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Computational Replication of the Abnormal Secondary Kinetic Isotope Effects in a Hydride Transfer Reaction in Solution with a Motion Assisted H-Tunneling Model

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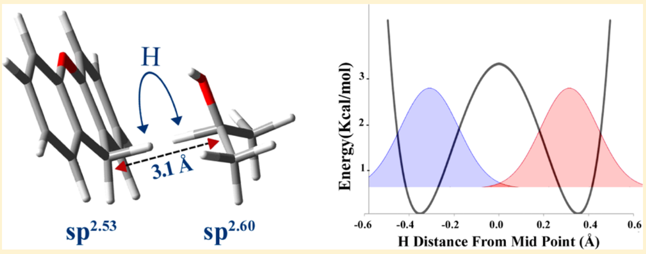
Supporting Information



ABSTRACT: We recently reported abnormal secondary deuterium kinetic isotope effects (2° KIEs) for hydride transfer reactions from alcohols to carbocations in acetonitrile (*Chem. Comm.* 2012, *48*, 11337). Experimental 2° KIE values were found to be inflated on the 9-C position in the xanthylium cation but deflated on the *β*-C position in 2-propanol with respect to the values predicted by the semi-classical transition-state theory. No primary (1°) isotope effect on 2° KIEs was observed. Herein, the KIEs were replicated by the Marcus-like H-tunneling model that requires a longer donor−acceptor distance (DAD) in a lighter isotope transfer process. The 2° KIEs for a range of potential tunneling-ready-states (TRSs) of different DADs were calculated and fitted to the experiments to find the TRS structure. The observed no effect of 1° isotope on 2° KIEs is explained in terms of the less sterically hindered TRS structure so that the change in DAD due to the change in 1° isotope does not significantly affect the reorganization of the 2° isotope and hence the 2° KIE. The effect of 1° isotope on 2° KIEs may be expected to be more pronounced and thus observable in reactions occurring in restrictive environments such as the crowded and relatively rigid active site of enzymes.

INTRODUCTION

The importance of quantum mechanical H-tunneling is increasingly recognized in chemical and biological reactions. Hydrogen's small mass and de Broglie uncertainty in position near the transition state (TS) contributes to the tunneling effect.[1] Observations of inflated primary kinetic isotope effects (1° KIEs) and temperature under- or overdependence of 1° KIEs versus values predicted by semi-classical transition state (TS) theory are often used to suggest H-tunneling.[2−14] Although relatively less used in the general chemistry field, abnormal secondary (2°) KIEs can also function as an indicator for H-tunneling.[15−18] These abnormalities include inflated 2° KIEs with respect to the values predicted by the semi-classical TS theory and larger 2° KIEs for H-transfer than for D-transfer, i.e., a 1° isotope effect on 2° KIEs.[17,19−23] Often, the inflated 2° KIEs were explained in terms of 1° H-tunneling and 1°/2° H coupled motions, in which part of the 2° H out of plane bending vibrational mode is converted to a translational mode, leading to an increase in 2° KIE.[22,24] Within this explanation, since H-tunneling is more significant than D-tunneling, the effects of 1°/2° H coupled motions are more significant for the former than the latter, leading to a larger 2° KIE in the former process. However, even if there is other evidence demonstrating H-tunneling, some solution and enzymatic H-transfers still show no 1° isotope effect on 2° KIEs.[7,25−28] Furthermore, we recently reported a *deflated* 2° KIE and no 1° isotope effect on 2° KIEs for a solution hydride transfer reaction that cannot be explained by the traditional theories.[29] In that work, semi-classical models predicted KIEs close to the equilibrium isotope effect (EIE) for a late TS, but the measured KIEs were closer to unity (typical of an early TS). Therefore, behavior of 2° KIEs in H-transfer reactions, especially how 1° H-tunneling affects 2° KIEs, is not well explained by semi-classical theories. This paper demonstrates that both inflated and deflated 2° KIEs that we observed can be replicated within a recently developed H-tunneling model and the model can also explain the observed absence of 1° isotope effect on 2° KIEs.

The abnormal 2° KIEs that we reported are for the hydride transfer reactions from aliphatic alcohols to xanthylium ion ($Xn^+ClO_4^-$) and 9-phenylxanthylium ion ($PhXn^+BF_4^-$) in acetonitrile (Scheme 1).[29] These hydride transfer processes strictly follow the second-order kinetics and are thus proper systems to study the relationship between the magnitude of 2° KIEs and 1° H-tunneling.[29−32] The 2° KIEs on both the *β*-CH_3/CD_3 position of the 2-propanol (*β*-D_6 2° KIE = 1.05) and on the *α*-9-H/D position of the Xn^+ (*α*-D 2° KIE = 0.99) (Scheme 1, R = R′ = CH_3, R″ = H) were found to be very close

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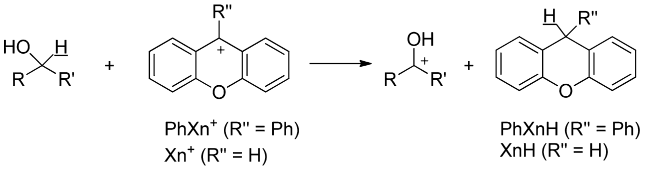
Scheme 1. Hydride Transfer Reaction from 2-Propanol to Carbocations



to unity and far from the corresponding EIEs (1.52 and 0.89, respectively).[29] They are expected to be closer to the corresponding EIEs, though, since Hammond's Postulate predicts a late TS for such reactions that produce a highly unstable α-hydroxy carbocation product (C^+−OH). Furthermore, the KIEs contradict the observed large negative Hammett constant (−2.66) for the hydride transfer reactions from substituted benzyl alcohols to $PhXn^+$, which is also consistent with a late TS.[28] Therefore, the observed inverse 2° KIEs on Xn^+ that accompanies a conversion from sp^2 to sp^3 is inflated in magnitude and the observed normal 2° KIEs on the alcohol that accompanies a conversion from sp^3 to sp^2 is deflated. While the former can possibly be explained in terms of H-tunneling and 1°/2° H coupled motions, the latter cannot. In the meantime, no 1° isotope effects on 2° KIEs were observed on either the alcohol or the cation, complicating the understanding of the hydride transfer chemistry.

In that communication, we used a new phenomenological model, called the Marcus-like H-tunneling model, to explain the observed 2° KIEs. Within that full tunneling model, H-tunneling takes place in tunneling ready states (TRSs) where the reactant and product potential wells are degenerate and the donor−acceptor distance (DAD) is short enough for efficient tunneling.[17,33,34] In that model, 2° KIEs arise from isotopic differences on the structural reorganization necessary to reach a TRS. Thus, the observed closer to unity 2° KIEs on both the alcohol and the cation were explained in terms of the small degree of reorganization of H/D toward the formation of the TRS, i.e., the small degrees of rehybridization of the donor and acceptor carbons. Moreover, the observed no 1° isotope effect on 2° KIEs was explained due to the loose TRS complex in solution, i.e., the difference in DAD for H- and D-tunneling (long vs short) does not affect the difference in reorganization of the 2° H/D to an extent that can be determined experimentally. While these explanations appear reasonable, a quantitative replication of the 2° KIEs by the Marcus-like model is needed to support the rationale. In this paper,

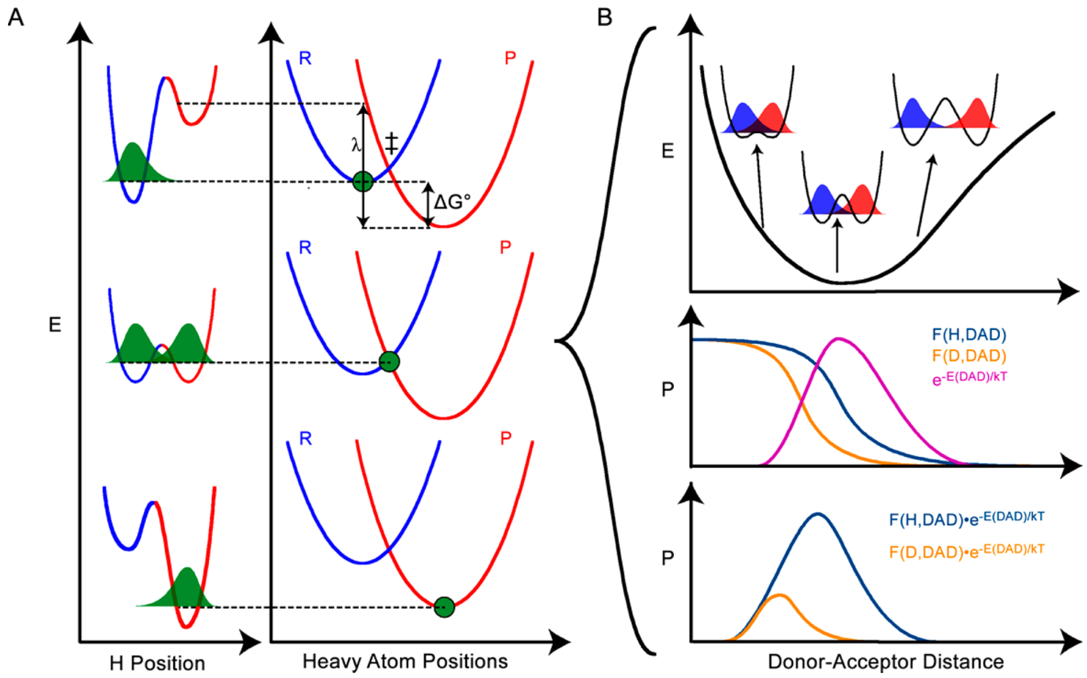


Figure 1. Marcus-like model of H-tunneling. (A) Top, middle, and bottom panels show three stages of the reaction in two designated coordinates: the H position and the positions of the heavy atoms that modulate the potential surfaces (reactant surface is blue and product surface is red) for the transferred H. In the top panel, the heavy atoms are in a position such that the ZPE of the H is lower in the reactant well, so the H-wave function (green) is localized there. In the middle panel, the heavy atoms have rearranged to a TRS (‡), where the ZPE for the transferred H is equal in the reactant and product wells and the H-wave function (including contributions from any motions coupled to the H-position) can tunnel through the barrier. The rate of reaching this tunneling ready state depends on the reaction driving force ($\Delta G°$) and the reorganization energy (λ). In the bottom panel, the heavy atoms have rearranged further, making the ZPE of the product lower than the reactant and thus trapping the transferred H in the product well. (B) At the TRS (middle panel of A) fluctuations of the DAD affect the tunneling probability. The top panel shows a free energy surface for the designated DAD coordinate, indicating the different levels of reactant−product wave function overlap at different DADs. At short enough DAD, the ZPE of the transferred particle may be above the barrier, but this leads to very small 1° KIEs and does not appear to be the case for ADH. The middle panel shows the Boltzmann probability distribution of the system being at any given DAD (magenta), along with the tunneling probabilities of H and D as a function of DAD (orange and purple, respectively). The bottom panel shows the product of the Boltzmann factor and the tunneling probability for each particle, yielding the probability of a reactive trajectory as a function of DAD. Panel B illustrates that the reactive trajectories for H and D go through different average DADs, constituting an isotope effect on TRS structure. In ADH, the difference in average DAD for hydride vs deuteride transfers, which was estimated as 0.2 Å, leads to differences in 2° KIEs when the transferred isotope is different. Figure and caption are reprinted with permission from ref 39. Copyright 2013 American Chemical Society.

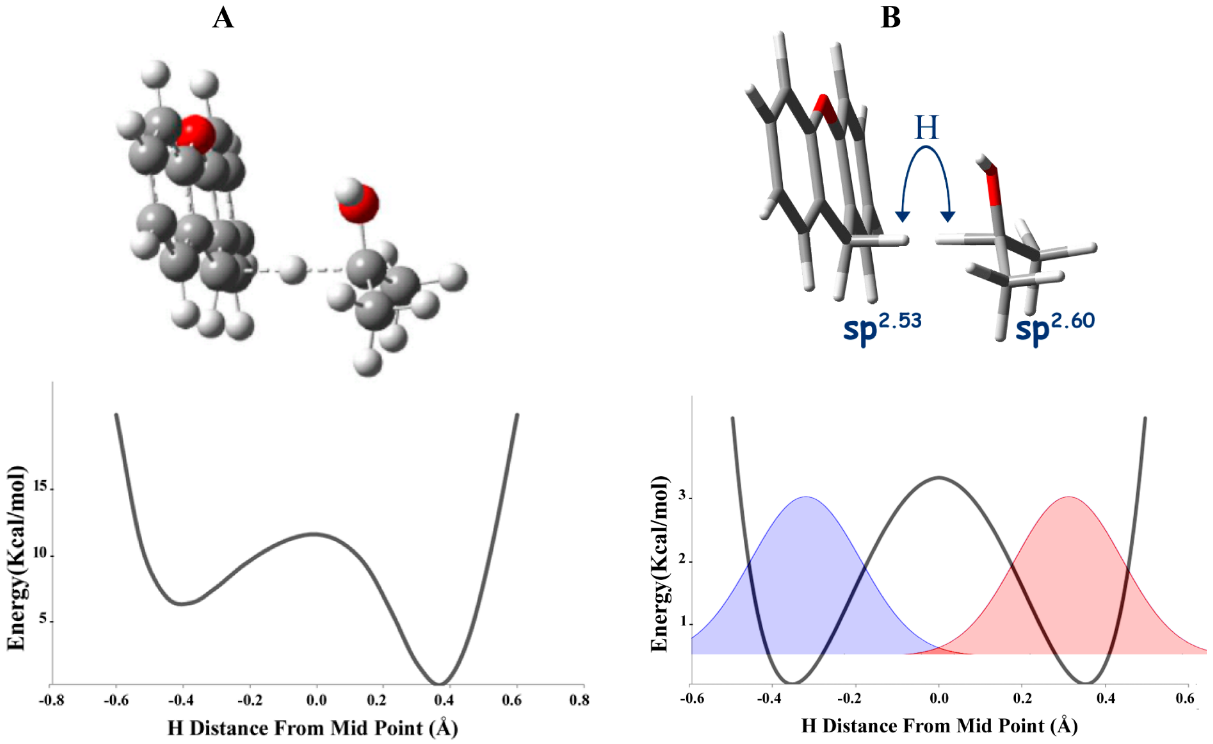


Figure 2. (A) Initial optimized TS-like structure with DAD = 3.1 Å and the corresponding asymmetric double-potential wells (left for Acceptor-H vibrations in product XnH; right for Donor-H vibrations in reactant 2-propanol). The structure is in a position close to the TRS (‡) on the heavy atom coordinate in Figure 1A. (B) TRS structure with DAD = 3.1 Å, and the corresponding degenerate double-potential wells. The calculated hybridization states are for the reacting carbons, respectively. Although the transferring hydride is shown in both the donor and acceptor positions (i.e., in $(\text{Donor-H})^{\text{TRS}}$ and $(\text{Acceptor-H})^{\text{TRS}}$), it is actually delocalized between the two.

calculation of the observed abnormal 2° KIEs was carried out using the Marcus-like model. A series of "TRS" structures were found, and their 2° KIEs were calculated to fit to the experimental values. Within the framework of the Marcus-like model, we conclude (1) 2° KIEs can be either inflated or deflated in this H-tunneling mechanism, and (2) 2° KIEs are larger when a lighter isotope tunnels, and the difference between the two KIEs for two transferred isotopes depends upon the isotope-sensitive structure of the TRS.

■ THEORETICAL MODEL AND CALCULATION METHOD

In the Marcus-like model, two processes are required to achieve a TRS: (i) the system must reach a point of energy degeneracy between the reactant and product, and (ii) the system must reach a short enough DAD for H-tunneling. Both processes are assisted by heavy atom motions. The former process is sometimes referred to as reorganization, and the latter as gating. Equation 1 gives the rate constant (k) in terms of both processes, and a pictorial description of the model that explains each term in eq 1 is included in Figure 1.[17,35−39]

$$k = Ce^{-(\Delta G^{\circ}+\lambda)^{2}/4k_{B}T\lambda}\int_{DAD1}^{DAD0} F(m,\,DAD)\;e^{-E(\mathrm{DAD})/k_{B}T}\,d\mathrm{DAD} \qquad (1)$$

In this equation, the first exponential is the Marcus term in which ΔG° is the reaction's exoergicity and λ is the heavy atom reorganization energy. This corresponds with the reorganization process that results in a TRS in which the energies of the reactant (R) potential well and the product (P) potential well match (the middle panel in Figure 1A). This term is 2° isotope sensitive but 1° isotope insensitive. The $F(m,$ DAD) is the Franck−Condon term that accounts for the 1° H wave function overlap (and thus the tunneling probability) between the two degenerate potential wells at a certain DAD (the left middle panel in Figure 1A). This term is 1° isotope sensitive with a lighter isotope having higher tunneling probability. It is also DAD sensitive with a shorter DAD resulting in more wave function overlap (the top panel in Figure 1B). The second exponential is the gating term relating the system energy to the thermal distribution of DADs (the middle panel in Figure 1B). The integration of the product of the last two terms over a range of DADs shows the probability of the trajectory of the tunneling H as a function of DAD (the bottom panel in Figure 1B). A heavier isotope requires a shorter average DAD for efficient tunneling (both the middle and bottom panels in Figure 1B).[39] While the last two terms concern the nucleus that tunnels and determine the 1° KIE, the 2° KIE is embedded in the Marcus term as isotopic differences in the rate of reaching a TRS.[19]

The calculation method is based on that of Roston and Kohen for modeling the hydride transfer from benzyl alcohol to NAD^{+} in alcohol dehydrogenase.[40] All geometry optimizations, potential energy surface (PES) scans, and frequency calculations were done with Gaussian09 at the B3LYP/6-31+G(d) level. To calculate the 2° KIE to fit to the experimental value, the structure of an assumed TRS is needed. To look for the "TRS" structure, the heavy atom skeleton structure at a certain DAD was first optimized with the hydrogen atom fixed at the midpoint of a straight line between the donor and acceptor carbons. This is a TS-like structure. The 180 °C_{donor}−H−C_{acceptor} angle was chosen for computational simplicity, and the quantum mechanical/molecular mechanical simulations demonstrate that the angle indeed does not substantially deviate from linearity.[41,42] The structure (**A**) in Figure 2 shows such for the hydride transfer reaction from 2-propanol to Xn^{+} at DAD = 3.1 Å. The corresponding C−H vibrational energy wells in reactant (2-propanol) and product (XnH) potential wells do not have the same energy (Figure 2A). The heavy atom positions of this skeletal structure should be close to those of the TRS (marked with ‡ in Figure 1A), but additional adjustment must be made to find a point of degeneracy.

To yield degenerate potential wells for efficient H-tunneling to occur, the energy of the XnH well needs to decrease and that of the 2-propanol well needs to increase (see double potential wells for **A**). The out-of-plane bending angles (θ's) of the groups attached to the reacting carbons are then adjusted to mediate the relative positions of the potential wells. The θ of the C_9—H in XnH was chosen for the purpose. This H group is the lightest group attached to the acceptor carbon, so its motion is much faster than other atoms such that the heavy atom skeleton could be approximated as rigid while changing the position of this H. This simplifies the structural reoptimization process. In the case of 2-propanol, however, there is no α-H. Therefore, the α-carbon hybridization was adjusted by altering the θ of the OH group. Since the mass of this group is comparable to those of other groups, the structure is relaxed (optimized) at each θ in order to reach the minimum energy. Changing the out-of-plane bending angles on both sides results in a range of structures that are examined for degeneracy of reactant and product potential wells.[40] The structure with the minimum energy degenerate double potential wells was chosen as the TRS at a given DAD. As an example, the degenerate double potential wells for a DAD of 3.1 Å as well as the corresponding TRS structure (**B**) are shown in Figure 2. Note that although the transferring hydride is shown in both the donor and acceptor positions in structure (**B**) (i.e., in $(\text{Donor-H})^{\text{TRS}}$ and $(\text{Acceptor-H})^{\text{TRS}}$), it is actually delocalized between the two. Therefore, the TRS is a quantum superposition of the two states.

The hybridization states (sp^H) of the donor and acceptor carbons in the TRS structure were calculated using eq 2:[40]

$$H = 2 + (180 - \theta)/(180 - \theta_0) \qquad (2)$$

In this equation, θ is the out-of-plane bending angle at the TRS, and θ_0 is the angle of the reduced form of each molecule. The calculated hybridizations of the reacting carbons from the donor and acceptor of the TRS with DAD = 3.1 Å is also included in structure (**B**).

The wave function of the transferring H at the TRS can then be approximated as the linear combination of the wave functions of H in each well (eq 3):[38,40]

$$\Psi_{\text{TRS}} = \frac{1}{\sqrt{2}}\psi_{\text{d}} + \frac{1}{\sqrt{2}}\psi_{\text{a}} \qquad (3)$$

ψ_{d} and ψ_{a} represent the wave functions of the 1° H localized in the donor and acceptor wells, respectively. Making this approximation allows us to calculate the 2° KIE as the geometric mean of the 2° KIEs calculated with the H localized in the donor and acceptor wells, i.e., 2° KIE = $k_{\text{H}}/k_{\text{D}} = (2°\ \text{KIE}_{\text{d}} \cdot 2°\ \text{KIE}_{\text{a}})^{1/2}$ (where the subscripts indicate the KIE calculated for the donor (d) or acceptor (a) states). The respective 2° KIE can be calculated using the ISOEFF program[43] that uses the full Bigeleisen equation (see eq 4 for the calculation of the donor state as an example).[44]

$$\begin{aligned} 2°\ \text{KIE}_{\text{d}} &= (\kappa_{\text{H}}/\kappa_{\text{D}})_{\text{d}} \cdot \text{MMI}_{\text{d}} \cdot \text{EXC}_{\text{d}} \cdot \text{ZPE}_{\text{d}} \\ &= (\kappa_{\text{H}}/\kappa_{\text{D}})_{\text{d}} \cdot \text{MMI}_{\text{d}} \cdot \text{EXC}_{\text{d}} \cdot \frac{\prod_i^{n_{\text{vib}}} \text{e}^{-(\beta h/2c)(\nu_{iHd}^{\text{TRS}} - \nu_{iH}^{\text{GS}})}}{\prod_i^{n_{\text{vib}}} \text{e}^{-(\beta h/2c)(\nu_{iDd}^{\text{TRS}} - \nu_{iD}^{\text{GS}})}} \end{aligned} \qquad (4)$$

In this equation, $(\kappa_{\text{H}}/\kappa_{\text{D}})$, MMI, and EXC represent isotope effects on transmission coefficient, mass moment of inertia, and vibrationally excited populations, respectively. ZPE represents the contribution to KIE from the isotopic difference in zero point energies between reactant/product ground state ($(\text{Donor(Acceptor)-H})^{\text{GS}}$) and TRS ($(\text{Donor(Acceptor)-H})^{\text{TRS}}$). The ν_i represents the frequency of any vibration mode (i) in the TRS and in the ground state; β is $1/k_{\text{B}}T$; h is Planck's constant; k_{B} is Boltzmann constant, and c is speed of light. ISOEFF07 uses all of the frequencies including the imaginary ones to calculate the KIEs (3N-6 vibrational modes by default[43] (see note 45)).

The same method was used to calculate the 2° KIEs for TRSs at a range of DADs. Changing the DADs had slight effect on the degeneracy of the double potential wells. Thus, only slight changes in out-of-plane bending angles were further needed. The resulting 2° KIEs at each DAD were compared to the experimental value to find the best-fit DAD, i.e., the best-fit TRS structure.

The fitting based on experimental KIEs allows us to find the TRS structure for the reaction in solution. We note that there is only one fitting parameter (the DAD) in this computational method. All other parameters are inherent to the model.

■ RESULTS AND DISCUSSION

Table 1 lists the calculated 2° KIEs as a function of DAD in the TRS for the hydride transfer reaction from 2-propanol to Xn^+.

Table 1. Calculated 2° KIEs as a Function of DAD in the TRSs for the 2-Propanol/Xn^+ Reaction

	DAD (Å)					
	2.9	3.0	3.1	3.2	3.3	3.5
β-D_6 2° KIE (on alcohol)[a]	1.030	1.043	1.053	1.065	1.074	1.075
α-D 2° KIE (on cation)[b]	0.973	0.981	0.986	0.990	0.992	0.995

[a] On 2-propanol. [b] On Xn^+

Table 2. Calculated and Experimental 2° KIEs and the Best-Fit DADs for the 2-Propanol/Xn^+ Reaction[a]

	2° KIE (expt)[b]	2° KIE (calcd (1))[c]	2° KIE (calcd (2))[d]
	Hydride Transfer		
β-D_6 2° KIE (on 2-propanol)	1.05 ± 0.02	1.053	
α-D 2° KIE (on Xn^+)	0.99 ± 0.02		0.990
corresponding DAD		3.1 Å	3.2 Å
best-fit DAD		3.15 Å[f]	
	Deuteride Transfer		
β-D_6 2° KIE (on 2-propanol)	1.04 ± 0.02	1.043	
α-D 2° KIE (on Xn^+)	0.98 ± 0.03		0.981
corresponding DAD		3.0 Å[e]	3.0 Å[e]
best-fit DAD		3.0 Å[f]	

[a] See texts for details. [b] From ref 29; within experimental error there is no 1° isotope effect on 2° KIEs. [c] Best-fit to the 2° KIE on alcohol. [d] Best-fit to the 2° KIE on cation. [e] 2° KIEs from TRS with DAD = 3.0 Å fit the 2° KIEs on both reactants. [f] A discussion of the difference in the two values may be meaningless; see footnote b and text.

Table 2 shows the values of 2° KIEs that best match the experiments and the corresponding best-fit DADs. The 1° KIE reported for this reaction is 2.8, indicating the hydride transfer is rate-determining.[29] In Table 2, the column (calcd (1)) shows the TRS/DAD with best-fit to the β-D_6 2° KIE on the 2-propanol moiety of the TRS, and the column (calcd (2)) with best-fit to the α-2° KIE on the Xn^+. Within expectation, the two corresponding DADs are quite close showing that one TRS structure fits to the experimental 2° KIEs on both reactants. The best-fit DAD is then taken as the average of the two *close* DADs. Since the 2° KIEs for the H- and D-transfer processes are the same within the experimental error (Table 2), a discussion about the difference between the two corresponding best-fit DADs to verify the prediction for the longer DAD in a lighter isotope transfer is meaningless (also see the subsequent discussion). Nevertheless, the simultaneous fit to the 2° KIEs on both reactants (inflated on Xn^+ and deflated on 2-propanol!) showcases the successful application of the

Marcus-like model in explaining the "abnormal" 2° KIEs observed in this reaction. The best-fit TRS structure that has a DAD of 3.1 Å is shown in Figure 2B.

Table 1 shows that the *magnitudes* of the normal β-D_6 2° KIE on 2-propanol and the inverse α-D 2° KIE on Xn^+ both decrease as DAD shortens. The trend for the β-D_6 2° KIE can be explained in terms of the decreased space between the two moieties at a TRS as a result of decreasing DAD so that both the vibrational freedom of the 2° H/D and the isotopic differences in hyperconjugation from the β-CH_3/CD_3 groups decreases, thus reducing the 2° KIE. While normally the α-D 2° KIE for an sp^2 to sp^3 process is inverse as the result of the restricted bending vibration of the α-CH/CD bond due to the increased number of coordination at the reaction center, a decrease of DAD will further restrict the bending vibrations thus making the 2° KIE more inverse. Since the isotopic substitution at the 1° position is expected to affect the DAD (shorter for D-tunneling than H-tunneling; see Figure 1B),[39] this provides an alternative theoretical explanation, in addition to the H-tunneling and 1°/2° H coupled motions,[24] for the 1° isotope effect on 2° KIEs observed in some systems. That is, the 2° KIE is greater in magnitude for H-transfer (with longer DAD) than for D-transfer. Note that this latter explanation following the Marcus-like model has been previously proposed for qualitatively understanding the observed effect of 1° isotope on α-type 2° KIEs (on benzyl alcohol) in alcohol dehydrogenases,[34,39,46] the results in Table 1 show its potential applicability to explain the effect of 1° isotope on the β-type ones (on 2-propanol) as well.

Interestingly, the data in Table 1 show that in this system the 2° KIE changes *only slightly* with DAD over the range around the fit DAD. For example, for a DAD change of 0.6 Å (from 2.9 Å to 3.5 Å), the changes in β-2° KIE on 2-propanol and α-2° KIE on Xn^+ are only 0.045 and 0.022, respectively. Therefore, although the DAD is expected to be longer for H- than for D-transfer by about 0.2 Å,[40] the expected difference in their 2° KIEs is so small that is not detectable experimentally. This is consistent with our observations of no significant 1° isotope effect on 2° KIEs (Table 2), which can be explained in terms of a loose TRS complex in a less restrictive solvent environment so that 1° isotope effect or the resulting steric effect on 2° KIEs are greatly minimized.[28,29] Notably, this effect is not in accord with the observations in the analogous hydride transfer reactions from benzyl alcohol to NAD^+ coenzyme mediated by alcohol dehydrogenases, which have repeatedly shown a significant 1° isotope effect on 2° KIEs.[20,34,40] The Marcus-like model suggests that the relatively spacious active site of enzyme is evolved for the transfer of the most abundant H isotope, so D-transfer, which requires a shorter DAD, leads to crowded/deformed active site and decreases the extent of rehybridization and thus suppresses the 2° KIE.[34,40]

The calculated hybridization states of $sp^{2.60}$ and $sp^{2.53}$ for the α-C of the alcohol and 9-C of the Xn^+ in the TRS (Figure 2B) would indicate that the geometry change of the reactants is about halfway completed at the TRS, whereas the charge transfer from the alcohol is nearly complete, as indicated by the Hammett correlation study on the donor alcohol (also see Introduction).[28] Additionally, the rehybridization of the donor and acceptor carbons are found to be nonsynchronous with that in alcohol (from sp^3 to $sp^{2.60}$) lagging behind that in Xn^+ (from sp^2 to $sp^{2.53}$). These results suggest a TRS with imbalanced development in charge and geometry in the alcohol moiety and nonsynchronous rehybridization between the two reacting carbons. Interestingly, an imbalanced TRS was also found in the yeast ADH reaction. The calculated rehybridization of the α-C's of the benzyl alcohol (from sp^3 to $sp^{2.76}$) and NAD^+ (from sp^2 to $sp^{2.34}$) in the TRS are also nonsynchronous with that in alcohol lagging behind that in NAD^+.[40] But, the Hammett study using the substituted benzyl alcohols gave a reaction constant of about zero, suggesting rehybridization precedes H-tunneling.[47] The quantitative comparison between the two systems manifests the role of protein motions in such catalysis: protein motions advance the geometry change of the system, providing favorable orbital conditions for H-tunneling to occur.[28,29]

In conclusion, the abnormal 2° KIEs observed in the hydride transfer reaction from 2-propanol to Xn^+ in solution were replicated from the Marcus-like H-tunneling model, a phenomenological model that emphasizes the link between environmental motions and H-tunneling. The model indicates an average TRS structure with imbalanced development in rehybridization and charge transfer in the alcohol moiety and nonsynchronous rehybridization between the donor and acceptor carbons. The computed relationship between DAD and both α- and β-2° KIEs reveals that the longer the DAD, the larger the magnitude of 2° KIE, reflecting steric effects (resulted from a 1° isotope effect) on the 2° KIEs. This 1° isotope effect on 2° KIEs may be expected to be more pronounced and thus observable in reactions occurring in restrictive environments such as the crowded and relatively rigid active site of enzymes.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Atom coordinates and absolute energies of the TRS structures with different DADs. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(45) Although there is no separation between vibrational and rotational modes at the nonstationary TRS point, we regard that it is still a valid approximation to calculate over 3N-6 modes. In order to examine the validity of this approximation, we calculated the 2° KIE for the TRS structure with the DAD of 3.1Å using the 3N modes. Our results show that there is no significant change to the computed 2° KIE.

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