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Substituent Effects on Temperature Dependence of Kinetic Isotope Effects in Hydride-Transfer Reactions of NADH/NAD$^+$ Analogues in Solution: Reaction Center Rigidity Is the Key

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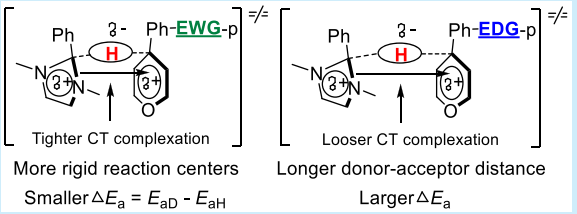
ABSTRACT: Substituent effects on the temperature dependence of primary kinetic isotope effects, characterized by $\Delta E_a = E_{aD} - E_{aH}$, for two series of the title reactions in acetonitrile were studied. The change from $\Delta E_a \approx 0$ for a highly rigid system to $\Delta E_a > 0$ for systems with reduced rigidities was observed. The rigidities were controlled by the electronic and steric effects. This work replicates the observations in enzymes and opens a new research direction that studies structure−ΔE_a relationship.



Kinetic isotope effect (KIE) is an important measure to study H-transfer reaction mechanisms. Within the semiclassical transition state (TS) theory, the maximum primary (1°) deuterium (D) KIE is about 9 and the isotopic activation energy difference ΔE_a (= $E_{aD} - E_{aH}$) is between 1.0 and 1.2 kcal/mol.[1,2] When KIE and ΔE_a are outside of their limits, the Bell model with a H-tunneling correction to the said theory is often used to rationalize them.[3] One extreme case of the Bell model is when ΔE_a is close to zero. That corresponds to a ground state tunneling where $E_{aH} = E_{aD} \approx 0$ and KIE is huge, which should happen at only extremely low temperature conditions.[1,2]

In the past two decades, however, it has been frequently observed that KIEs (both small and large) are temperature independent ($\Delta E_a \approx 0$) in the wild-type enzymes (*wt*-enzymes) around physiological temperature conditions, but they become temperature dependent to various extents with enzyme variants ($\Delta E_a > 0$ or even above the semiclassical limit).[4−15] A few contemporary H-tunneling theories have been established or used to explain the unusually small ΔE_a and its change relating to enzyme structures and further to attempt to provide information for the possible role of protein thermal motions in catalysis.[8,11,16−22] One largely used is the vibration-assisted activated H-tunneling (VA-AHT) model, which could include the Marcus-like model and TS theory extension, both of which involve a full H-tunneling process.[11,17,23] These phenomenological models presume that heavy atom motions bring H-donor and -acceptor to a tunneling-ready-state (TRS) where the activated reactant and product moieties have matching energy, allowing H-tunneling to occur over a range of donor−acceptor distances (DADs) sampled by the constructive heavy atom vibrations. Within that model, KIE is a function of DAD_{TRS}, and its temperature dependence is related to the density of DAD_{TRS} distributions.[21,24] Therefore, the $\Delta E_a \approx 0$ with *wt*-enzymes has been explained in terms of the well-organized reaction coordinate in which DAD_{TRS} is short and the range of DAD_{TRS}'s sampled is narrow. This could reason as the *wt*-enzyme has a densely packed active site whose heavy atom motions press the two reactants close to each other prohibiting them from being separated. In enzyme variants, however, the active site structure is impaired, the DAD_{TRS} becomes longer, and its fluctuation range becomes broader, leading to $\Delta E_a > 0$.

The link of DAD_{TRS} distributions to ΔE_a's has prompted us to start a new research direction to study the structure−ΔE_a relationship for the H-transfer reactions in solution.[25] Understanding of this relationship could not only provide insight into the above explanations for the observed trends of ΔE_a's in enzymes but also help find the appropriate models for H-transfer chemistry. Our hypothesis is, the more rigid the reaction centers, the more densely distributed the DAD_{TRS}'s, the weaker will be the temperature dependency of the KIEs (i.e., smaller ΔE_a). To investigate the hypothesis, the effect of system rigidity on ΔE_a's needs to be studied. In our research, we use the electronic and steric effects to control the rigidity of the reaction centers (Scheme 1). That is, a TRS with rigid reaction centers could be a tightly associated reactive complex with strong electronic interactions/attractions between H-donor and -acceptor, or with steric factors that minimize the flexibility of the reaction centers.[25]

As a preliminary work, we have recently reported the structural effects on ΔE_a's for hydride-transfer reactions of four

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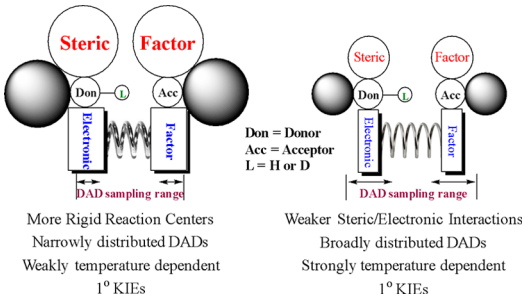
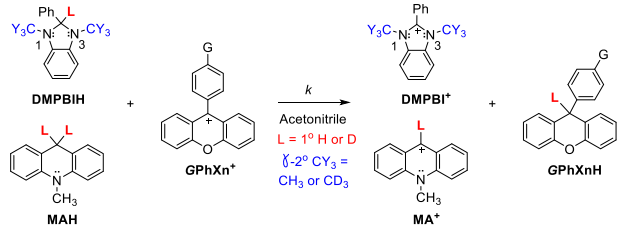
Scheme 1. Hypothesis Following the VA-AHT Model[25a]



[a]Two factors are used to control the rigidities of the reaction centers, electronic and steric effects. The thicker spring represents a stronger donor−acceptor electronic interaction.

very different NADH analogues with the same hydride acceptor (NAD^+ analogue) in acetonitrile.[25] One reason to choose these reactions to study is that they are enzyme model reactions so that results can be more directly compared with those from enzymes to provide insight into the possible role of enzyme thermal motions or DAD_{TRS} sampling in catalysis. The other reason is that these reactions are known to take place in charge-transfer (CT) complexes so that use of the electronic effects between reactants to control the rigidity could be managed.[25−27] The lowest and highest ΔE_a values were 0.37 and 1.52 kcal/mol, respectively, which correspond with the reactions of 1,3-dimethyl-2-phenylbenzimidazoline (DMPBIH) and 1-benzyl-1,4-dihydronicotinamide (BNAH) with 10-methylacridinium cation ($MA^+BF_4^-$). We found that a more rigid system, which corresponds to more narrowly distributed DAD_{TRS}'s, gave rise to a smaller ΔE_a, supporting the above explanations for enzymes and thus our hypothesis.[25] In that paper, we also raised a question as to whether $\Delta E_a \approx 0$ is unique to only *wt*-enzymes that the nature gives or a solution system can also be designed to make $\Delta E_a \approx 0$ happen. There, the four systems have large variations in donor structures and their electronic and steric effects are not clearly separated. To isolate the electronic effect for study, in this paper, we systematically studied the substituent effect on the ΔE_a's for the two series of hydride-transfer reactions from DMPBIH and 10-methylacridine (MAH) to the 9-*para*-substituted (*G*) phenylxanthylium ions ($GPhXn^+BF_4^-$, G = CN, CF_3, Br, H, CH_3O, $N(CH_3)_2$), respectively, in the same solvent (Scheme 2). We expect that the $GPhXn^+$ with an electron-withdrawing group (EWG), as compared to electron-donating group (EDG), will form a tighter CT/TRS complex and thus give a smaller ΔE_a. In the meantime, since DMPBIH has higher hydride releasing ability (by 15.4 kcal/mol[28,29]) and quite larger steric requirement than MAH at the reaction center, the reactions of the former donor are expected to be much more

Scheme 2. Hydride-Transfer Reactions Studied in This Work (G = CN, CF_3, Br, H, CH_3O, $N(CH_3)_2$)



rigid and give smaller ΔE_a values. It should be noted that one initial reason to choose $GPhXn^+$ as hydride acceptors is that they are severely sterically hindered and are strong electron acceptors for CT complexation.[29] Since we have reported a small ΔE_a of 0.37 kcal/mol for the reaction of DMPBIH with MA^+ of lower steric requirement and less electron affinity than $PhXn^+$,[25,29] the reaction of DMPBIH with $GPhXn^+$ would be expected to form a more rigid TRS and produce a smaller and possibly close-to-zero ΔE_a, especially when the substituent is a strong EWG.

Figure 1 shows the Arrhenius plots of the reactions of DMPBIH with selected $GPhXn^+$. The KIEs at 25 °C, E_{aH}

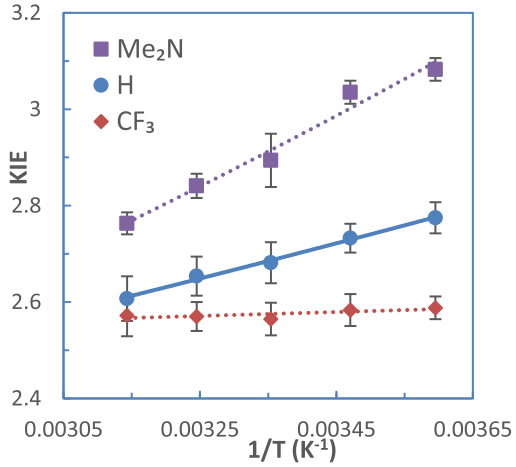


Figure 1. Arrhenius plots of the KIEs for the reactions of DMPBIH with selected $GPhXn^+$ (from 5 to 45 °C). Lines represent nonlinear regression to an exponential equation.

values, and ΔE_a values for the two series of reactions are listed in Table 1. Several features are immediately recognized: (1)

Table 1. Substituent Effects on Kinetics of Hydride-Transfer Reactions in Acetonitrile[a]

substituent (*G*)	$KIE^{25\ °C}$	E_{aH} (kcal/mol)	ΔE_a (kcal/mol)
	DMPBIH/$GPhXn^+$		
CN	2.62 (0.03)	2.83 (0.10)	0.04 (0.18)
CF_3	2.56 (0.03)	3.33 (0.05)	0.03 (0.07)
Br	2.55 (0.03)	3.70 (0.05)	0.07 (0.07)
H	2.68 (0.04)	4.13 (0.05)	0.27 (0.06)
CH_3O	2.74 (0.03)	4.58 (0.05)	0.55 (0.06)
$(CH_3)_2N$	2.89 (0.06)	7.52 (0.04)	0.50 (0.08)
	MAH/$GPhXn^+$		
CN	3.85 (0.03)	7.48 (0.05)	0.85 (0.06)
CF_3	4.06 (0.04)	7.59 (0.06)	0.89 (0.07)
Br	4.04 (0.03)	7.91 (0.06)	0.89 (0.07)
H	4.08 (0.03)	8.11 (0.04)	0.88 (0.05)
CH_3O	4.18 (0.04)	8.79 (0.08)	0.92 (0.16)
$(CH_3)_2N$	4.45 (0.05)	11.12 (0.08)	0.96 (0.18)

[a]Numbers in parentheses are standard deviations.

EWG facilitates the reaction; (2) the reactions of DMPBIH are faster than those of MAH; (3) both KIE and ΔE_a increase from reactions of $GPhXn^+$ with EWGs to EDGs; (4) the reactions of DMPBIH have smaller ΔE_a than those of MAH; and (5) $\Delta E_a \approx 0$ was found from the reactions of DMPBIH with $GPhXn^+$ of strong EWGs. It is important to note here that the small KIEs with $\Delta E_a \approx 0$ but $E_a \neq 0$ determined at around

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room temperature, like observed in *wt*-enzymes, strongly suggest H-tunneling mechanism but cannot be explained by the Bell model.

We have reported the CT absorptions of many similar systems that include DMPBIH and MAH as hydride donors as well.[25,27] Although it appears reasonable to expect that EWGs in GPhXn$^+$ would favor a tighter CT-complex in the TRS than EDGs (due to a more favorable ΔG°), we have determined the substituent effect in GPhXn$^+$ (G = CF_3, H, $(CH_3)_2N$) on the γ-2° KIEs at the N,N-$2CH_3/2CD_3$ position of DMPBIH for their reactions to attempt to verify the expectation. The 2° KIE originates from a decrease in negative hyperconjugation between the lone-pair electrons on N and σ^* orbital of the attached C-H/D bond due to the loss of electron density on N in the reaction.[30,31] This process with electron density loss tightens the C-H/D bonds, leading to an inverse 2° KIE. It is expected that an EWG would make a tighter CT complex so that the DMPBIH moiety at the TRS ends up with more electron density loss, equivalent to more positive charge gain, producing a more inverse 2° KIE. On the other hand, we are aware that the positive charge accumulation on DMPBIH is not solely from the CT complexation, the hydride-transfer from its 2-C-H bond cleavage also contributes to the accumulation of the positive charge at DMPBIH. Under the latter circumstances, however, according to the Hammond's postulate, GPhXn$^+$ with an EWG would form an early TRS so that less positive charge would be developed on DMPBIH producing less inverse 2° KIE. Our results in Table 2 show that

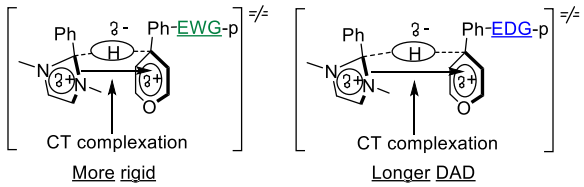
Table 2. Γ-$2CH_3/2CD_3$ 2° KIEs on DMPBIH and Charges at the DMPBIH Moiety of the TRS[a]

acceptor	γ-$2CH_3/2CD_3$ 2° KIEs on DMPBIH[b]	charge (ζ) carried at DMPBIH at the TRS
CF_3PhXn$^+$	0.89 (0.01)	0.58+ (0.05)
PhXn$^+$	0.91 (0.01)	0.47+ (0.05)
$(CH_3)_2$NPhXn$^+$	0.94 (0.02)	0.32+ (0.11)

[a]At 25 °C. [b]Numbers in parentheses are standard deviations.

the 2° KIEs are indeed inverse and the value increases from GPhXn$^+$ with CF_3 (0.89) to H (0.91) to $(CH_3)_2N$ (0.94). They strongly suggest that the *EWG*PhXn$^+$ forms a tighter CT complexation in the TRS structure (Scheme 3). By comparison

Scheme 3. CT Complexation at the TRS of Reactions of DMPBIH with GPhXn$^+$[a]



[a]Only the reactive rings of the reactants are drawn. The oval-shaped H represents a H-wave packet.

of the 2° KIEs with the equilibrium isotope effect (2° EIE = 0.81) for the conversion from DMPBIH to DMPBI$^+$ that reflects a gain of a full positive charge on N, the partial positive charge carried by the DMPBIH moiety at the TRS is calculated (ζ = (1−2° KIE)/(1−2° EIE)) and listed in Table 2 as well.[31] It decreases from the reactions of GPhXn$^+$ with CF_3 (0.58+) to H (0.47+) to $(CH_3)_2N$ (0.32+).

The above analyses suggest that the reactions of GPhXn$^+$ of EWGs with both DMPBIH and MAH, as compared to EDGs, would have more narrowly distributed DAD_{TRS}'s. Correlations of the trend of the DAD_{TRS} distributions with the observed smaller ΔE_a's in the reactions with EWGs and larger ΔE_a's with EDGs in both systems (Table 1) clearly indicate that a smaller ΔE_a results from a greater rigidity of the donor−acceptor centers. Moreover, the reactions of DMPBIH would produce the more rigid TRS's than the reactions of MAH due to the greater steric requirement and higher electron/hydride donating ability of the DMPBIH donor (see introduction). The observed smaller ΔE_a's in the reactions of DMPBIH (0−0.55 kcal/mol) than in the reactions of MAH (0.85−0.96 kcal/mol) also suggest that a more rigid system gives a smaller ΔE_a. All of these correlations between the reaction center rigidity and ΔE_a strongly support our hypothesis. Furthermore, we note that the extent of change in ΔE_a is much greater in the reactions of DMPBIH than in the reactions of MAH over the same range of substituents (Table 1). This suggests that ΔE_a is more sensitive to the electronic effect in a more rigid system. Importantly, as expected, the $\Delta E_a \approx 0$ was found in the reactions of DMPBIH with GPhXn$^+$ of a strong EWG (CN or CF_3). While the $\Delta E_a \approx 0$ is rarely seen in solution reactions, perhaps the more important discovery is that the result is associated with the most rigid TRS among the reactions.

To summarize, substituent/electronic effects on ΔE_a's for the two series of NADH/NAD$^+$ model reactions were studied to investigate the hypothesis that a more rigid system gives a smaller ΔE_a. Reactions with a tighter CT complexation between H-donor and acceptor and more crowded reaction centers give a smaller ΔE_a. $\Delta E_a \approx 0$ was found in the most rigid system. Therefore, $\Delta E_a \approx 0$ is not unique to the *wt*-enzyme catalyzed H-transfer reactions, and modification of the system rigidity could make $\Delta E_a \approx 0$ for the reactions in solution. All of the results strongly support our hypothesis. The change from $\Delta E_a \approx 0$ for a highly rigid system to $\Delta E_a > 0$ for systems with reduced rigidities in solution well replicates the trends of ΔE_a's observed in *wt*-enzymes versus variants. This supports the explanations in terms of the DAD_{TRS} sampling difference in relation to the densely packed active site in *wt*-enzymes and impaired loosely packed active site in their variants within the VA-AHT model. One other prediction from the latter model is that a longer DAD_{TRS} leads to a larger KIE.[32,33] This has indeed been observed in this work. In both series of reactions, both DAD_{TRS} and KIE increase from EWGs to EDGs (Table 1). Studies of the other predictions from the model is continuing in this lab.[34−37] Note that other contemporary H-transfer/tunneling theories have also been used to simulate the ΔE_a's observed in enzymes,[20,32,38−40] but none of them could predict a straightforward structure−ΔE_a relationship beforehand. We have not excluded the possibility that our results could be explained by these latter theories, but they can certainly add to the current debates on the appropriateness of models to describe H-transfer reactions in enzymes and solution.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c02049.

General procedures including syntheses and kinetic determinations, detailed kinetic data (PDF)

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Notes

The authors declare no competing financial interest.

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