

Supplementary Information

Imbalanced Tunneling Ready States in Alcohol Dehydrogenase Model Reactions: Rehybridization Lags behind H-Tunneling

Blake Hammann, Mortezaali Razzaghi, Sadra Kashfolgheta and Yun Lu*

Department of Chemistry, Southern Illinois University Edwardsville, Edwardsville, IL
62025, USA

General procedures

9-Phenylxanthylum tetrafluoroborate ($\text{PhXn}^+\text{BF}_4^-$) and xanthylum perchlorate ($\text{Xn}^+\text{ClO}_4^-$) were synthesized from the reactions of the corresponding alcohol precursors with HBF_4 or HClO_4 in propionic anhydride or acetic anhydride according to a published procedure.¹ 2-Propanol- β - d_6 ($(\text{CD}_3)_2\text{CHOH}$), 2-Propanol- α - d_1 ($(\text{CH}_3)_2\text{CDOH}$) and 2-Propanol- α - d_1 - β - d_6 ($(\text{CD}_3)_2\text{CDOH}$) were prepared by the reduction of acetone or acetone- d_6 with NaBH_4 or NaBD_4 according to a procedure described in literature.² 2-propanol-O-d ($(\text{CH}_3)_2\text{CHOD}$) was prepared by deuterium exchange of the OH group in the normal 2-propanol with DCl in D_2O in the N_2 atmosphere. The D content was determined by NMR to be 99.4% per C-D bond. In order to perform direct kinetic comparison, normal 2-propanol was also synthesized by reduction of acetone with NaBH_4 . The 9-deuterated xanthylum ion ($\text{Xn}(\text{D})^+$) was synthesized from 9-deuterated xanthidrol that was made from the reduction of xanthone by NaBD_4 in methanol/THF ($v/v = 1$). The D-content was found to be 96% by NMR. Acetonitrile was distilled over $\text{KMnO}_4/\text{K}_2\text{CO}_3$ and then P_2O_5 before use.

Kinetic measurement and data analysis

The procedure for the kinetic determination of the reactions of PhXn^+ was reported previously,³ and that for the reactions of Xn^+ is as follows. 2 mL of alcohol solution of certain concentration (e.g. 0.012 M) in MeCN was placed in a cuvette sitting in a thermostated UV-Vis cell holder. 32 μL of 0.0025 M Xn^+ stock solution (in MeCN) was rapidly transferred to the alcohol solution and the decay in absorbance (A) at 372 nm due to Xn^+ with time (t) was recorded for about 2 half-lives (Figure S1). The obtained $\ln(A) - t$ data were fitted to the pseudo-first-order rate law and the slope of the linear correlation ($R^2 > 0.997$, mostly > 0.999) was taken as the pseudo-first-order rate constant (k^{pfo}). Second-order rate constant ($k = k^{\text{pfo}} / [\text{alcohol}]$) and KIE ($=k_{\text{H}}/k_{\text{D}}$) were calculated. For the determination of small secondary KIEs, a back to back determination of kinetics of the reactions of H- and D- compounds was carried out in order to keep the consistent kinetic conditions. Typical determinations of the second-order rate constants and the 1° and 2° KIEs for each reaction are shown in Table S1.

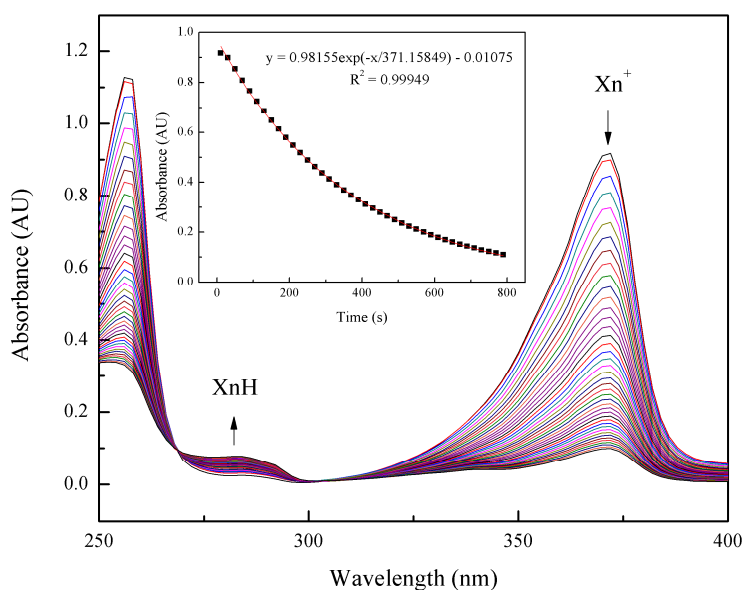


Figure S1. Kinetic scans for the reaction of 2-propanol (0.012 M) and Xn^+ (4×10^{-5} M) in MeCN at 60 °C. The inset shows the first-order exponential fit of the decay of absorbance at 372 nm with time.

Table S1. A typical determination of the 1° and 2° KIEs for the reactions in MeCN at 60°C^a

	k ($M^{-1}s^{-1}$)	1° KIE	β -D ₆ 2° KIE on 2-propanol	α -2° KIE on Xn^+
<u>Determination of the β-D₆ 2° KIEs on 2-propanol^b</u>				
<i>For the reaction of $CL_3CY(OH)CL_3$ with $PhXn^+$</i>				
Y = H, L = H	3.26×10^{-4}	3.39 (L = H)	1.05 (Y = H)	
Y = H, L = D	3.10×10^{-4}			
Y = D, L = H	9.63×10^{-5}		0.97 (Y = D)	
Y = D, L = D	9.64×10^{-5}			
<i>For the reaction of $CL_3CY(OH)CL_3$ with Xn^+</i>				
Y = H, L = H	0.256	2.76 (L = H)	1.05 (Y = H)	
Y = H, L = D	0.244			
Y = D, L = H	0.0927		1.06 (Y = D)	
Y = D, L = D	0.0870			

Determination of the α -2° KIEs on Xn(L)⁺c

For the reaction of CH₃CY(OH)CH₃ with Xn(L)⁺

Y = H, L = H	0.282	2.50 (L = H)	0.98 (Y = H)
Y = D, L = H	0.113		0.97 (Y = D)

Y = H, L = D	0.289		
Y = D, L = D	0.116		

For the reaction of CH₃CY(OH)CH(CH₃)₂ with Xn(L)⁺

Y = H, L = H	0.289	2.59 (L = H)	1.01 (Y = H)
Y = D, L = H	0.112		0.99 (Y = D)

Y = H, L = D	0.287		
Y = D, L = D	0.113		

For the reaction of cyclo-(CH₂)₅CYOH with Xn(L)⁺

Y = H, L = H	0.825	2.26 (L = H)	1.00 (Y = H)
Y = D, L = H	0.365		0.97 (Y = D)

Y = H, L = D	0.823		
Y = D, L = D	0.373		

^a from 3 to 4 repetitions under the consistent reaction conditions (same solutions and at the same day, back to back determinations of the reactions of H- and D- substrates), the KIEs reported in the *Communication* (Table 1) are the average of many (4 to 8) such determinations; ^b by Blake Hammann; ^c by Mortezaali Razzaghi.

Correction of 2° KIE_{obs} on Xn⁺

Since 9-deuterated xanthylum ion (Xn(D)⁺) contains 4% normal Xn⁺ (Xn⁺(H)) (from ¹H NMR), a correction of the observed 2° KIE_{obs} needs to be made. Using $k_{H(D)}$ to represent the rate constants of the reactions with Xn(H)⁺ and Xn(D)⁺, respectively, according to $2^\circ \text{KIE}_{\text{obs}} = k_{\text{H}} / (0.04k_{\text{H}} + 0.96k_{\text{D}})$, $2^\circ \text{KIE} = k_{\text{H}} / k_{\text{D}}$ was calculated and reported.

1. Dauben Jr, H. J.; Honnen, L. R.; Harmon, K. M. *J. Org. Chem.* **1960**, *25*, 1442-1445.
2. Thompson, M. S.; Meyer, T. J. *J. Am. Chem. Soc.* **1982**, *104*, 4106-4115.

3. Lu, Y.; Qu, F; Zhao, Y.; Small, A. M. J.; Bradshaw, J.; Moore, B. *J. Org. Chem.* **2009**, *74*, 6503-6510.