar{R} = 2.7$ Å and $\Omega = 180$ cm$^{-1}$ for the first system and $\bar{R} = 2.8$ Å and $\Omega = 140$ cm$^{-1}$ for the second system. As shown in this figure, the first system has a higher KIE for higher temperatures, while the second system has a higher KIE for lower temperatures. This range of temperatures is close enough to room temperature to be experimentally accessible for many systems. From the experimental perspective, the KIE could be observed to increase or decrease with $\bar{R}$, depending on the temperature, change in frequency, and other characteristics of the system.

We emphasize that the general trends based on the analysis of Eqs. (2-7) and (2-8) will not always be followed. As discussed above, excited vibronic states often contribute significantly to the overall PCET rate, but Eqs. (2-7) and (2-8) are based on the assumption that only the ground vibronic states are contributing. Since the contributions from excited states depend on a complex interplay among several factors, including the Boltzmann probability, the vibronic coupling, and the free energy barrier, the relative contributions will vary with temperature and will differ for hydrogen and deuterium. In this case, the temperature dependence of the KIE is determined by summing over all pairs of states for hydrogen, summing over all pairs of states for deuterium, and subsequently taking the ratio of these two rate constants. In addition, Eqs. (2-7) and (2-8) are only valid in the high-temperature (low-frequency) regime for the $R$-mode. For
systems that are not in the high-temperature or low-temperature regime, we must use Eq. (2-2) to evaluate the rate constants for hydrogen and deuterium, and the prediction of general qualitative trends is more difficult.

In some cases, the KIE has been observed experimentally to increase with temperature.\textsuperscript{15} For the rate constant expressions in the high-temperature (low-frequency) limit for the $R$-mode (i.e., Eqs. (2-4) and (2-6)), the KIE tends to decrease with temperature because of the exponential prefactor that depends on temperature, as evident in Eq. (2-7). Even accounting for contributions from excited vibronic states does not seem to reverse this qualitative trend. On the other hand, for the more general rate constant expression given in Eq. (2-2), the KIE will increase with temperature for certain choices of parameters. Figure 2-8 depicts the temperature dependence of the KIE determined from Eq. (2-2) for a system with $\lambda = 3$ kcal/mol, $\Delta G^0 = -6.5$ kcal/mol, $\bar{R} = 2.7 \text{ Å}$, $\Omega = 600 \text{ cm}^{-1}$, and $M = 100$ amu.

Figure 2-8: Temperature dependence of the KIE calculated with $k^\text{quant}$ for a model system with $\lambda = 3$ kcal/mol, $\Delta G^0 = -6.5$ kcal/mol, $\bar{R} = 2.7 \text{ Å}$, $\Omega = 600 \text{ cm}^{-1}$, and $M = 100$ amu.
remaining contributions arising from other pairs of states. A detailed analysis of a specific system exhibiting this type of temperature dependence of the KIE will be discussed elsewhere.48

Finally, we analyze the dependence of the rate constant and KIE on the driving force $\Delta G^0$. Figure 2-9 depicts the dependence of the rate constant and the corresponding $\ln[KIE]$ on driving force for a model system with $\lambda = 20$ kcal/mol, $\overline{R} = 2.7 \, \text{Å}$, $\Omega = 150 \, \text{cm}^{-1}$, $M = 100 \, \text{amu}$, and $T = 303 \, \text{K}$. In (a) and (b), the red curve corresponds to the calculation including only the ground reactant and product vibronic states, and the black curve corresponds to the calculation that is converged with respect to excited vibronic states. In (c), the color code for the pairs of reactant/product vibronic states is as follows: 0/0 (black), 1/0 (blue), 2/0 (magenta), 0/1 (red), and 0/2 (green).

**Figure 2-9:** Driving force dependence of (a) the rate constant $k_{Hi}^{\text{highT}}$, (b) the associated KIE, and (c) the contributions of pairs of reactant/product vibronic states for a model system with $\lambda = 20$ kcal/mol, $\overline{R} = 2.7 \, \text{Å}$, $\Omega = 150 \, \text{cm}^{-1}$, $M = 100 \, \text{amu}$, and $T = 303 \, \text{K}$. In (a) and (b), the red curve corresponds to the calculation including only the ground reactant and product vibronic states, and the black curve corresponds to the calculation that is converged with respect to excited vibronic states. In (c), the color code for the pairs of reactant/product vibronic states is as follows: 0/0 (black), 1/0 (blue), 2/0 (magenta), 0/1 (red), and 0/2 (green).
kcal/mol in this model system, the curvature is so small for ln[KIE] that the quadratic dependence of ln[KIE] on driving force is barely visible when only the ground states are included.

When excited vibronic states are included, however, the qualitative behavior of both the rate constant and ln[KIE] is altered. The rate constant does not decrease as $\Delta G^0$ becomes more negative because excited vibronic product states associated with low free energy barriers and relatively large vibronic couplings become accessible. For this reason, Figure 2-9a exhibits an increase in the rate constant as the driving force becomes more negative. The KIE also exhibits qualitatively different behavior when excited vibronic states are included. Typically the contributions from excited vibronic states increase as $\Delta G^0$ becomes more positive or more negative (i.e., as the reaction becomes more asymmetric). Figure 2-9c illustrates that the $(0/0)$ pair dominates at $\Delta G^0 = 0$, the $(1/0)$ pair dominates as the reaction becomes more endergonic, and the $(2/0)$ pair dominates as the reaction becomes even more endergonic. Similarly, the $(0/1)$ pair dominates as the reaction becomes more exergonic, and the $(0/2)$ pair dominates as the reaction becomes even more exergonic. As discussed above and illustrated in Figure 2-1b, the KIE tends to decrease as the contributions of excited vibronic states increase because the excited vibronic states are associated with larger overlaps and therefore a smaller ratio of hydrogen to deuterium overlaps. As a result, the ln[KIE] decreases more rapidly for both positive and negative driving forces when excited vibronic states are included. Figure 2-9b illustrates this phenomenon for the model system studied. Note that the maximum of ln[KIE] is not at exactly $\Delta G^0 = 0$ because the proton transfer interface is asymmetric (i.e., different Morse potentials are used for reactant and product states). For this model, the ground state contributions are greatest for slightly negative driving forces due to the complex interplay of various factors in the rate constant expression. Qualitatively similar behavior is observed in the high-frequency regime for the $R$-mode using $k_{\text{low}T}$. In this regime, the KIE is rigorously independent of driving force when
only the ground reactant and product vibronic states are included, as indicated by Eq. (2-13), but the KIE depends on driving force in a similar manner as shown in Figure 2-9b when excited vibronic states are included.

Similar behavior of the KIE was observed previously for vibrationally nonadiabatic proton transfer (PT) reactions.\textsuperscript{45,46} An important difference between vibrationally nonadiabatic PT and the PCET reactions discussed here is that typically vibrationally nonadiabatic PT is electronically adiabatic (i.e., the reaction occurs on the electronic ground state). In contrast, the PCET reactions discussed here are electronically nonadiabatic and occur on two different electronic surfaces corresponding to the two electron transfer states. As a result, the nature of the nonadiabatic coupling is different for these two types of reactions.\textsuperscript{38} In addition, the solvent reorganization is typically much smaller for PT than for PCET because the proton is transferred a shorter distance than the electron. This difference in solvent reorganization energies leads to qualitative differences in the dependence of the KIE on driving force, particularly when only the ground states contribute to the rates.

**Conclusions**

In this chapter, we have compared the KIEs obtained with different rate constant expressions for PCET reactions. The KIEs obtained from rate constants $k_{\text{highT}}$ and $k_{\text{lowT}}$ rigorously approach those obtained from $k_{\text{quant}}$ in the low-frequency and high-frequency $R$-mode limits, respectively. The KIEs obtained from the rate constant $k_{\text{UK}}$ behave qualitatively similar to those obtained from $k_{\text{highT}}$ and $k_{\text{quant}}$ in the low-frequency $R$-mode regime. Analytical expressions for the dependence of the KIE on both temperature and driving force were derived including only the ground reactant and product vibronic states.
While these simple analytical expressions are useful for predicting general trends, our calculations have illustrated the importance of converging the rate constants with respect to the numbers of both reactant and product vibronic states. Excited vibronic state contributions are typically greater for deuterium than for hydrogen and decrease the magnitude of the KIE because of the smaller ratio of the hydrogen to deuterium overlaps for excited states. Although the excited reactant vibronic state has a smaller Boltzmann probability than the ground state, the free energy barrier and coupling terms may be large enough to result in a significant contribution from the excited reactant state. Moreover, the inclusion of a sufficient number of product vibronic states is particularly important when studying the driving force dependence of the KIE for very negative driving forces.

In addition, we analyzed the dependence of the magnitude of the KIE on the physical properties of the PCET system. Typically the magnitude of the KIE increases as the equilibrium proton donor-acceptor distance increases if all other parameters remain fixed. Moreover, typically the magnitude of the KIE increases as the proton donor-acceptor mode frequency increases if all other parameters remain fixed. In experimentally studied systems, often the proton donor-acceptor mode frequency increases as the equilibrium R value decreases, however, leading to the possibility of observing the magnitude of the KIE to either increase or decrease with the equilibrium R value. The magnitude of the KIE is relatively insensitive to changes in the solvent reorganization energy within a physically reasonable range.

Furthermore, we studied the dependence of the KIE on the temperature. In the low-frequency regime for the R-mode, the KIE decreases as the temperature is increased, and the temperature dependence of the ln[KIE] typically decreases as the proton donor-acceptor mode frequency increases. In the high-frequency regime for the R-mode, the KIE is independent of the temperature when only the ground reactant and product vibronic states contribute to the rates, but
contributions from excited vibronic states could lead to either an increase or a decrease of the KIE with temperature.

We also studied the dependence of the rate constant and the KIE on the driving force. In contrast to the inverted Marcus region behavior predicted and observed experimentally for electron transfer, our calculations indicate that the PCET nonadiabatic rate constant increases as the driving force becomes more negative because excited vibronic product states associated with low free energy barriers and relatively large vibronic couplings become accessible. Moreover, the ln[KIE] is a maximum near zero driving force and decreases significantly as the driving force becomes more positive or negative because the contributions from excited vibronic states increase as the reaction becomes more asymmetric, and contributions from excited vibronic states decrease the KIE.

These calculations provide physical insights that are directly relevant to experimental data. For example, recently the magnitude of the KIE was observed experimentally to decrease with increasing proton donor-acceptor distance in phenol-base complexes, where the proton donor-acceptor distance was increased by structural modification. Our calculations suggest a possible explanation for this observation in terms of a decrease in the proton donor-acceptor frequency as the proton donor-acceptor distance increases. These two effects impact the KIE in opposite directions, and the KIE may be observed to decrease with increasing proton donor-acceptor distance when the lower frequency dominates the observed trend. In another set of experiments, the KIE for the PCET reaction associated with oxidation of a ubiquinol analogue in acetonitrile was observed to increase as the temperature increases. Our calculations provide an explanation for this observation in terms of a rigid hydrogen bond, which corresponds to a high proton donor-acceptor frequency, in conjunction with the low dielectric constant of acetonitrile, which corresponds to small solvent reorganization energy. These two properties allow the possibility of this type of unusual temperature dependence. Finally, the dependence of the rate
constant and KIE on the driving force has been studied experimentally for a wide range of PCET systems.\textsuperscript{11,50-52} Our model calculations provide a theoretical framework for the interpretation of these types of experimental studies. Overall, these calculations on model systems assist in the interpretation of experimental data and provide predictions of trends in the KIE that can be tested experimentally.

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References

Chapter 3

Driving force dependence of rates for nonadiabatic proton and proton-coupled electron transfer: Conditions for inverted region behavior


The driving force dependence of the rate constants for nonadiabatic electron transfer (ET), proton transfer (PT), and proton-coupled electron transfer (PCET) reactions are examined. Inverted region behavior, where the rate constant decreases as the reaction becomes more exoergic (i.e., as $\Delta G^0$ becomes more negative), has been observed experimentally for ET and PT. This behavior was predicted theoretically for ET but is not well understood for PT and PCET. The objective of this chapter is to predict the experimental conditions that could lead to observation of inverted region behavior for PT and PCET. The driving force dependence of the rate constant is qualitatively different for PT and PCET than for ET because of the high proton vibrational frequency and substantial shift between the reactant and product proton vibrational wavefunctions. As a result, inverted region behavior is predicted to be experimentally inaccessible for PT and PCET if only the driving force is varied. This behavior may be observed for PT over a limited range of rates and driving forces if the solvent reorganization energy is low enough to cause observable oscillations. Moreover, this behavior may be observed for PT or PCET if the proton donor-acceptor distance increases as $\Delta G^0$ becomes more negative. Thus, a plausible explanation for experimentally observed inverted region behavior for PT or PCET is that varying the driving force also impacts other properties of the system, such as the proton donor-acceptor distance.
According to standard Marcus theory of nonadiabatic electron transfer (ET),\(^1\) the dependence of the logarithm of the ET rate constant on the driving force \(\Delta G^0\) is represented by an inverted parabola. The maximum rate corresponds to the activationless regime with \(-\Delta G^0 = \lambda\), and the inverted region is defined as \(-\Delta G^0 > \lambda\), where \(\lambda\) is the reorganization energy of the reaction. The existence of the inverted region, where the ET rate constant decreases as the reaction becomes more exoergic (i.e., as \(\Delta G^0\) becomes more negative), has been confirmed experimentally.\(^1,2\) The inverted region behavior has also been experimentally observed for proton transfer (PT) reactions,\(^3-5\) although the theoretical basis for these experimental observations has not been well understood.\(^6\) To our knowledge, the inverted region behavior has not yet been experimentally observed for proton-coupled electron transfer (PCET) reactions, in which an electron and proton transfer simultaneously with no stable intermediate.\(^7-9\) The objectives of this chapter are to elucidate the fundamental differences in the driving force dependence of the rate constants for ET, PT, and PCET and to identify the experimental conditions that could lead to observation of inverted region behavior for PT and PCET.

Our analysis is based on the following form of the rate constant for a vibronically nonadiabatic charge transfer reaction:\(^9-11\)

\[
\begin{align*}
k = \sum_{\mu} P_{\mu} \sum_{\nu} \frac{V_{\mu\nu}^2}{\hbar} \sqrt{\frac{\pi}{\lambda k_B T}} \exp \left[ -\frac{\left( \Delta G^0 + \lambda + \epsilon_{\nu} - \epsilon_{\mu} \right)^2}{4\lambda k_B T} \right]
\end{align*}
\]

where \(P_{\mu}\) is the Boltzmann probability for the reactant state \(\mu\) and \(V_{\mu\nu}\) is the nonadiabatic coupling between the reactant and product vibronic states \(\mu\) and \(\nu\) with vibrational energies \(\epsilon_{\mu}\) and \(\epsilon_{\nu}\) relative to their ground states. In the vibronically nonadiabatic limit, \(V_{\mu\nu} \ll k_B T\) and the solvent timescale is much faster than the timescale associated with the nonadiabatic transitions.
For PCET reactions, typically the proton transfer is electronically nonadiabatic (i.e., the proton tunneling timescale is much faster than the electronic transition timescale). In this case, the vibronic coupling is the product of the electronic coupling $V_{\mu\nu}^{el}$ and the overlap integral $S_{\mu\nu}$ between the reactant and product proton vibrational wavefunctions:\(^9,12,13\)

$$V_{\mu\nu} = V_{\mu\nu}^{el} S_{\mu\nu}$$  \hspace{1cm} (3-2)

For nonadiabatic ET reactions that are coupled to intramolecular vibrational quantum modes, the rate constant is also given by Eq. (3-1) with the vibronic coupling of the form in Eq. (3-2).\(^11\)

In general, vibronically nonadiabatic PT reactions could be either electronically adiabatic or electronically nonadiabatic. The form of the rate constant in Eq. (3-1) is valid for both cases, but the form of the vibronic coupling will correspond to Eq. (3-2) for the electronically nonadiabatic case and to a tunneling matrix element for the electronically adiabatic case.\(^14\) The latter case is related to hydrogen atom transfer reactions, which may be viewed as vibronically nonadiabatic PCET reactions in which the proton transfer is electronically adiabatic.\(^13\) Note that PT reactions could also be completely adiabatic, where the system remains in the ground electronic and vibrational states throughout the reaction. In this case, the rate constant is given by a standard transition state theory expression modified to account for the zero point energy of the transferring proton.\(^15\)

We investigated model systems corresponding to vibronically nonadiabatic ET, PT, and PCET. In the first set of calculations, we assumed that the proton transfer reaction was electronically nonadiabatic, so the vibronic coupling was of the form given in Eq. (3-2). The solvent reorganization energy was chosen to be 20 kcal/mol for ET and PCET and 5 kcal/mol for PT. For simplicity, the vibrational modes were represented by harmonic potentials with the minima for the reactant and product states separated by $\delta \xi$. The frequency of the proton vibrational mode was chosen to be 3000 cm$^{-1}$ with $\delta \xi = 0.5$ Å for both PT and PCET, and the
The dependence of the rate constant on the driving force for these three models is depicted in Figure 3-1. The ET model exhibits the typical inverted region behavior with a maximum corresponding to $-\Delta G^0 = \lambda = 20 \text{ kcal/mol}$. The PCET and PT models exhibit significant asymmetry with a substantial shift in the maximum, resulting in maxima at $-\Delta G^0 \approx -125 \text{ kcal/mol}$ and $-100 \text{ kcal/mol}$ for PCET and PT, respectively. Thus, observation of the inverted region behavior is predicted to be experimentally inaccessible for the PCET and PT models.

The free energy dependence of the rate constants can be understood by analysis of the contributions to the expression given in Eq. (3-1). The free energy curves along the collective solvent coordinate for the PCET model with two different driving forces are illustrated in Figure 3-2. The free energy curves corresponding to the ground reactant and the ground and first two excited product vibronic states are depicted. The relative contributions of the pairs of reactant/product vibronic states to the overall rate constant are determined primarily by a balance between the free energy barrier, $\Delta G^2 = \left( \Delta G^0 + \lambda + \epsilon_v - \epsilon_p \right)^2 / 4\lambda$, and the vibronic coupling $V_{\mu\nu}$.

The Boltzmann probability for the reactant state is also relevant when excited reactant states are
considered, but for simplicity we consider only the ground reactant vibronic state in this analysis, although the excited reactant states are included in the calculations. In the normal Marcus region, where $-\Delta G^0 < \lambda$, the free energy barrier is lowest for the ground product vibronic state, but in the inverted Marcus region, the free energy barrier becomes lowest for an excited product vibronic state.

In general, the vibronic coupling varies significantly for different pairs of reactant/product vibronic states due to differences in the overlap integrals. Figure 3-3 depicts the dependence of the overlap integral on the vibrational quantum number of the product state for the ET and PCET models. For the ET model, the overlap integral decreases monotonically as the vibrational quantum number of the product state increases because of partial destructive interference effects arising from the nodal structure of the excited vibrational states. As a result,
the ET rate constant decreases as $\Delta G^0$ becomes more negative because the dominant product state corresponds to a higher vibrational quantum number with smaller vibronic coupling. The participation of such a vibrational quantum mode in ET has been found to lead to asymmetry and shifting of the maximum in the driving force dependence.\(^{11}\) For the PCET and PT models, however, initially the overlap increases as the vibrational quantum number of the product state increases because the overlap integral is dominated by the tails of the vibrational wavefunctions, and the excited proton vibrational wavefunctions are more delocalized. For highly excited states, the oscillations of the proton vibrational wavefunctions lead to cancellation effects that result in a decrease in the overlap integral, as in the ET model. Due to this behavior of the overlap integrals, the PCET and PT rate constants continue to increase as $-\Delta G^0$ becomes greater than $\lambda$ because the excited product states with greater vibronic coupling become accessible. Eventually the rate constant decreases when the vibronic coupling decreases for highly exoergic reactions.

The qualitatively different behavior that arises for PT and PCET compared to ET is due to the higher frequency of the proton and the larger shift between the reactant and product proton vibrational wavefunctions. To further analyze the relative significance of these properties, we performed calculations for different combinations of the vibrational frequency $\omega$ and shift $\delta \alpha$. For $\omega = 400$ cm$^{-1}$ and $\delta \alpha = 0.5$ Å, the free energy dependence of the rate constant is very similar to that for the ET model. For $\omega = 3000$ cm$^{-1}$ and $\delta \alpha = 0.1$ Å, the curve is much more asymmetric than that for the ET model, but the maximum is still at $-\Delta G^0 \approx \lambda$. These figures are provided in Appendix A. Thus, the combination of a high vibrational frequency and a relatively large shift leads to the qualitative driving force dependence depicted in Figure 3-1 for the PT and PCET models.

We also investigated the impact of other vibrational modes on the free energy dependence of the PCET and PT rates. We performed calculations with two uncoupled quantum
modes, where one mode is the proton and the second mode is either another dimension of the proton motion or another intramolecular mode. The second mode was assigned a frequency of $\omega = 400 \text{ cm}^{-1}$ and a shift of $\delta \kappa = 0.1 \text{ Å}$. The resulting free energy dependence of the rate constant is virtually indistinguishable from that of the original model with only the proton vibrational mode because the overlap integrals between the ground reactant and excited product vibrational states of the second mode are very small, therefore, these excited states do not contribute significantly to the overall rate constant. Thus, the driving force dependence is dominated by the high-frequency mode and is not significantly affected by the inclusion of additional low-frequency modes. In addition, we analyzed the rate constant expressions that include the proton donor-acceptor motion and determined that the inclusion of this mode should not impact the qualitative driving force dependence of the rate constant.

Since many PT reactions are electronically adiabatic, we also investigated the free energy dependence of the PT rate constant in this regime. For the proton vibrational states below the barrier of the electronically adiabatic proton potential, the vibronic coupling is a tunneling matrix element that can be estimated using the semiclassical expression for tunneling through a parabolic barrier. For the proton vibrational states above the barrier, the vibronic coupling can be estimated as half of the splitting between the adiabatic vibrational energy levels. As shown in Figure 3-4a, the driving force dependence of the rate constant for this type of electronically adiabatic PT model exhibits a plateau as $\Delta G^0$ becomes more negative because the coupling becomes virtually constant for the excited vibrational states. The oscillations exhibited in Figures 3-1 and 3-4a for the PT models are due to the relatively small reorganization energy for PT. These oscillations arise from effects that occur when the dominant product state is changing from state $\nu$ to state $\nu + 1$ as $\Delta G^0$ becomes more negative. When the width $\sqrt{2\lambda k_B T}$ of the Gaussian term in Eq. (3-1) is smaller than the splitting between adjacent vibrational energy levels, the
driving force dependence of the total rate constant exhibits oscillations with a period that is approximately equal to the vibrational energy level splitting. As illustrated in Figure 3-4a, the amplitude of these oscillations increases as the reorganization energy decreases.

As mentioned above, the inverted region behavior has been observed experimentally for PT reactions. This discrepancy between theory and experiment has been denoted a “conundrum” in the literature. The calculations presented above also suggest that the inverted region behavior is not expected to be observed experimentally for vibronically nonadiabatic PT in either the electronically adiabatic or electronically nonadiabatic regime. For reasons discussed above, the inclusion of the three-dimensional motion of the transferring proton, low-frequency intramolecular modes, or the proton donor-acceptor mode is not expected to impact the qualitative driving force dependence of the PT rate constant. If the additional modes are strongly
coupled to the proton vibrational mode, however, multidimensional tunneling effects could potentially impact the driving force dependence. Moreover, additional modes corresponding to substantial geometrical changes associated with high frequencies and large shifts between the reactant and product states could impact the relative weightings of the pairs of reactant/product vibronic states and thus the driving force dependence. In general, PT reactions could be completely adiabatic in the normal region, and the conversion to the nonadiabatic regime or to alternative mechanisms in the inverted region could lead to a decrease in the overall rate constant.

A plausible explanation for some of these observations is that the experiments could be probing one of the oscillations observed in the PT curves of Figures 3-1 and 3-4a. This explanation could apply to the experimental data on PT within benzophenone/aniline contact radical ion pairs in a series of different solvents. In this case, the inverted region behavior is observed experimentally for rates that typically vary by only a factor of ~2 over a relatively small range of driving forces. Moreover, the inverted region behavior is observed for nonpolar solvents corresponding to lower solvent reorganization energies but is not observed for polar solvents corresponding to higher solvent reorganization energies. These experimental data are consistent with Figure 3-4a, which illustrates that the amplitude of the theoretically predicted oscillations is larger for lower solvent reorganization energies. According to the theoretical calculations, the oscillations may not be experimentally observable for highly polar solvents. Furthermore, the maximum of the first oscillation will occur at a more negative value of $\Delta G^0$ for higher solvent reorganization energies. Note that the more pronounced inverted region behavior observed experimentally in Ref. for rates that vary by more than two orders of magnitude over a range of driving forces spanning ~10 kcal/mol does not appear to be consistent with the theoretically predicted period and amplitude of these oscillations, particularly because these PT reactions were studied in a polar solvent.
An alternative explanation for the experimentally observed inverted region behavior in PT reactions is that the driving force is varied in a manner that also impacts another property of the system, such as the proton donor-acceptor distance or vibrational frequency, the proton vibrational frequencies, or the electronic coupling. This explanation could apply to the experimental data on PT between acids and diphenylmethyl carbanions in dimethylformamide studied in Ref. 3. In this case, the driving force is varied by altering the pK\textsubscript{a} values of the proton donor for a fixed proton acceptor. As discussed in the literature,\textsuperscript{20,21} ΔpK\textsubscript{a}, the difference in pK\textsubscript{a} values between the proton donor and acceptor, is strongly correlated with the hydrogen bond length and energy. Specifically, the proton donor-acceptor distance is expected to increase as the magnitude of ΔpK\textsubscript{a} increases (i.e., as $\Delta G^0$ becomes more negative for the series of complexes studied in Ref. 3). This effect can be incorporated into the electronically adiabatic PT model by increasing the intrinsic proton transfer barrier, which depends strongly on the proton donor-acceptor distance, as $\Delta G^0$ becomes more negative. As depicted in Figure 3-4a, this modified PT model clearly exhibits inverted region behavior. Similarly, this effect can be incorporated into the electronically nonadiabatic PT and PCET models by increasing the proton donor-acceptor distance as $\Delta G^0$ becomes more negative. Figure 3-4b illustrates that this modified PCET model also exhibits inverted region behavior. In these models, initially the rate increases as $\Delta G^0$ becomes more negative, despite the increase in the proton donor-acceptor distance, but eventually the increasing donor-acceptor distance causes the rate to decrease. Thus, the inverted region behavior could be observed experimentally for both PT and PCET systems if the driving force is varied in a manner that also impacts the properties of the proton transfer interface. In contrast to PT and PCET, varying the driving force experimentally for ET reactions is not expected to significantly impact the overlap integrals corresponding to the intramolecular vibrational modes or the electronic coupling.
The calculations in this chapter elucidate the underlying physical principles dictating the driving force dependence of the rate constants for ET, PT, and PCET reactions and predict the experimental conditions that could lead to observation of inverted region behavior for PT and PCET. The driving force dependence of the rate constant is qualitatively different for PT and PCET than for ET because the overlap integrals corresponding to excited vibronic states initially increase for PT and PCET but decrease monotonically for ET. This difference in the overlap integrals is due to a combination of the higher proton vibrational frequency and the larger shift between the reactant and product proton vibrational wavefunctions. As a result, these types of theories predict that the inverted region behavior will not be experimentally accessible for PT and PCET reactions if only the driving force is varied. The experiments measuring inverted region behavior for PT over a limited range of rates and driving forces could be probing an oscillation arising from the relatively small reorganization energy. Another plausible explanation for the experimentally observed inverted region behavior for PT is that varying the driving force also impacts other properties of the system, such as the proton donor-acceptor distance. Our analysis suggests that this possibility should be considered when inverted region behavior is observed experimentally for PT or PCET reactions.

Acknowledgments

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Supporting information

Illustration of proton vibrational wavefunctions associated with the PCET model; illustration of oscillations in driving force dependence associated with PT model; illustration of the driving
force dependence of the nonadiabatic rate constant with two uncoupled modes, where \( \lambda = 20 \) kcal/mol, \( \omega = 3000 \) cm\(^{-1}\) and \( \delta x = 0.5 \) Å for the first mode, and \( \omega = 400 \) cm\(^{-1}\) and \( \delta x = 0.1 \) Å for the second mode; illustration of the impact of reactant and product excited states on the driving force dependence; and illustration of the driving force dependence of the nonadiabatic rate constant with \( \lambda = 20 \) kcal/mol and \( \omega \) and \( \delta x \) varied.

References

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Chapter 4

Impact of mutation on hydrogen transfer interface and substrate conformation in soybean lipoxygenase


The impact of distal mutation on the hydrogen transfer interface properties and on the substrate mobility, conformation, and orientation in soybean lipoygenase-1 (SLO) is examined. SLO catalyzes a hydrogen abstraction reaction that occurs by a proton-coupled electron transfer mechanism. Mutation of isoleucine 553 to less bulky residues has been found experimentally to increase the magnitude and temperature dependence of the kinetic isotope effect for this reaction. This residue borders the linoleic acid substrate but is ~15 Å from the active site iron. In the present study, we model these experimental data with a vibronically nonadiabatic theory and perform all-atom molecular dynamics simulations on the complete solvated wild-type and mutant enzymes. Our calculations indicate that the proton transfer equilibrium distance increases and the associated frequency decreases as residue 553 becomes less bulky. The molecular dynamics simulations illustrate that this mutation impacts the mobility, geometrical conformation, and orientation of the linoleic acid within the active site. In turn, these effects alter the proton donor-acceptor equilibrium distance and frequency, leading to the experimentally observed changes in the magnitude and temperature dependence of the kinetic isotope effect. This study provides insight into how the effects of distal mutations may be transmitted in enzymes to ultimately impact the catalytic rates.
Introduction

Lipoxygenases are non-heme metalloenzymes that catalyze the oxidation of fatty acids. Mammalian lipoxygenases are medically significant in terms of immune response\textsuperscript{1} and cancer prevention.\textsuperscript{2-5} Soybean lipoxygenase-1 (SLO) is often utilized as a model for mammalian lipoxygenases and has been studied extensively with a wide range of both experimental\textsuperscript{6-14} and theoretical\textsuperscript{13-30} methods. The reaction catalyzed by SLO is depicted in Figure 4-1. In the first step, the pro-S hydrogen from C11 of the natural substrate, linoleic acid, transfers to the Fe(III)-OH cofactor, forming a radical intermediate substrate and Fe(II)-OH\textsubscript{2}. The radical intermediate substrate subsequently reacts with molecular oxygen to ultimately form hydroperoxyoctadecadienoic acid and Fe(III)-OH. The first step, which involves hydrogen abstraction, has been shown to be rate-limiting above 5 °C.\textsuperscript{13,14} Moreover, quantum mechanical calculations,\textsuperscript{20} as well as analysis of experimental measurements of thermodynamic quantities,\textsuperscript{13,17} imply that the hydrogen abstraction occurs by a proton-coupled electron transfer (PCET) mechanism. In this mechanism, the electron transfers from the \(\pi\)-system of the substrate to the iron of the cofactor, while the proton transfers from the C11 of the substrate to the hydroxyl ligand of the cofactor.

Figure 4-1: Proposed mechanism of SLO. Figure reproduced with permission from Ref. 17.
Kinetic studies of wild-type (WT) SLO indicate that the deuterium kinetic isotope effect (KIE) is unusually large, with a value of 81 at room temperature, and that the temperature dependences of the catalytic rate constants and KIEs are relatively weak. These observations have been interpreted to imply a substantial degree of hydrogen tunneling with significant contributions from vibrational promoting modes that modulate the hydrogen tunneling distance. Numerous SLO mutants have also been characterized structurally and kinetically. Of particular interest is the mutation of the hydrophobic residue isoleucine 553 (I553) to valine, leucine, alanine, and glycine. As depicted in Figure 4-2, residue 553 borders the bound linoleic acid substrate but is ~15 Å from the active site iron. The experimental data show almost no structural changes due to these mutations but indicate that the magnitude and temperature dependence of the KIE increase as residue 553 becomes less bulky (i.e., along the series depicted in Figure 4-3). These results have been interpreted to suggest an increased

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**Figure 4-2**: Depiction of linoleic acid substrate, I553 residue, and Fe-OH portion of cofactor obtained from a snapshot of an MD trajectory. Regions I, II, and III are identified.

**Figure 4-3**: Series of SLO mutants studied in the present chapter. Figure modeled after scheme in Ref. 14.
importance of donor-acceptor distance sampling as the size of the side chain at position 553 decreases.\textsuperscript{14}

The SLO reaction has been studied with a broad spectrum of theoretical methods.\textsuperscript{13-30} The experimental data have been analyzed in terms of various semiclassical and quantum mechanical rate models.\textsuperscript{13-19} In addition, reduced models of the active site region have been studied with density functional theory\textsuperscript{20-23} and mixed quantum/classical dynamical approaches.\textsuperscript{24,25} Furthermore, the full enzymatic system has been studied with all-atom molecular dynamics simulations.\textsuperscript{26-29} Olsson\textit{et al.}\textsuperscript{26,27} used an empirical valence bond potential energy surface to generate free energy profiles and included nuclear quantum effects with path integral methods. This work reproduced the magnitude but not the temperature dependence of the experimentally measured KIE for WT SLO. Tejero\textit{et al.}\textsuperscript{28} used a hybrid semiempirical quantum mechanical/molecular mechanical potential energy surface and ensemble-averaged variational transition state theory with multidimensional tunneling to calculate the rate constants for WT SLO and the I553A mutant. This work reproduced the magnitude of the KIE after applying a correction to the potential energy barrier but did not attempt to reproduce the temperature dependence. In these simulations, the proton donor-acceptor distance was observed to become larger and more flexible in the mutant reactant complex simulation.

Our previous molecular dynamics study of WT SLO\textsuperscript{29} was based on a vibronically nonadiabatic theoretical formulation\textsuperscript{31,32} that includes the quantum mechanical effects of the active electrons and the transferring proton, as well as the motions of all atoms in the entire solvated enzyme. In this theory, the rate constant is represented by the time integral of a probability flux correlation function. We performed classical molecular dynamics simulations of the complete solvated enzyme system to obtain the time correlation functions of the energy gap and the proton donor-acceptor mode, which were used to calculate the rate constant and KIE.\textsuperscript{29} These calculations reproduced the experimentally observed magnitude and temperature
dependence of the KIE for WT SLO without fitting any parameters directly to the experimental kinetic data. Furthermore, we found that the time-dependence of the probability flux correlation function is dominated by equilibrium properties, thereby validating the use of a simpler analytical rate constant expression for this system.

The objective of the present chapter is to apply this vibronically nonadiabatic theoretical formulation to the series of I553 mutants of SLO in order to understand the impact of this distal mutation at a molecular level. We use a two-pronged approach to address this issue. In the first approach, we use the rate constant expressions derived within the framework of the vibronically nonadiabatic theory to model the experimental data for the WT and four mutant SLO enzymes. This modeling provides insight into the fundamental physical principles underlying the experimentally observed trends in the magnitude and temperature dependence of the KIE upon mutation. Specifically, we find that relatively subtle changes in the proton donor-acceptor equilibrium distance and frequency lead to the experimentally observed trends. In the second approach, we perform all-atom classical molecular dynamics simulations of the entire solvated enzyme system for the WT and two mutant SLO enzymes. These simulations provide insight into how this distal mutation that borders the linoleic acid substrate but is ~15 Å from the active site iron could influence the properties of the proton transfer interface. In particular, we find that this mutation impacts the mobility, geometrical conformation, and orientation of the linoleic acid within the active site.

An outline of this chapter is as follows. In “Modeling the kinetic data,” we summarize the vibronically nonadiabatic theory and present the results obtained from modeling the experimental kinetic data for the WT and mutant SLO enzymes. In “Molecular dynamics simulations,” we describe the molecular dynamics methodology and present the analysis of the simulations for the WT and mutant SLO enzymes. In “Summary and concluding remarks,” we summarize the significant findings of this study and provide concluding remarks.
Modeling the kinetic data

Theory

In this chapter, we model the experimental data using the vibronically nonadiabatic rate constant expressions derived previously. In this formulation, the PCET reaction is described in terms of nonadiabatic transitions between pairs of reactant and product mixed electron-proton vibronic states. The nonadiabatic transitions are induced by thermal fluctuations of the solvent/protein environment, and these thermal fluctuations are assumed to occur on a much faster time scale than the nonadiabatic transitions. The vibronically nonadiabatic treatment is applicable to systems for which the nonadiabatic coupling between vibronic states is much less than the thermal energy. Our previous calculations indicate that the PCET reaction catalyzed by SLO is vibronically nonadiabatic. In addition, the motion of the proton donor-acceptor distance $R$ is treated explicitly, where $M$ and $\Omega$ are the $R$-mode effective mass and frequency, respectively. The derivations of the nonadiabatic PCET rate constants are based on Fermi’s golden rule and linear response theory for the solvent/protein environment.

In these derivations, the nonadiabatic coupling between reactant and product vibronic states is assumed to be of the form:

$$V_{\mu\nu}(R) = V_{\mu\nu}^{(0)} \exp[-\alpha_{\mu\nu}(R - \bar{R}_\mu)]$$  \hspace{1cm} (4-1)

when $R$ is near its equilibrium value. Here $V_{\mu\nu}^{(0)}$ is the vibronic coupling between vibronic states $\mu$ and $\nu$ at distance $R = \bar{R}_\mu$, and $\alpha_{\mu\nu}$ is the exponential decay parameter. This form of the coupling is a reasonable approximation in the region of $R$ near its equilibrium value, as illustrated by expanding $\ln\left[ V_{\mu\nu}/V_{\mu\nu}^{(0)} \right]$ in a Taylor series around $R = \bar{R}_\mu$ and retaining only the linear terms. In the electronically nonadiabatic limit for proton transfer, Eq. (4-1) can be expressed as
\[ V_{\mu \nu}(R) = V^{el} \cdot S_{\mu \nu}(R), \] where \( V^{el} \) is the electronic coupling and \( S_{\mu \nu}(R) \) is the overlap between the reactant and product proton vibrational wavefunctions for states \( \mu \) and \( \nu \) at a distance \( R \).\(^{35,36} \) In this limit, \( V_{\mu \nu}^{(0)} = V^{el} \cdot S_{\mu \nu}^{(0)} \), where \( S_{\mu \nu}^{(0)} \) is the overlap at the distance \( \bar{R} \), and the parameter \( \alpha_{\mu \nu} \) describes the approximately exponential decay of the overlap with \( R \) near \( \bar{R} \).

Using the short-time, high-temperature approximation for the solvent/protein environment and representing the \( R \)-mode as a quantum mechanical harmonic oscillator, the rate constant can be expressed as:\(^3\)

\[
k^{\text{quant}} = \sum_{\mu} P_{\mu} \sum_{\nu} \left| \frac{V_{\mu \nu}^{(0)}}{\hbar^2 \Omega} \right|^2 \exp \left[ \frac{2 \lambda_{\mu \nu}^{(a)}}{\hbar \Omega} \right] \int d\tau \exp \left[ -\frac{1}{2} \chi \tau^2 + p (\cos \tau - 1) + i(q \sin \tau + \theta \tau) \right] \tag{4-2}
\]

with the dimensionless parameters defined as:

\[
\zeta = \coth \left( \frac{\beta \hbar \Omega}{2} \right); \quad \chi = \frac{2 \lambda}{\beta \hbar^2 \Omega}; \quad \theta = \frac{\Delta G_{\mu \nu}^{(a)} + \lambda}{\hbar \Omega}; \quad p = \zeta \frac{\lambda_{\mu \nu}^{(a)}}{\hbar \Omega}; \quad q = \frac{\lambda_{\mu \nu}^{(a)}}{\hbar \Omega} \tag{4-3}
\]

In this expression, the summations are over reactant states \( \mu \) and product states \( \nu \), \( P_{\mu} \) is the Boltzmann probability for reactant state \( \mu \), \( \Delta G_{\mu \nu}^{(a)} \) is the free energy of reaction for states \( \mu \) and \( \nu \), \( \lambda \) is the reorganization energy, \( \lambda_{\mu \nu}^{(a)} = \hbar^2 \alpha_{\mu \nu}^2 / 2M \), and \( \beta = 1/k_B T \). This short-time, high-temperature approximation for the solvent/protein environment is valid when \( \lambda \) is large enough to ensure that the solvent/protein fluctuation dynamics are fast relative to the coherent nonadiabatic transitions. This expression also assumes that the reorganization energy and equilibrium proton donor-acceptor distance are the same for all pairs of reactant and product vibronic states.
The rate constant given in Eq. (4-2) can be further simplified in the high-temperature (low-frequency) regime for the R-mode, where \( \hbar \Omega \ll k_B T \). In this regime, the rate constant has the form:

\[
k_{\text{high}\text{T}} = \sum_p \sum \frac{|V_p^{(p)}|^2}{\hbar} \exp \left[ \frac{2 k_B T \alpha_{\mu \nu}^2}{M \Omega^2} \right] \sqrt{\left( \frac{\pi}{\lambda + \lambda_{\mu \nu}^{(a)}} \right) k_B T} \exp \left[ - \frac{\left( \Delta G_{\mu \nu}^0 + \lambda + \lambda_{\mu \nu}^{(a)} \right)^2}{4 \left( \lambda + \lambda_{\mu \nu}^{(a)} \right) k_B T} \right]
\] (4-4)

This expression is derived by performing a short-time expansion of the trigonometric functions in Eq. (4-2) up to second order and evaluating the time integral analytically. Eq. (4-4) leads to a simplified expression for the KIE when the reorganization energy and driving force are independent of isotope, \( \lambda_{\mu \nu}^{(a)} \ll \lambda \), and only the ground reactant and product vibronic states contribute to the rates. Under these conditions:

\[
\text{KIE} \approx \left[ \frac{|S_H|^2}{|S_D|^2} \right] \exp \left[ - \frac{2 k_B T}{M \Omega^2} \left( \alpha_D^2 - \alpha_H^2 \right) \right]
\] (4-5)

and:

\[
\frac{d}{dT} \ln[\text{KIE}] \approx - \frac{2 k_B T}{M \Omega^2} \left( \alpha_D^2 - \alpha_H^2 \right)
\] (4-6)

Here \( S_H \) and \( S_D \) are the overlaps of the hydrogen and deuterium ground state wavefunctions, and \( \alpha_H \) and \( \alpha_D \) describe the approximately exponential dependence of these overlaps on \( R \). In the application to SLO described below, we calculate the KIEs using both Eqs. (4-2) and (4-4), converging the results with respect to the excited reactant and product vibronic states. The simplified forms for the KIE and its temperature dependence given in Eqs. (4-5) and (4-6) are used only to analyze the physical principles dictating the qualitative trends.

An alternative rate constant expression attributed to Kutznetsov and Ulstrup\textsuperscript{37} has been applied to SLO by Klinman and coworkers\textsuperscript{13,14,30,38}. This expression is of the form:
\[ k^\text{UK} = \sum_{\mu} P_{\mu} \sum_{\nu} \frac{|V^{\text{ad}}|}{\hbar} \sqrt{\frac{\pi}{\lambda k_B T}} \exp \left[ -\frac{(\Delta G_{\mu\nu}^0 + \lambda)^2}{4\lambda k_B T} \right] P(R) \left[ S_{\mu\nu}(R) \right]^2 dR \]  

(4-7)

where \( P(R) = \sqrt{\frac{M \Omega^2}{2\pi k_B T}} \exp \left[ -M \Omega^2 \left( R - \bar{R} \right)^2 / \left( 2k_B T \right) \right] \) is the Boltzmann probability for a classical harmonic oscillator representation of the \( R \)-mode. In principle, the harmonic approximation for the \( R \)-mode could be avoided by using a more realistic probability distribution \( P(R) \). Note that the integration limits are chosen differently in some implementations, but \( P(R) \) and therefore the integrand are negligible for values of \( R \) deviating significantly from \( R = \bar{R} \).

Similar to the other rate constant expressions given above, this expression relies on Fermi’s golden rule formalism and linear response theory for the solvent/protein environment. In addition, this expression is based on the assumptions that the proton vibrational motion is in the low-temperature (high-frequency) regime, the proton motion is not coupled to the solvent, and the \( R \)-mode is in the high-temperature (low-frequency) regime.\(^{39-41}\) The rate constants \( k^\text{UK} \) and \( k^\text{highT} \) become identical when the following three conditions are satisfied: the proton transfer is electronically nonadiabatic (i.e., \( V_{\mu\nu} = V^{\text{ad}} S_{\mu\nu} \)), the overlap decreases exponentially with \( R \) near its equilibrium value (i.e., \( S_{\mu\nu} = S_{\mu\nu}^{(0)} \exp \left[ -\alpha_{\mu\nu} \left( R - \bar{R} \right) \right] \)), and \( \lambda^{(0)}_{\mu\nu} \ll \lambda. \)\(^{42}\) Both \( k^\text{UK} \) and \( k^\text{highT} \) are only strictly valid in the low-frequency regime for the \( R \)-mode, where \( \hbar \Omega < k_B T, \) but \( k^\text{quant} \) given in Eq. (4-2) is valid for general \( R \)-mode frequencies. A detailed comparison of these expressions for a series of model systems has been presented elsewhere.\(^{43}\)

**Results of modeling**

We used the vibronically nonadiabatic theory of PCET described above to model the experimental kinetic data for the series of I553 mutants of SLO. For the purposes of this
modeling study, the solvent reorganization energy is 39 kcal/mol, as calculated previously from molecular dynamics simulations of WT SLO, and the driving force is –5.4 kcal/mol, as estimated previously on the basis of experimental data.\textsuperscript{29} The reactant and product proton potential energy curves are described by Morse potentials corresponding to C–H and O–H vibrational modes, respectively, with the parameters given in Ref.\textsuperscript{29}. The Morse potentials are positioned so that the minima of the reactant and product potentials are separated by a distance of $R - R_{\text{CH}} - R_{\text{OH}}$, where $R_{\text{CH}}$ and $R_{\text{OH}}$ correspond to the equilibrium C–H and O–H bond lengths of 1.09 and 0.96 Å, respectively. The hydrogen and deuterium vibrational wavefunctions are calculated by solving a one-dimensional Schrödinger equation for the hydrogen or deuterium moving in the reactant and product Morse potentials. Analytical solutions are available for the eigenfunctions of Morse potentials.\textsuperscript{44} The overlaps $S_{\mu \nu}^{(0)}$ between the reactant and product vibrational wavefunctions at $\bar{R}$ are calculated numerically. The parameters $\alpha_{\mu \nu}$ are determined for each pair of vibronic states by calculating the numerical derivatives of the natural logarithm of the corresponding overlap integrals with respect to $R$ at $\bar{R}$. Note that a three-dimensional description of the hydrogen vibrational wavefunction with a more realistic proton potential would impact the quantitative results of this modeling study but is not expected to alter the qualitative trends. The value of $\nu_{\text{el}}$ is assumed to be isotopically independent and therefore is not required for the calculation of the KIE.

For each SLO mutant, we determined the values of the equilibrium proton donor-acceptor distance, $\bar{R}$, and the effective $R$-mode frequency, $\Omega$, by minimizing the root-mean-square deviation (RMSD) between the calculated and experimental values for the ln[KIE]. Thus, we minimized the function:
where \( N \) is the number of experimental data points, \( T_i \) is the temperature for data point \( i \), and \( \text{KIE}_{\text{calc}} \) and \( \text{KIE}_{\text{expt}} \) are the calculated and experimental KIEs, respectively. The values of \( \text{KIE}_{\text{calc}} \) were determined using both \( k_{\text{quant}} \) and \( k_{\text{highT}} \). The values of \( \bar{R} \) and \( \Omega \) obtained from this fitting procedure are given in Table 4-1. To generate these results, the effective \( R \)-mode mass \( M \) was chosen to be 100 amu, as used in previous modeling studies.\(^{43} \) The results with two other masses, as well as a brief discussion of the impact of the mass \( M \) on the results, are given in Appendix B.

The qualitative trends are the same for all three values of the masses.

Figure 4-1 depicts the experimental data points and the curves calculated using the rate constant \( k_{\text{quant}} \) with values of \( \bar{R} \) and \( \Omega \) given in Table 4-1. As shown in Figure 4-4, the magnitude and temperature dependence of the KIE increases as residue 553 becomes less bulky. Moreover, Table 4-1 indicates that the proton donor-acceptor equilibrium distance increases and the proton donor-acceptor mode frequency decreases as residue 553 becomes less bulky. Note that this trend is not followed by I553L and I553A because the experimental data for these two mutants are so similar that the theoretical modeling is unable to distinguish between them. Table 4-1 also shows that similar results are obtained with both \( k_{\text{quant}} \) and \( k_{\text{highT}} \), thereby indicating that this system is in the low-frequency \( R \)-mode regime for these parameters. We emphasize that the specific values of \( \bar{R} \) and \( \Omega \) obtained from this procedure are not directly related to

### Table 4-1: Proton donor-acceptor equilibrium distances and frequencies determined for WT SLO and series of I553 mutants. \( \bar{R} \) given in Å and \( \Omega \) given in cm\(^{-1} \). Fitting procedure for \( \ln[\text{KIE}] \) was done with either \( k_{\text{quant}} \) from Eq. (4-2) or \( k_{\text{highT}} \) from Eq. (4-4).

<table>
<thead>
<tr>
<th>enzyme</th>
<th>( k_{\text{quant}} )</th>
<th>( k_{\text{highT}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \bar{R} )</td>
<td>( \Omega )</td>
</tr>
<tr>
<td>WT</td>
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<td>174</td>
</tr>
<tr>
<td>I553V</td>
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<td>140</td>
</tr>
<tr>
<td>I553G</td>
<td>2.96</td>
<td>127</td>
</tr>
</tbody>
</table>
experimentally measurable quantities due to the approximate nature of the model. On the other hand, the trends in these effective distances and frequencies provide useful mechanistic insights, as well as predictions concerning the impact of mutation on the KIE.

The correlation between the changes in proton donor-acceptor equilibrium distance and frequency and the magnitude and temperature dependence of the KIE can be analyzed in terms of the simplified expressions given in Eqs. (4-5) and (4-6). As indicated by Eq. (4-5), the magnitude of the KIE will increase as the ratio of the hydrogen to deuterium overlaps increases. In general, this ratio increases as the equilibrium proton donor-acceptor distance increases. Thus, if all other quantities remain the same, the magnitude of the KIE is expected to increase as $\overline{R}$ increases, as observed in Figure 4-4 and Table 4-1. As shown in Table 4-1, however, the frequency decreases as the equilibrium proton donor-acceptor distance increases. Eq. (4-5) indicates that the magnitude of the KIE will decrease as the frequency $\Omega$ decreases, so these two effects impact the KIE in opposite directions. For this system, the increase in the equilibrium proton donor-acceptor distance dominates over the decrease in the frequency, but the magnitude of the KIE for the mutants is moderated by these opposing factors. In addition, Eq. (4-6) indicates that the temperature dependence of ln[KIE] will increase as the frequency $\Omega$ decreases, as observed in Figure 4-4 and Table 4-1.

**Figure 4-4:** Plot of ln[KIE] versus 1000/T for WT SLO and the series of I553 mutants. Lines were obtained from calculations using $k_{\text{quant}}$ with parameters given in text and Table 4-1. Closed circles are the experimental data from Ref. 14. Color scheme is the same as in Figure 4-3: WT (black), I553V (purple), I553L (green), I553A (red), and I553G (blue).
Klinman and coworkers observed similar trends using $k^{UK}$, but their equilibrium distances were significantly higher and frequencies were significantly lower.\textsuperscript{14,45} Specifically, they found that $\bar{R}$ increases from 3.29 Å to 4.60 Å and $\Omega$ decreases from 64 cm\textsuperscript{-1} to 38 cm\textsuperscript{-1} over the series of mutants spanning I553V to I553G. They used a similar effective mass of $M = 110$ amu, reorganization energy of $\lambda = 19$ kcal/mol, driving force of $\Delta G^0 = -6.0$ kcal/mol, and similar Morse parameters for the proton potentials. Using $k^{\text{highT}}$ with the exact same parameters as used by Klinman and coworkers, we obtained virtually identical values for $\bar{R}$ and $\Omega$ as given in Table 4-1 and similar level of agreement with the experimental data as shown in Figure 4-4. These results are given in Appendix B. Thus, the discrepancies in the values of $\bar{R}$ and $\Omega$ do not arise from differences in the other parameters. Using $k^{UK}$, we found that multiple combinations of $\bar{R}$ and $\Omega$ lead to reasonable agreement with the experimental data and that the specific values obtained depend on the details of the fitting procedure. In other words, the surface generated by plotting $\chi$ as a function of $\bar{R}$ and $\Omega$ is relatively flat in this regime. Note that the expressions $k^{\text{highT}}$ and $k^{\text{quant}}$ are not valid for the very low frequencies obtained by Klinman and coworkers because the approximation that the overlap between the reactant and product proton vibrational wavefunctions decreases exponentially with $R$ in the relevant regime near the equilibrium distance does not apply for such low frequencies. Moreover, the expression $k^{UK}$ is also not reliable for very large equilibrium distances and low frequencies due to the anharmonicity of the proton donor-acceptor mode and numerical difficulties arising from the extremely small overlap integrals in this regime. As a result, the fitting procedure is more robust with $k^{\text{highT}}$ and $k^{\text{quant}}$ than with $k^{UK}$. In any case, the general trends observed in the two studies are in complete agreement.
Molecular dynamics simulations

Computational methods

To elucidate the molecular basis for the trends described in the previous section, we performed molecular dynamics simulations for the WT SLO and the I553A and I553G mutants. The initial coordinates for the WT SLO were obtained from the X-ray crystallographic structure (PDB code 1YGE). The simulations for the I553A and I553G mutants were each performed with two different initial structures. The first pair of initial structures was based on the X-ray crystallographic structure of wild-type SLO (PDB code 1YGE), and the profix utility in the JACKAL protein modeling package was used to perform the I553A or I553G mutation. The second pair of initial structures was based on the X-ray crystallographic structures of the I553A and I553G mutants (PDB codes 3BNE and 3BNC, respectively), where the unresolved residues were added with the profix utility in JACKAL. These initial structures are denoted Structure 1 and Structure 2, respectively, for each mutant. The root-mean-square deviation (RMSD) between the backbone residues of Structure 1 and Structure 2 was calculated with the VMD program to be 1.73 Å for the I553A mutant and 0.97 Å for the I553G mutant. Performing the MD simulations with two different starting structures allowed us to analyze the sensitivity of the results to the starting structure. The H++ database was used to determine the protonation states of the residues in the enzyme. The enzyme was solvated in a periodically replicated truncated octahedral box with dimensions of 110 × 110 × 110 Å with ~18,000 explicit TIP3P water molecules. To neutralize the overall charge of the system, peripheral water molecules were replaced with sodium cations.

Currently a crystal structure of SLO with bound linoleic acid substrate is not available, leading to uncertainty about the positioning of the substrate during catalysis. We obtained the
initial conformation of the linoleic acid substrate for our simulations from previous quantum mechanical and docking calculations. This conformation is in the reverse orientation compared to that postulated on the basis of experimental kinetic studies. On the other hand, the orientation we are using is consistent with the orientation determined crystallographically for the purple form of soybean lipoygenase-3 with bound product (PDB 1IK3). It is also consistent with the orientation used for the simulations in Ref. where the active sites of the two lipoygenase structures were overlapped, and the product was transformed to linoleic acid by superimposing the common atoms. Furthermore, a number of experiments on lipoygenases suggest that the substrate could bind in both orientations. These issues are directions for future study.

We performed the molecular dynamics simulations with a modified version of DLPROTEIN. The potential energy surface was based on the AMBER 99 forcefield with modifications described in Ref. to model the linoleic acid substrate and Fe cofactor. The entire solvated enzyme system, which consists of ~67,000 atoms, was allowed to move during the molecular dynamics simulations. Long-range electrostatic interactions were calculated with the Smooth Particle Mesh Ewald method. All bonds involving hydrogen were constrained to their equilibrium lengths with the SHAKE algorithm. The classical equations of motion were integrated with the velocity Verlet algorithm with a time step of 1 fs. The NPT and NVT simulations were both performed with Nosé-Hoover thermostats.

Each system was thoroughly equilibrated prior to data collection. To prevent the linoleic acid substrate from dissociating, the distance between C11 of the linoleic acid substrate and the oxygen of the hydroxyl ligand was constrained to 3.2 Å, the sum of the van der Waals radii of carbon and oxygen, during equilibration. Initially, the energy of the entire system was minimized in a three step procedure: (1) the solvent water molecules were optimized while the entire enzyme was fixed; (2) the mutated or added residues were optimized while the rest of the enzyme was
fixed; (3) the entire system, including the enzyme, substrate, and solvent molecules, was optimized. Subsequently, molecular dynamics trajectories were propagated for 50 ps at 100, 200, and 300K with the NPT ensemble. In the final phase of equilibration, molecular dynamics trajectories were propagated for 100 ps at 300K with the NVT ensemble.

The data collection for each initial structure consisted of three parallel molecular dynamics trajectories with different initial velocities. These simulations were performed at 303 K for 1 ns starting from Structures 1 and 2 of the I553A and I553G mutants, as well as the WT structure used in previous studies, yielding a total of 3 ns of data for each of the five initial structures. In order to prevent substrate dissociation, the proton donor-acceptor distance was constrained to 2.72 Å in the WT SLO, 2.83 Å in the I553A mutant, and 2.96 Å in the I553G mutant. These values were determined from the modeling of the kinetic data described in “Modeling the kinetic data.” Although these constraints may impact the results, they were necessary to prevent substrate dissociation over the relatively long nanosecond time scales of the MD trajectories. Our analysis focuses on properties that are not expected to be directly impacted by this constraint.

Molecular dynamics results

We analyzed the mobility, geometrical conformation, and orientation of the linoleic acid substrate in the active site of the WT and mutant enzymes. For the purposes of analysis, we divide linoleic acid into three regions, as depicted in Figure 4-2. Region I consists of backbone carbons 1 through 8, Region II consists of backbone carbons 9 through 13, and Region III consists of backbone carbons 14 through 18. As illustrated in Figure 4-2, the proton donor carbon 11 is contained in Region II, and the mutation site 553 is closest to Region I. For completeness, we present the data obtained with both initial structures for each mutant, where Structure 1
corresponds to the mutated WT crystal structure, and Structure 2 corresponds to the crystal structure of the mutant. The data from Structures 1 are biased toward the initial WT structure and hence may not provide a reliable representation of the mutant enzymes. In many cases, the results obtained with both initial structures are qualitatively similar, but when they differ, we focus our analysis mainly on the data obtained from Structures 2.

We analyzed the root-mean-square fluctuation (RMSF) of the linoleic acid backbone atoms to examine the substrate flexibility and motion. For this purpose, we aligned the active site residues, without including the linoleic acid substrate itself, of all configurations from each set of molecular dynamics trajectories. The active site was defined to include all residues with at least one atom within 5 Å of the linoleic acid in the initial WT structure, as well as the Fe cofactor with ligands. The residues included in the active site are: GLU 256, THR 259, LYS 260, VAL 354, SER 491, HID 494, GLN 495, HID 499, TRP 500, HID 504, ILE 538, LEU 541, ALA 542, SER 545, LEU 546, ILE 552, ILE/ALA/GLY 553, THR 556, PHE 557, HID 690, ASN 694, GLN 697, THR 709, SER 747, VAL 750, ILE 751, LEU 754, ILE 839. Elimination of the linoleic acid substrate in the alignment procedure allowed us to analyze the motion of the substrate within the active site.

Figure 4-5 depicts the RMSF of the linoleic acid backbone carbon atoms. This figure indicates that Region II is less mobile than Regions I or III. The smaller mobility of Region II is due to several factors, including the restrictions associated with being located in the middle of the molecule, the π-bonding in this region, and the constraint applied to the distance between the substrate C11 and the oxygen of the hydroxyl ligand on the iron cofactor. A comparison between Figures 4-5a and 4-5b indicates that the RMSFs for the two different initial structures for each mutant are similar in Regions I and II but are different in Region III. In both figures, the substrate motions are qualitatively similar for the WT and mutants in Regions I and II, and the substrate fluctuations are greater in Region I than in Region II. According to the more reliable
In addition to analyzing the substrate RMSFs, we also analyzed the thermally averaged distances between the backbone carbons at the ends of Regions I, II, and III. These thermally averaged distances, along with the standard deviations, are depicted in Figure 4-6. The observed trends are not indicative of changes in carbon-carbon bond distances, which are maintained by harmonic potentials, but rather represent more global conformational changes in the linoleic acid substrate backbone. The standard deviations in Figure 4-6 are consistent with Figure 4-5 in that Region I exhibits the greatest deviation and Region II exhibits the smallest deviation.
Figure 4-6 illustrates the changes in the average end-to-end distances of the three regions as residue 553 becomes less bulky. Regions I and III do not exhibit clear trends. In Region II, however, the C9–C13 distance exhibits a distinct increase as I553 becomes less bulky. Although the increase in this average distance is of similar magnitude to that of the RMSF in this region, the trend appears to be meaningful based on the standard deviations provided in Figure 4-6. This increase in the C9–C13 distance indicates a stretching of the linoleic acid backbone in Region II that could lead to an increase in the proton donor-acceptor distance between C11 and the hydroxyl oxygen. These molecular dynamics simulations do not provide any information about causal effects, but the modeling in the previous section suggests that the stretching of the linoleic acid backbone in Region II is correlated with the increase in the proton donor-acceptor distance.

We also calculated the thermally averaged distances between the linoleic acid backbone carbon atoms and the α-carbon of residue 553. These distances are depicted in Figure 4-7. This figure suggests that the placement and orientation of the substrate within the active site changes upon mutation of I553. The linoleic acid backbone carbon closest to the α-carbon of residue 553 is C3 in WT and is shifted to C5 in both mutants for the initial Structures 2 and to an even higher-numbered substrate carbon in both mutants for the initial Structures 1. As implied by Figure 4-2, this shifting of the substrate relative to residue 553 is consistent with an increase in the
equilibrium proton donor-acceptor distance upon mutation. Figure 4-7 also indicates that the substrate is closer to the $\alpha$-carbon of residue 553 for the I553G mutant than for the I553A mutant. This trend is consistent with a larger equilibrium proton donor-acceptor distance for the less bulky residue glycine than for alanine. Thus, this analysis suggests that mutation at residue 553 may lead to reorientation of the substrate in the active site.

**Summary and concluding remarks**

In this chapter, we examined the impact of distal mutation on the hydrogen transfer interface properties and on the substrate mobility, conformation, and orientation in SLO. This study is based on experimental data showing that the magnitude and temperature dependence of the KIE increase as the distal residue 553 becomes less bulky. Modeling of this experimental data with a vibronically nonadiabatic theory indicates that the proton transfer equilibrium distance increases and the associated frequency decreases as residue 553 becomes less bulky. Molecular dynamics simulations of the complete solvated enzyme for WT and mutant SLO enzymes provide insight into how this distal mutation could affect these properties of the proton transfer interface. The simulations suggest that the replacement of I553 with a less bulky residue may lead to a decrease in the mobility of the opposite end of the substrate and a stretching of the middle region of the substrate backbone, which includes the proton donating carbon atom. Furthermore, the simulations also suggest a shifting of the substrate relative to residue 553 upon mutation of this residue, as well as a movement of the substrate closer to residue 553 as this residue becomes less bulky. These changes in mobility, conformation, and orientation of the substrate are correlated with the increase in the proton donor-acceptor equilibrium distance and decrease in the associated frequency. Distinguishing between cause and effect, however, is not possible due to limitations of the MD simulations.
The insights that have been generated from the vast body of experimental and theoretical studies of SLO have implications beyond this specific system. In general, distal mutations have been observed to significantly impact catalytic rates for many enzymes. Such effects are particularly apparent for reactions involving hydrogen tunneling, which is extremely sensitive to the proton donor-acceptor distance and frequency. For these types of reactions, measurement of the magnitude and temperature dependence of the KIE provides an exquisitely sensitive probe of the impact of mutation. A combination of structural and kinetic studies, in conjunction with theoretical modeling and computer simulations, will continue to enhance our understanding of how the effects of mutations are transmitted through proteins to ultimately impact the catalytic rates.

Acknowledgements

We thank Judith Klinman for helpful discussions about this project. We are grateful for financial support from NIH grant GM56207.

Supporting information

Tables of proton donor-acceptor equilibrium distances and frequencies determined for WT SLO and series of I553 mutants with two different values of the effective mass $M$ and with the parameters used by Klinman and coworkers;\textsuperscript{14} plot of ln[KIE] versus 1000/T from calculations using $k^{\text{highT}}$ with the parameters used by Klinman and coworkers.\textsuperscript{14}
References

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Chapter 5

Conclusions

The goal of this dissertation is to analyze and understand the impact of mutation on the proton-coupled electron transfer reaction catalyzed by soybean lipoxygenase. Using our theoretical framework, we have gained insight into the experimentally observed changes in kinetic isotope effect (KIE) magnitude and temperature dependence for a series of soybean lipoxygenase mutants. In order to do this, we studied the dependence of the KIE on the properties of model systems. We then refined our model system to more fully represent soybean lipoxygenase and studied the proton transfer interface in detail.

In studying the impact of input parameters on the rate expressions, we found a complex interplay among solvent reorganization energy, driving force, and effective proton donor-acceptor mass, distance, and vibrational frequency. Trends in the magnitude and temperature dependence of the KIE are related to changes in donor-acceptor distance and frequency. If both the donor-acceptor distance and vibrational frequency change, the KIE could be observed to increase or decrease as the donor-acceptor distance increases. We show that the magnitude and temperature dependence of the KIE are affected by both parameters. Trends in the temperature dependence of the KIE are determined by the combination of all system properties. With a low solvent reorganization energy (i.e. a nonpolar solvent) and a high proton donor acceptor vibrational frequency (i.e. a rigid hydrogen bond), the KIE either increase or decrease with temperature, depending on all other system properties. Trends in the driving force dependence are related to trends in the contributions of excited vibronic states. As the reaction becomes more asymmetric,
contributions from excited vibronic states increase, decreasing the KIE. Our theory is able to predict the physical basis for experimentally observed trends.

The apparent discrepancy between the theoretically predicted driving force dependence and the experimentally observed driving force dependence for proton transfer reactions has been called a “conundrum” in the literature.\textsuperscript{1,2} Marcus inverted region behavior has been observed for proton transfer when, according to theory, it should not be experimentally accessible. We explain this discrepancy in two ways. First, if another parameter is also varied with driving force, such as proton donor-acceptor distance or proton transfer barrier, the rate could decrease as the driving force becomes more negative. Second, when the solvent reorganization energy is low, oscillations appear in the driving force dependence. Apparent inverted region behavior would be observed if the experimental driving force range was such that one of these oscillations was probed.

Understanding the relationship between different system properties allowed us to predict the trends in proton donor-acceptor distance and vibrational frequency for a series of soybean lipoxygenase mutants. The mutated residue, isoleucine 553, is \textasciitilde 15 Å from the active site Fe but still borders the linoleic acid substrate. As it becomes less bulky, the magnitude and temperature dependence of the KIE both increase.\textsuperscript{3-5} By fitting our theory to experimental KIE data, we found that as the residue becomes less bulky the proton donor-acceptor distance increases and vibrational frequency decreases. Molecular dynamics simulations performed on the wild-type and two representative mutants show that the substrate mobility, conformation, and orientation also change with mutation.

In this dissertation we applied a vibronically nonadiabatic theory of proton-coupled electron transfer to a series of soybean lipoxygenase mutants. By studying the dependence of the rate and KIEs on system properties, we were able to predict the trends in proton donor-acceptor distance and vibrational frequency for a series of soybean lipoxygenase mutants. We used
molecular dynamics simulations to understand the impact of mutation on the substrate. On a broader scope, we were able to explain general experimental trends in rate and KIE of PCET reactions.

References

Appendix A

Supporting information: Chapter 3

Figure A-1: Proton potential energy curves and associated proton vibrational wavefunctions for the PCET model, where $\lambda = 20$ kcal/mol, $\omega = 3000$ cm$^{-1}$ and $\delta x = 0.5$ Å, with (a) the ground reactant and product vibrational states degenerate, (b) the ground reactant and tenth excited product vibrational states degenerate, and (c) the ground reactant and twentieth excited product vibrational states degenerate. The overlap integral is largest for (b), and the overlap is smallest for (c) because of cancellation effects due to the oscillations of the excited proton vibrational wavefunctions.

Figure A-2: Illustration of the oscillations in the driving force dependence of the nonadiabatic rate constant for PT with $\lambda = 20$ kcal/mol (black), $\lambda = 10$ kcal/mol (green), $\lambda = 5$ kcal/mol (red), and $\lambda = 2.5$ kcal/mol (blue). The reduced mass of the vibrational mode is 1 amu, $\omega = 3000$ cm$^{-1}$, $\delta x = 0.5$ Å, $T = 300$ K, and $k^0_{\text{PT}}$ is the rate constant for $\Delta G^0 = 0$. When all other variables are held constant and $\lambda$ increases, the oscillations become less prominent. This indicates that the oscillations in the $\Delta G^0$ dependence of PT are due to the lower solvent reorganization energy.
Figure A-3: Driving force dependence of the vibronically nonadiabatic rate constant for the PCET model with two uncoupled modes, where $\lambda = 20 \text{ kcal/mol}$, $\omega = 3000 \text{ cm}^{-1}$ and $\delta \tilde{x} = 0.5 \text{ Å}$ for the first mode, and $\omega = 400 \text{ cm}^{-1}$ and $\delta \tilde{x} = 0.1 \text{ Å}$ for the second mode. The red curve corresponds to this model with two uncoupled modes, and the blue curve corresponds to the original PCET model with only the first mode. The proton transfer is assumed to be electronically nonadiabatic, the temperature is 300 K, and $k_{H}^{0}$ is the rate constant for $\Delta G^{0} = 0$. The reduced mass of both vibrational modes is 1 amu.

Figure A-4: Illustration of the impact of reactant and product excited states on the driving force dependence of the nonadiabatic rate constant for the PCET (black), PT (red), and ET (blue) models. In the PCET model, $\lambda = 20 \text{ kcal/mol}$, $\omega = 3000 \text{ cm}^{-1}$, and $\delta \tilde{x} = 0.5 \text{ Å}$. In the PT model, $\lambda = 5 \text{ kcal/mol}$, $\omega = 3000 \text{ cm}^{-1}$, and $\delta \tilde{x} = 0.5 \text{ Å}$. In the ET model, $\lambda = 20 \text{ kcal/mol}$, $\omega = 400 \text{ cm}^{-1}$, and $\delta \tilde{x} = 0.1 \text{ Å}$. The reduced mass of the vibrational mode is 1 amu for PCET and PT and 10 amu for ET. The temperature is 300 K, and $k_{H}^{0}$ is the rate constant for $\Delta G^{0} = 0$. Including only the ground reactant state and converging with respect to only product states (a) gives the same driving force dependence as converging with respect to both reactant and product excited states (Figure 5-1). Including only ground reactant and ground product states (b) gives an incomplete illustration of the driving force dependence. In general, excited product states become more important for more negative driving forces.
Figure A-5: Driving force dependence of the vibronically nonadiabatic rate constant for three models with $\lambda = 20$ kcal/mol and (1) $\omega = 400$ cm$^{-1}$ and $\delta \zeta = 0.1$ Å (black); (2) $\omega = 400$ cm$^{-1}$ and $\delta \zeta = 0.5$ Å (blue); (3) $\omega = 3000$ cm$^{-1}$ and $\delta \zeta = 0.1$ Å (red). The first model corresponds to the ET model discussed in Chapter 3. The temperature is 300 K, and $k_H^0$ is the rate constant for $\Delta G^0 = 0$. The reduced mass of the vibrational mode is 1 amu for all of these models.

Figure A-6: Driving force dependence of the vibronically nonadiabatic rate constant for three models with $\lambda = 20$ kcal/mol and (1) $\omega = 3000$ cm$^{-1}$ and $\delta \zeta = 0.5$ Å (black); (2) $\omega = 3000$ cm$^{-1}$ and $\delta \zeta = 0.4$ Å (blue); (3) $\omega = 2500$ cm$^{-1}$ and $\delta \zeta = 0.5$ Å (red). The first model corresponds to the PCET model discussed in Chapter 3. The temperature is 300 K, and $k_H^0$ is the rate constant for $\Delta G^0 = 0$. The reduced mass of the vibrational mode is 1 amu for all of these models.
Appendix B

Supporting information: Chapter 4

Discussion of impact of effective mass $M$ on results

Table B-1: Proton donor-acceptor equilibrium distances and frequencies determined for WT SLO and series of I553 mutants with parameters given in Chapter 4 except $M = 14$ amu. $\bar{R}$ given in Å and $\Omega$ given in cm$^{-1}$. Fitting procedure for ln[KIE] was done with either $k_{\text{quant}}$ or $k_{\text{highT}}$.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$k_{\text{quant}}$</th>
<th>$k_{\text{highT}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{R}$</td>
<td>$\Omega$</td>
</tr>
<tr>
<td>WT</td>
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</tr>
<tr>
<td>I553V</td>
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<td>373</td>
</tr>
<tr>
<td>I553G</td>
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</tbody>
</table>

Table B-2: Proton donor-acceptor equilibrium distances and frequencies determined for WT SLO and series of I553 mutants with parameters given in Chapter 4 except $M = 6.86$ amu. $\bar{R}$ given in Å and $\Omega$ given in cm$^{-1}$. Fitting procedure for ln[KIE] was done with either $k_{\text{quant}}$ or $k_{\text{highT}}$.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$k_{\text{quant}}$</th>
<th>$k_{\text{highT}}$</th>
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</tr>
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<tr>
<td>I553G</td>
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<td>480</td>
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</table>

In Tables 4-1, B-1, and B-2, the equilibrium proton donor-acceptor distance $\bar{R}$ and the frequency $\Omega$ were determined by the fitting procedure described in Chapter 4, thereby reproducing the experimental KIEs. The results are given here for $M = 14$ amu, as determined in Ref. 1, and $M = 6.86$ amu, the reduced mass of carbon and oxygen. In the limit that $\lambda_{\text{pr}}^{(a)} \ll \lambda$, $k_{\text{highT}}$ depends only on $M\Omega^2$, so the impact of altering the mass $M$ is a direct scaling of the
frequency $\Omega$. The dependence of $k^{\text{quant}}$ and the associated KIE on the mass $M$ and the frequency $\Omega$ is more complicated, as indicated by Eqs. (4-2) and (4-3), but empirically the overall impact of altering the mass is also predominantly the scaling of the frequencies in this regime.

The qualitative trends are the same for all three values of the mass. Note that the discrepancies between $k^{\text{quant}}$ and $k^{\text{highT}}$ are larger for smaller mass $M$ because the frequency $\Omega$ increases, and the high-temperature (low-frequency) approximation for the $R$-mode is no longer valid. Thus, the results for $k^{\text{highT}}$ are not reliable in this regime. The results for $k^{\text{quant}}$ are valid for these masses and frequencies. The equilibrium proton donor-acceptor distance $\bar{R}$ obtained with $k^{\text{quant}}$ is virtually independent of the choice of mass, but the magnitude of the frequency $\Omega$ increases as the mass $M$ decreases.

**Fitting with alternate parameters**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$\bar{R}$ (Å)</th>
<th>$\Omega$ (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
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</tr>
<tr>
<td>I553V</td>
<td>2.77</td>
<td>151</td>
</tr>
<tr>
<td>I553L</td>
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</tr>
<tr>
<td>I553A</td>
<td>2.83</td>
<td>136</td>
</tr>
<tr>
<td>I553G</td>
<td>2.96</td>
<td>122</td>
</tr>
</tbody>
</table>

*Table B-3*: Proton donor-acceptor equilibrium distances and frequencies determined for WT SLO and series of I553 mutants with parameters from Ref. 2. $\bar{R}$ given in Å and $\Omega$ given in cm$^{-1}$. Fitting procedure for ln[KIE] was done with $k^{\text{highT}}$. 
**Figure B-1**: Plot of ln[KIE] versus 1000/T for WT SLO and the I553A and I553G mutants. Lines were obtained from calculations using $k_{\text{high}}^T$ with parameters from Ref. 2 and Table B-3. Closed circles are the experimental data from Ref. 2. Color scheme is the same as in Figure 4-3: WT (black), I553A (red), and I553G (blue).

**References**

Appendix C

Summary of rate constant expressions

Equations

In the following rate constant expressions, we assume that the vibronic coupling decreases exponentially with \( R \):

\[
V_{\mu \nu} = V_{\mu \nu}^{(0)} \exp\left[-\alpha_{\mu \nu} (R - \overline{R}_\mu)\right]
\]

where \( \overline{R}_\mu \) is the average value of \( R \) in the reactant state \( \mu \). Moreover, we define \( \delta R = \overline{R}_\nu - \overline{R}_\mu \) to be the difference between the product and reactant equilibrium values of \( R \). For simplicity, we assume that \( \overline{R}_\mu \) and \( \delta R \) are the same for all pairs of reactant and product states, although the \( \mu \nu \) subscript could be included for generality. In the electronically nonadiabatic limit for proton transfer, the vibronic coupling takes the form \( V_{\mu \nu} (R) = V^{el} S_{\mu \nu} (R) \), where \( V^{el} \) is the constant effective electronic coupling and \( S_{\mu \nu} (R) \) is the overlap between reactant \( \mu \) and product \( \nu \) proton vibrational wavefunctions at distance \( R \). In the limit where \( R \) is near its equilibrium value, \( S_{\mu \nu} = S_{\mu \nu}^{(0)} \exp\left[-\alpha_{\mu \nu} (R - \overline{R}_\mu)\right] \) and \( V^{(0)}_{\mu \nu} (R) = V^{el} S_{\mu \nu}^{(0)} \) where \( S_{\mu \nu}^{(0)} \) is the proton vibrational wavefunction overlap at \( \overline{R}_\mu \). The parameter \( \alpha_{\mu \nu} \) describes the exponential decay of the overlap with \( R \) near \( \overline{R}_\mu \).

The general form of the rate expression is given as:

\[
k = \sum_{\mu} P_{\mu} \sum_{\nu} k_{\mu \nu}
\]

(C-2)
where $P_\mu$ is the Boltzmann probability for reactant state $\mu$ and $k_{\mu\nu}$ is the partial nonadiabatic rate constant for transition from reactant state $\mu$ to product state $\nu$.

Using the short-time, high-temperature approximation for the solvent and representing the $R$-mode as a quantum mechanical harmonic oscillator, the rate constant can be given by Eq. (50) in Ref. 1:

$$k_{\mu\nu} = \left[ \frac{P_{\mu}(0)}{\hbar^2 \Omega} \right] \exp \left[ \frac{2\lambda_{\mu\nu}(a)}{\hbar \Omega} \right] \int d\tau \exp \left[ -\frac{1}{2} \chi \tau^2 + p \left( \cos \tau - 1 \right) + i \left( q \sin \tau + \theta \tau \right) \right]$$

with the dimensionless parameters defined as:

$$\zeta = \coth \left( \frac{1}{2} \beta \hbar \Omega \right); \quad \chi = \frac{2\lambda_r}{\beta \hbar^2 \Omega^2}; \quad \theta = \frac{\Delta G^0_{\mu\nu} + \lambda_\mu}{\hbar \Omega}$$

$$p = \zeta \frac{\lambda_r + \lambda_{\mu\nu}(a)}{\hbar \Omega} - 2\sqrt{\frac{\lambda_r \lambda_{\mu\nu}(a)}{\hbar \Omega}}; \quad q = \frac{\lambda_r + \lambda_{\mu\nu}(a)}{\hbar \Omega} - 2\zeta \frac{\sqrt{\lambda_r \lambda_{\mu\nu}(a)}}{\hbar \Omega}$$

(C-4)

Here $\beta = 1/k_B T$, $\lambda_s$ is the solvent reorganization energy, $\lambda_r$ is the $R$-mode reorganization energy defined as $\lambda_r = \frac{1}{2} M \Omega^2 \delta R^2$, where $M$ and $\Omega$ are the $R$-mode effective mass and frequency, respectively, and $\lambda_{\mu\nu}$ is the coupling energy defined as $\lambda_{\mu\nu}(a) = \frac{\hbar^2 \alpha_{\mu\nu}}{2M}$. For simplicity, we assume that the solvent and $R$-mode reorganization energies are the same for all pairs of reactant and product states, although the $\mu \nu$ subscript could be included for generality. $\Delta G^0_{\mu\nu}$ is the free energy of reaction for the reactant $\mu$ and product $\nu$ states (i.e., the driving force for these states).

Alternatively, if we assume that the equilibrium value of $R$ is the same for reactants and products (i.e. $\delta R = 0$), the dimensionless parameters defined in Eq. (C-4) become:

$$\zeta = \coth \left( \frac{1}{2} \beta \hbar \Omega \right); \quad \chi = \frac{2\lambda}{\beta \hbar^2 \Omega^2}; \quad \theta = \frac{\Delta G^0_{\mu\nu} + \lambda}{\hbar \Omega}; \quad p = \zeta \frac{\lambda_{\mu\nu}}{\hbar \Omega}; \quad q = \frac{\lambda_{\mu\nu}}{\hbar \Omega}$$

(C-5)
Using the classical harmonic oscillator approximation for the R-mode, we get:

\[
k_{\mu \nu} = \frac{|V^{(0)}_{\mu \nu}|^2}{\hbar \Omega} \exp \left[ \frac{2\alpha_{\mu \nu}^2}{\beta M \Omega^2} \right] \int d\tau \exp \left[ -\frac{1}{2} \chi \tau^2 + \frac{\alpha_{\mu \nu}^2}{\beta M \Omega^2} \left( \cos \tau - 1 \right) + i \delta \right]
\]  

(C-6)

There are several different ways to make the high-temperature approximation for the R-mode. If we represent the R-mode time correlation function by that of a classical harmonic oscillator and do a stationary phase and short-time approximation, we obtain Eq. 52 in Ref. 1. If we represent the R-mode time correlation function by that of a quantum mechanical harmonic oscillator (i.e. Eq. (C-3)) and do a short-time expansion of the trigonometric functions followed by analytical integration, we obtain Eq. 35 in Ref. 2:

\[
k = \sum_\mu P_\mu \sum_v \frac{|V^{(0)}_{\nu \mu}|^2}{\hbar} \left[ \frac{2k_{\mu \nu} T \alpha_{\mu \nu}^2}{M \Omega} \right] \sqrt{\frac{\pi}{\lambda_{\mu \nu} k_{\mu \nu} T}} \exp \left[ -\frac{\left( \Delta G_{\mu \nu}^0 + \Delta G_{\mu \nu}^1 + \frac{1}{4} \left( \lambda_{\mu \nu} + \lambda_{\mu \nu}^{(a)} \right)^2 \right)}{4 \lambda_{\mu \nu} k_{\mu \nu} T} \right]
\]  

(C-7)

where the total reorganization energy is defined as \( \Lambda_{\mu \nu} = \lambda_{\mu \nu} + \lambda_{\mu \nu}^{(a)} \). If we assume that \( \delta \mathcal{R} = 0 \), the expression simplifies to:

\[
k = \sum_\mu P_\mu \sum_v \frac{|V^{(0)}_{\nu \mu}|^2}{\hbar} \left[ \frac{2k_{\mu \nu} T \alpha_{\mu \nu}^2}{M \Omega} \right] \sqrt{\frac{\pi}{\lambda_{\mu \nu}^{(a)} k_{\mu \nu} T}} \exp \left[ -\frac{\left( \Delta G_{\mu \nu}^0 + \lambda_{\mu \nu}^{(a)} \right)^2}{4 \lambda_{\mu \nu}^{(a)} k_{\mu \nu} T} \right]
\]  

(C-8)

If we represent the R-mode time correlation function by that of a classical harmonic oscillator (i.e. Eq. (C-6)) and do a short-time expansion of the trigonometric functions followed by analytical integration assuming that \( \delta \mathcal{R} = 0 \), we obtain Eq. 9 in Ref. 3:

\[
k = \sum_\mu P_\mu \sum_v \frac{|V^{(0)}_{\nu \mu}|^2}{\hbar} \left[ \frac{2k_{\mu \nu} T \alpha_{\mu \nu}^2}{M \Omega^2} \right] \sqrt{\frac{\pi}{\lambda_{\mu \nu}^{(a)} k_{\mu \nu} T}} \exp \left[ -\frac{\left( \Delta G_{\mu \nu}^0 + \lambda_{\mu \nu}^{(a)} \right)^2}{4 \lambda_{\mu \nu}^{(a)} k_{\mu \nu} T} \right]
\]  

(C-9)
The only difference between Eqs. (C-8) and (C-9) is the \( \lambda_{\mu\nu}^{(a)} \) in the numerator of Eq. (C-8). For both Eqs. (C-8) and (C-9), if we further assume that the \( R \)-mode time correlation function is equal to its value at zero time, or assume that \( \lambda_{\mu\nu}^{(a)} \ll \lambda_i \), then the expression simplifies to:

\[
k = \sum_{\mu} P_{\mu} \sum_{\nu} \frac{V_{\mu\nu}^{(0)}}{\hbar} \frac{1}{4 \lambda_i k_i T} \exp \left[ -\frac{\left( \Delta G_{\mu\nu}^{(a)} + \lambda_i \right)^2}{4 \lambda_i k_i T} \right]
\]

(C-10)

If we make the low-temperature approximation for the \( R \)-mode, using the stationary phase approximation, the analytical rate expression is Eq. A1 in Ref. 2 which is exactly the same as Eq. 53 in Ref. 1:

\[
k = \sum_{\mu} P_{\mu} \sum_{\nu} \frac{V_{\mu\nu}^{(0)}}{\hbar} \frac{1}{4 \lambda_i k_i T} \exp \left[ -\frac{\left( \Delta G_{\mu\nu}^{(a)} + \lambda_i \right)^2}{4 \lambda_i k_i T} \right]
\]

(C-11)

The expression for the rate constant based on correlation functions in the absence of the harmonic approximation is given in Eqs. 16, 17, and 27 of Ref. 1. This expression with the quantum mechanical harmonic approximations for the solvent and the \( R \)-mode is given in Eq. 29 of Ref. 2.
Tests

Note: Unless otherwise specified, cflux is Eq. (C-6) and red, qflux is Eq. (C-3) and blue, and highT is Eq. (C-9) and black. $R = 2.87\,\text{Å}$, $\lambda_s = 39\,\text{kcal/mol}$, and $\Delta G^\circ = -5.4\,\text{kcal/mol}$.

Figure C-1: Plot of KIE vs. $\Omega$ for $M = 100$ amu (a) and $M = 14$ amu (b). At $M = 100$ amu and in the range of 100-150 cm$^{-1}$, cflux and highT are very similar but qflux is different. By 100 cm$^{-1}$, cflux, qflux, and highT are identical. The plot for $M = 14$ amu is similar to that for $M = 100$ amu but for a frequency range of 225-375 cm$^{-1}$. By 225 cm$^{-1}$ cflux, qflux and highT are identical.

Figure C-2: Plot of KIE vs. $M$ for $M\Omega^2$ held constant. For range 10-100 amu, cflux and highT are very similar throughout (with slightly better agreement at low mass), and cflux, qflux, and highT become very similar for $M \sim 100$ amu. Note that the mass dependence of highT rate constant comes from the $\lambda_{s\mu}^{(a)}$ terms, which becomes very small for larger masses, so KIE plateaus at high mass.
Figure C-3: Plot of KIE vs. $1/T$ for $M = 100$ amu (a) and $M = 14$ amu (b), keeping $M\Omega^2$ constant. For $M = 100$ amu, qflux, cflux, and highT are nearly identical, with the best agreement at high temperatures. For $M = 14$ amu, cflux and highT are nearly identical, and qflux agrees at high temperatures but diverges at lower temperatures. At $T = 303$ K, qflux gives KIE bigger by about 20. Note: At $T = 303$ K, for $M = 14$ the KIE is around 60, and for $M = 100$ the KIE is around 90. This difference is due to $\lambda_{\mu\nu}^{(a)}$ effects.

Figure C-4: Plot of KIE vs. $1/T$ for $M = 100$ and 14 amu, keeping $M\Omega^2$ constant and using Eqs. (C-8-10). For Eq. (C-10), where $\lambda_{\mu\nu}^{(a)} = 0$, both masses give the same curve (this must be the case since $M\Omega^2$ is the same). For $M = 100$ amu, Eqs. (C-8-10) give similar curves, which agree better for higher $T$. At $T = 270$ K, the difference between Eqs. (C-8) and (C-9) for the KIE is about 20. For $M = 14$, the difference is much bigger, greater than 300 at 270 K, and the KIEs do not even match at $T = 370$ K. Green = 14 amu. Magenta = 100 amu. Dashed = Eq. (C-8). Solid = Eq. (C-9). Black = Eq. (C-10).

References

Appendix D

Docking simulations of wild-type soybean lipoxygenase and linoleic acid: Heads versus tails

Background

Polyunsaturated fatty acids form multiple enantiomeric pairs of mono-hydroperoxide derivatives in non-enzymatic reactions. In lipoxygenase catalyzed reactions, generally one hydroperoxide enantiomer is favored.\(^1\) The first step of the enzymatic reaction is hydrogen atom abstraction by a proton-coupled electron transfer mechanism from a 1,4-pentadienyl position on the substrate to form a substrate derived radical intermediate. Molecular oxygen then binds to the radical and forms a fatty acid hydroperoxide.\(^1\) In the case of soybean lipoxygenase 1 (SLO-1), this reaction is highly stereoselective for 9,12-(Z,Z)-octadecadienic acid (linoleic acid) reacting to form 13(S)-hydroperoxyoctadecadienic acid (HPOD),\(^2\) as shown in Figure 4-1.\(^3\) The substrate can occupy the active site in one of two possible docking orientations. In the head first orientation, the carboxyl group of the fatty acid is buried in the active site. This orientation has been used in previous studies of SLO-1\(^3-7\) and similar mammalian lipoxygenases.\(^8\) This orientation is also found in the crystal structure of SLO-3 with the substrate derived radical intermediate bound.\(^9\) In the tail first orientation, the hydrocarbon tail of linoleic acid is buried in the active site. This is the orientation favored by Kliman et al.\(^2\) There is experimental support for both orientations.

The tail first orientation is more intuitively acceptable based on the apparent challenge of accommodating a charged carboxyl group in the active site. However, even near physiological pH, a significant portion of linoleic acid molecules may be uncharged.\(^1\) Further support for the
tail first orientation comes from site directed mutagenesis of two active site residues. Mutation of both Leu 546 and Leu 754 to the less bulky Ala causes a change in product distribution consistent with a tail first binding orientation.\(^2\) However, steric contributions from these amino acids alone can not explain the product distribution of SLO-1 as SLO-3 is significantly less stereoselective and both residues are conserved between the two structures.\(^9\) Based on the generalized model of Coffa et al for the impact of substrate orientation on product chirality in lipoxygenases, product stereochemistry is regulated by substrate orientation and depth of $\text{O}_2$ penetration into the active site.\(^1\) Figure 5 in Ref\(^1\) shows the different substrate binding orientations for arachidonic acid. In linoleic acid, carbons 9 and 13 are analogous to carbons 8 and 12 in arachidonic acid. As such, this model predicts a tail first binding orientation based on observed product stereochemistry.

The head first binding is supported by experimental evidence of a purple SLO-1 intermediate. Spectroscopic studies of the purple SLO-1 intermediate show that it is a catalytically competent Fe-O-O-L complex, where L is the linoleic acid derived radical intermediate.\(^9\) Crystal structures of the purple SLO-3 intermediate show that the substrate is bound in the head first orientation in this complex.\(^9\) Unfortunately, there is no crystal structure of SLO-1 with linoleic acid bound. The active sites of SLO-1 and SLO-3 are similar, but not identical. An important point of comparison between SLO-1 and SLO-3 is that while the 13(S) product is still dominant, SLO-3 is not as stereoselective as SLO-1, possibly because the active site of SLO-1 is not as flexible as that of SLO-3.\(^9\)

The goal of this appendix is to use docking simulations provide insight into the question of whether linoleic acid binds in the head or tail first orientation in SLO-1. Here we describe the setup and procedure of our docking simulations and present our results.
Simulations

Protein preparation

The initial structure for the protein was obtained from the X-ray crystallographic structure (PDB codes 1YGE). Using Maestro’s\textsuperscript{10} protein preparation features we added hydrogens and optimized the hydrogen bonding network with the OPLS 2005 force field. During hydrogen bonding network optimization, hydroxyl groups, water molecules, the amide groups of Asn and Gln, and the imidazole rings of His residues are reoriented and the appropriate protonation states and orientations are assigned. All other atoms remain at their initial crystal structure positions. Prior to optimization, the Fe-O-H angle was set to a value of 126°, a value based on parameters used in molecular dynamics simulations.\textsuperscript{3,4}

Ligand preparation

We created two libraries of linoleic acid structures: one library with the carboxyl group of linoleic acid protonated and one library with the carboxyl group of linoleic acid deprotonated. Each library started with ten initial structures of linoleic acid. Structure 1 was created in GaussView 4.1.2 using built-in bond lengths, angles, and dihedrals. Structure 2 is a crystal structure of oleic acid modified to have a second double bond, becoming linoleic acid.\textsuperscript{11} Structure 3 is a crystal structure of linoleic acid.\textsuperscript{12} Structure 4 is the result of previous docking simulations.\textsuperscript{3} Structure 5 comes from the crystal structure of the substrate derived radical intermediate in SLO-3.\textsuperscript{9} Form A of each structure is simply the initial coordinates, be they from a crystal structure or simulation. Form B of each structure is Form A geometry optimized at the B3LYP level of theory with the 6-31G** basis set using Gaussian 03.\textsuperscript{13} A conformational search on each of the ten initial library structures was performed using MacroModel\textsuperscript{14} to generate
multiple structures to dock. The protonated library contained 5100 structures, and the deprotonated library contained 6295 structures.

**Glide docking**

Glide\textsuperscript{15} determines the best docking conformation for a ligand by evaluating the interactions between a given ligand pose and the receptor. Glide's conformation generator, which is different than MacroModel, creates poses by performing a broad search of torsional space to find local minima. These local minima in torsional space correspond ligand poses. During docking simulations, the receptor is fixed, and the structure and characteristics of the receptor are represented on a grid. All poses are evaluated on this grid to determine the best placement of the ligand within the receptor. The best pose corresponds to the lowest Glide score. For the purposes of this study, the receptor is the active site of the enzyme, defined as the Fe-OH complex and the following residues: GLU 256, THR 259, LYS 260, VAL 354, SER 491, HID 494, GLN 495, HID 499, TRP 500, HID 504, ILE 538, LEU 541, ALA 542, SER 545, LEU 546, ILE 552, ILE/ALA/GLY 553, THR 556, PHE 557, HID 690, ASN 694, GLN 697, THR 709, SER 747, VAL 750, ILE 751, LEU 754, ILE 839. These residues were selected because they are either directly ligated to the active site Fe or are within 5 Å of linoleic acid in a previously docked structure.\textsuperscript{3,4}
Results

Illustrations

Figure D-1: Left: Previously docked structure of linoleic acid in SLO-1.\textsuperscript{3,4} Right: Crystal structure of product docked in SLO-3.\textsuperscript{9} Color-coded residues are consistent between the two figures. This comparison shows that previous\textsuperscript{3,4} docking orientation in SLO-1 is consistent with the crystal structure of docked product in SLO-3.

Figure D-2: Left: Illustration of previously docked structure, head first orientation.\textsuperscript{3,4} Right: Docking result, also in the head first conformation, with a proton donor-acceptor distance of 3.27 Å and a Glide score of -0.84.
Evaluation of initial library structures

Figure D-3: Plot of Glide score versus proton donor-acceptor distance for the ten initial library structures. Black = Structure 1, built in GaussView; red = Structure 2, derived from oleic acid crystallographic structure; blue = Structure 3, linoleic acid crystallographic structure; green = Structure 4, previously docked structure; and purple = Structure 5, modeled after docked product in SLO-3. Closed symbols = geometry optimized structure; open symbols = initial structures. Circle = head first docking orientation; square = tail first docking orientation.

Evaluation of full library of structures

Figure D-4: Plot of Glide score versus proton donor-acceptor distance for the full library of structures. Black open circles indicate head first docking orientation; black closed squares indicate tail first docking orientation.

Conclusions

We emphasize that there is no crystal structure of SLO-1 with docked linoleic acid. All results are without the assistance or bias of an initial experimental docking conformation. Both the initial library of structures and the full library of structures gave similar results. Poses with the head first docking orientation tended to be correlated to lower proton donor-acceptor
distances and Glide scores; however, poses with the tail first orientation also had reasonable proton donor-acceptor distances and low Glide scores. If not terribly conclusive, this study was consistent with the presence of support in the literature for both docking orientations.

References

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