Evidence from Multiple Isotope Effect Determinations for Coupled Hydrogen Motion and Tunneling in the Reaction Catalyzed by Glucose-6-phosphate Dehydrogenase

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We recently described a method for determining the intrinsic isotope effects on the bond-breaking step of an enzyme-catalyzed reaction by measuring the ^{13}C isotope effect with substrate that was either unlabeled or deuterated in positions giving a primary or α-secondary isotope effect.[1] Together with the primary and α-secondary deuterium isotope effects and the various equilibrium isotope effects on the reaction, these values allow simultaneous solution of five equations for the intrinsic ^{13}C and primary and α-secondary deuterium isotope effects and the forward and reverse commitments for the bond breaking step.[2] We illustrated the use of this method with data for the oxidation of glucose 6-phosphate by TPN catalyzed by glucose-6-phosphate dehydrogenase, and the values we obtained in H_2O are in Table I. We have now repeated these experiments in D_2O, and these values are also in Table I. The intrinsic ^{13}C isotope effect is not appreciably changed, but there is a large decrease in the primary deuterium isotope effect and also an apparent decrease in the α-secondary deuterium isotope effect, although the errors in these latter values do not allow firm conclusions to be drawn concerning the secondary isotope effect.

We interpret these data in terms of the transition-state structure shown in Figure 1. We presume that three hydrogens are in motion in this transition state: (1) the hydrogen at C-1 of glucose 6-phosphate, which is transferred to TPN as a hydride ion, (2) the hydrogen of the hydroxyl at C-1, which is transferred as a proton to the base on the enzyme which has been identified from pH profiles as a carboxyl group,[4] and (3) the hydrogen at C-4 of the nicotinamide ring of TPN, which undergoes at 54° bend to the position it occupies in the product, TPNH. The decrease in the primary and α-secondary deuterium isotope effects when the hydroxyls of glucose 6-phosphate contain deuterium instead of hydrogen is indicative of (1) coupled motion of the three hydrogens in the transition state, with all three motions being part of the reaction coordinate motion, and (2) tunneling. When all three of the atoms in flight are hydrogen, they tunnel efficiently, but substitution of deuterium for any of the three drastically decreases the efficiency of tunneling, and thus the rate. Further deuterium substitutions then have less of an effect than the first.[5]

Table I. Intrinsic Isotope Effects and Commitments for Glucose-6-phosphate Dehydrogenase in H_2O and D_2O

parameter[a]	H_2O[b]	D_2O[c]
^{13}k	1.041 ± 0.002	1.044 ± 0.004
^{D}k	5.3 ± 0.3	3.7 ± 0.3
$^{\alpha\text{-}D}k$	1.054 ± 0.035	1.00 ± 0.04
c_f	0.75 ± 0.26	1.9 ± 0.6
c_r	0.49 ± 0.27	0.65 ± 0.40
$c_f + c_r$	1.24 ± 0.14	2.5 ± 0.3

[a] ^{13}k, ^{D}k, and $^{\alpha\text{-}D}k$ are intrinsic primary ^{13}C, primary deuterium, and α-secondary deuterium isotope effects on the hydride-transfer step, while c_f and c_r are forward and reverse commitments. [b] The values are from ref 1. [c] Values determined by the method in ref 1 from experimental ^{13}C isotope effects (from changes in the natural abundance at C-1 of glucose 6-phosphate) of 1.0110 ± 0.0001 with unlabeled substrates, 1.0242 ± 0.0006 with 1-deuterated glucose-6-phosphate, and 1.0114 ± 0.0006 with TPN deuterated at C-4 of the nicotinamide ring. The primary and α-secondary deuterium isotope effects on V/K for glucose 6-phosphate were 1.81 ± 0.11 and 0.98 ± 0.01.

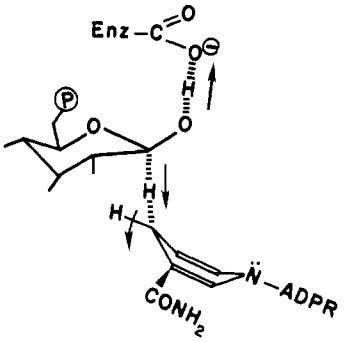

Figure 1. Transition-state structure for the glucose-6-phosphate reaction. The arrows show coupled hydrogen motions. During the reaction, C-1 of glucose becomes coplanar with the two oxygens and the carbon attached to it, and the bond order to the nascent carbonyl oxygen increases. The boat form of the ring with bond order 3 for N-1 is suggested by the large ^{15}N isotope effects (up to 1.07) seen at N-1 of the nicotinamide ring of DPN with liver alcohol[12] and formate[3] dehydrogenases and the fact that we have seen a similar ^{15}N isotope effect of 1.03 ± 0.01 in the glucose-6-phosphate dehydrogenase reaction with subsaturating glucose as the substrate in the presence of 30% dimethyl sulfoxide and 50 mM phosphate.

Similar examples of coupled hydrogen motions where the first deuterium substitution causes a larger isotope effect than the second include the proton exchanges between acetic acid and methanol when these molecules form a cyclic dimer in tetrahydrofuran (isotope effects of 5.1 and 3.1), and the rotation of the two protons in the central hole of tetraphenylporphine (isotope effects of 10 and 1).[6] In the oxidation of formate by DPN or its analogues catalyzed by formate dehydrogenase,[3] the α-secondary deuterium isotope effects at C-4 of the nicotinamide ring of the nucleotides showed values varying from 1.23 with DPN to 0.99 with the very slow substrate pyridinecarboxaldehyde–DPN, but in every case the use of deuterated formate decreased the α-secondary deuterium isotope effect halfway to the equilibrium isotope effect, which is 0.89 for such a reaction.[7] The normal values of the α-secondary deuterium isotope effects demonstrate that the motions of the primary and α-secondary hydrogens are coupled in the transition state (although the degree of coupling varies), but the effect of deuteration on the isotope effects shows that tunneling is involved. Similar α-secondary deuterium isotope effects that are decreased by primary deuteration have also been seen for nonenzymatic hydride-transfer reactions,[8] so the phenomenon is not unique to enzyme-catalyzed reactions.

(1) Hermes, J. D.; Roeske, C. A.; O'Leary, M. H.; Cleland, W. W. *Biochemistry* **1982**, *21*, 5106.

(2) An intrinsic isotope effect is the ratio of the rate constant (k_{light}/k_{heavy}) for the individual isotope sensitive step. For example, the intrinsic primary deuterium isotope effect, ^{D}k, is the ratio of rate constants k_H/k_D for the hydride transfer step. A commitment is the ratio of the rate constant for bond breaking to the net rate constant for release from the enzyme of a reactant (the labeled substrate when natural abundance ^{13}C labels are used or the one whose concentration is varied when rates with deuterated and unlabeled reactants are compared, the first product released for the reverse commitment). See: Cook, P. F.; Cleland, W. W. *Biochemistry* **1981**, *20*, 1790. It should be noted that our calculation of the intrinsic isotope effects and the commitments using the methodology of ref 1 assumes that the intrinsic ^{13}C isotope effect is independent of primary deuterium substitution of the substrate. This seems reasonable, since in the case of formate dehydrogenase, where no commitments exist and one sees the intrinsic isotope effects directly,[3] the ^{13}C isotope effect was the same with deuterated and unlabeled formate.

(3) Hermes, J. D.; Morrical, S. W.; O'Leary, M. H.; Cleland, W. W. *Biochemistry*, in press.

(4) Viola, R. E. *Arch. Biochem. Biophys.* **1984**, *228*, 415.

(5) It might be argued that proton removal from the OH of glucose 6-phosphate occurs prior to or following the hydride transfer step, so that in D_2O hydride transfer is somewhat less rate limiting. However, the primary deuterium isotope effect is reduced in D_2O to a greater extent than the ^{13}C ones, which is inconsistent with such a model. Further it is difficult to imagine proton transfer from a hydroxyl group to a carboxylate to give an alkoxide or, in the reverse direction of the other possibility, proton transfer from a carboxylic acid to the carbonyl oxygen of the lactone. We thus conclude that proton transfer is concerted with hydride transfer.

(6) Limbach, H.-H.; Hennig, J.; Gerritzen, D.; Rumpel, H. *Faraday Discuss. Chem. Soc.* **1982**, *74*, 229.

(7) Cook, P. F.; Blanchard, J. S.; Cleland, W. W. *Biochemistry* **1980**, *19*, 4853.

(8) Ostovic, D.; Roberts, R. M. G.; Kreevoy, M. M. *J. Am. Chem. Soc.* **1983**, *105*, 7629.

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Huskey and Schowen,[9] Saunders,[10] and Limbach et al.[6] have all published calculations which confirm that coupled motion plus tunneling are required to explain effects similar to those in Table I. We have also modeled the glucose-6-phosphate hydrogenase reaction by use of the BEBOVID-IV program of Sims et al.[11] In order to predict the values of the deuterium isotope effects in Table I and maintain realistic bond orders in the C···H···C system for hydride transfer, it was necessary to let the imaginary frequency be $\sim$1000 cm^{-1} so that tunneling became appreciable. Such a model could predict the deuterium isotope effects seen in H_2O and D_2O but also predicted a different ^{13}C isotope effect in the latter solvent as the result of tunneling. Further theoretical work is clearly needed to determine the reasons why the hydrogen motions appear to involve tunneling, while that of carbon does not.

The data in Table I show one other striking effect, namely, an *increase* in the commitments in D_2O. Since one expects a solvent isotope effect on the proton transfer which accompanies the hydride migration, the commitments are predicted to *decrease* unless D_2O causes larger effects on the steps that set the stage for catalysis than on the proton-transfer step itself. These data emphasize the danger of assuming that D_2O solvent isotope effects will not be seen on conformation changes of enzymes![13]

Acknowledgment. This work was supported by NIH Grant GM 18938.

(9) Huskey, W. P.; Schowen, R. L. *J. Am. Chem. Soc.* **1983**, *105*, 5704.
(10) Saunders, W. H.; Jr. *J. Am. Chem. Soc.* **1984**, *106*, 2223.
(11) Sims, L. B.; Burton, G.; Lewis, D. E., BEBOVIB-IV, Program 337, Quantum Chemistry Exchange Program, Department of Chemistry, Indiana University, Bloomington, IN.
(12) Cook, P. F.; Oppenheimer, N. J.; Cleland, W. W. *Biochemistry* **1981**, *20*, 1817.
(13) Actually it is not at all uncommon for D_2O to change the rates of conformation changes in proteins. See: Schowen, K. B. In "Transition States of Biochemical Processes"; Gandour, R. D., Schowen, R. L., Eds.; Plenum Press: New York, 1978; pp 225-283.

New Organoiron Synthons. *cis*- and *trans*-Vinylene Dication Equivalents

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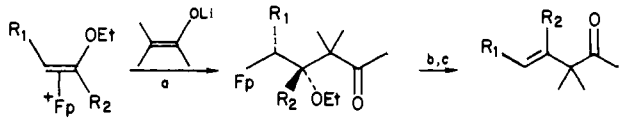
We have reported the use of vinyl ether-iron complexes (**1**) as synthetic surrogates for vinyl cations.[1] These reagents are easily prepared from α-halo acetals or ketals by successive metalation with NaFp (Fp = η^5-$C_5H_5Fe(CO)_2$) and acid-promoted elimination of alcohol[2] and may be stored without decomposition at 0 °C. They react rapidly with enolates at −78 °C to give adducts **2** resulting from stereospecific trans addition to the Fp–olefin bond. These are in turn transformed to vinyl, *trans*-propenyl, and isopropenyl ketones by successive acid-promoted elimination of ethanol and demetalation.

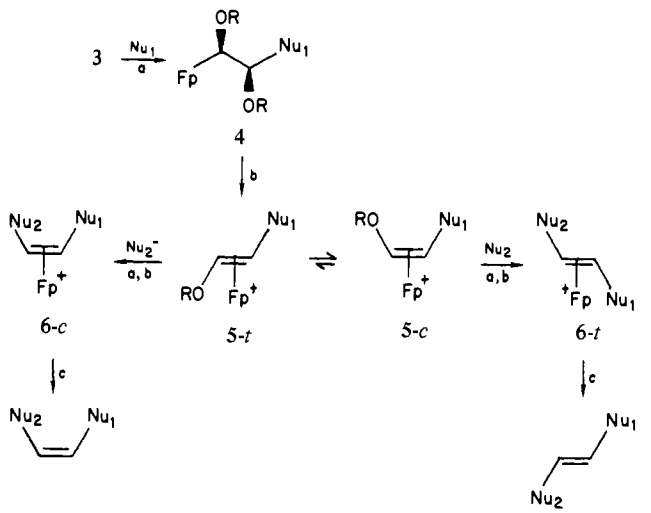


R_1, R_2 = H; R_1 = H, R_2 = Me; R_1 = Me, R_2 = H

(a) THF, −78 °C, 1 h; (b) HBF_4·Et_2O, CH_2Cl_2, −78 °C; (c) NaI, acetone, 25 °C, 0.5 h

(1) Rosenblum, M.; Bucheister, A.; Chang, T. C. T.; Cohen, M.; Marsi, M.; Samuels, S. B.; Scheck, D.; Sofen, N.; Watkins, J. C. *Pure Appl. Chem.* **1984**, *56*, 129 and references therein.
(2) Cutler, A.; Raghu, S.; Rosenblum, M. *J. Organomet. Chem.* **1974**, *77*, 381.

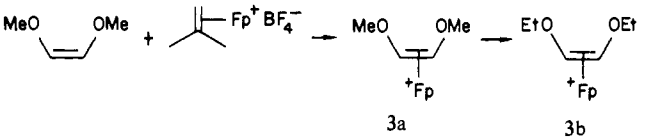
Scheme I[a]



[a] (a) THF, −78 °C, 0.5–1.0 h; (b) HBF_4·Et_2O, −78 °C then Et_2O; (c) CH_3CN, Δ, 2 h or NaI, acetone, 25 °C, 0.5 h.

We now find that the closely related (1,2-dialkoxyethylene)iron complexes **3** may be made to serve as either *cis*- or *trans*-vinylene dication equivalents, thus providing a new and more general route to *cis*- as well as *trans*-alkenyl cation equivalents.

Complex **3a**[3] may be prepared in multigram quantities by exchange complexation of *cis*-1,2-dimethoxyethylene[4] with Fp(isobutylene)BF_4 (CH_2Cl_2, 40 °C, 2 h, 95%).[5] This salt is transformed quantitatively to **3b** by briefly slurring in ethanol at room temperature and reprecipitating with ether.[6]



Both **3a** and **3b** are yellow crystalline, nonhygroscopic, air-stable solids, which may be stored at 0 °C. They react rapidly in THF suspension or in solutions of CH_2Cl_2 at −78 °C with a broad range of carbon nucleophiles (Table I) to give neutral adducts **4**.[7] These, on exposure to HBF_4 etherate at −78 °C, are converted to *trans*-alkenyl ether complexes **5**-*t* (Scheme I).[8] The *trans*-alkenyl complexes **5**-*t* may then be subjected to a second nucleophilic addition to give the *cis*-Fp(olefin) complex **6**-*c* and finally the demetalated cis olefin.

Alternatively, since the barrier to rotation about the double bond in Fp(vinyl ether) cations is comparatively low[9] and since cis metal–olefin complexes are in general thermodynamically more stable than their trans isomers,[10] isomerization of **5**-*t* to **5**-*c* may be effected by allowing solutions of **5**-*t* to stand at room tem-

(3) Weinberg, E. L.; Burton, T. J.; Baird, M. C.; Herberhold, M. *Z. Naturforsch. B: Anorg. Chem., Org. Chem.* **1981**, *36B*, 485.
(4) McElvain, S. M.; Stammer, C. H. *J. Am. Chem. Soc.* **1951**, *73*, 915.
(5) Cutler, A.; Ehntholt, D.; Giering, W. P.; Lennon, P.; Raghu, S.; Rosan, A.; Rosenblum, M.; Tancrede, J.; Wells, D. *J. Am. Chem. Soc.* **1976**, *98*, 3495. Exchange complexation of *trans*-1,2-dimethoxyethylene is reported[3] to give **3a** but in low yield.
(6) This exchange is effected to avoid potential nucleophilic attack at the alkyl center. Complex **3b** cannot be obtained directly by exchange complexation of 1,2-diethoxyethylene.
(7) The threo structure assigned to **4** is supported by the NMR spectra of the adducts derived from the addition of phenylmagnesium bromide or cyclohexanonelithium enolate to **3b**, which show J_{ab} = 3 Hz, in accord with the structure **4** (Nu_1 = Ph, 2-cyclohexanone).
(8) These all show J_{ab} = 11 Hz, while the cis isomers show J_{ab} = 6 Hz.
(9) Chang, T. C. T.; Foxman, B. M.; Rosenblum, M.; Stockman, C. *J. Am. Chem. Soc.* **1981**, *103*, 7361.
(10) Herberhold, M. "Metal π-Complexes"; Elsevier: Amsterdam, 1972; Vol. II. Hartley, F. R. *Chem. Rev.* **1973**, *73*, 163.

0002-7863/84/1506-7264$01.50/0