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Transition-State Structure for the Quintessential S_N2 Reaction of a Carbohydrate: Reaction of α-Glucopyranosyl Fluoride with Azide Ion in Water

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Supporting Information

ABSTRACT: We report that the S_N2 reaction of α-D-glucopyranosyl fluoride with azide ion proceeds through a loose (exploded) transition-state (TS) structure. We reached this conclusion by modeling the TS using a suite of five experimental kinetic isotope effects (KIEs) as constraints for the calculations. We also report that the anomeric ^{13}C-KIE is not abnormally large (k_{12}/k_{13} = 1.024 ± 0.006), a finding which is at variance with the previous literature value (Zhang et al. *J. Am. Chem. Soc.* **1994**, *116*, 7557).

The transfer of a carbohydrate moiety from one molecule to another is a fundamental biochemical transformation that occurs in all biological systems. Enzymes that catalyze these reactions are ubiquitous; typically, about 1−3% of an organism's genome encodes carbohydrate-transferring proteins,[1] a statistic that underscores the importance of this function. Determining the mechanisms of catalysis used by these enzymes, which include glycoside hydrolases and glycosyltransferases, is an active area of research. The mechanistic details of non-enzymatic reactions of glycosides provide a valuable foundation for understanding how enzymes accelerate these essential reactions.[2,3] A critical mechanistic question relevant to reactions at carbohydrate acetal centers is whether the reaction involves formation of an oxacarbenium ion intermediate.[4] In particular, does the reaction of a glycosyl derivative occur through a concerted S_N2 mechanism[5] to give an inverted product or via a stepwise mechanism in which product formation occurs at the stage of intimate,[6] solvent-separated,[7] or solvent-equilibrated[6] ions?

Increasingly, scientists are using transition-state (TS) structure modeling to study enzyme-catalyzed reactions.[8,9] This approach relies on the notion that the optimal experimental approach for TS structure determination is to measure multiple kinetic isotope effects (KIEs) and to use these values as constraints for theoretical modeling of the TS.[10,11] This strategy gives information about key TS features including geometry, charge development, and extent of bond cleavage and bond formation at the reaction center.[8]

The measurement of reaction center carbon KIEs has been used as a diagnostic tool for distinguishing between $D_N + A_N$ (S_N1) and A_ND_N (S_N2) mechanisms.[12] Specifically, the first measured carbon-KIE for a S_N1 reaction, the solvolysis of *tert*-butyl chloride, exhibits a low ^{14}C-KIE value (k_{12}/k_{14} = 1.027,[13] which equates to a ^{13}C-KIE of ∼1.014; see eqn S1 in the Supporting Information),[12] whereas S_N2 mechanisms such as the concerted reaction of methyl halide with cyanide typically exhibit much larger reaction center ^{13}C-KIE values (k_{12}/k_{13} = 1.07−1.08).[14,15] These early studies led to the generalization as outlined by Melander and Saunders, "Carbon isotope effects in S_N2 reactions are usually fairly large with k_{12}/k_{13} and k_{12}/k_{14} values of 1.03−1.08 and 1.06−1.15, respectively."[12]

Nucleophilic substitution reactions for most acetals (R′O−CHR−OR′′), which contain few electron-withdrawing groups, generally proceed via discrete oxacarbenium ion intermediates (S_N1 mechanisms) due to stabilization of positively charged species by the second oxygen atom.[16−19] In contrast, nucleophilic substitution reactions on carbohydrate acetal linkages straddle the mechanistic border between S_N1 and S_N2 reactions by virtue of the inherently short lifetimes of oxacarbenium ion intermediates; as a result, reactions via ion-pairs become important.[20] For instance, in water the glucopyranosylium ion has an estimated lifetime of approximately 1−3 ps.[7,18] In addition, studies have shown that, during alcoholysis of alkyl furanosides, cyclic oxacarbenium ions are not intermediates (these reactions involve a concerted S_N2 ring-opening mechanism).[21−23]

For reactions of *N*- and *O*-glycosides in aqueous media, the measured reaction center (anomeric carbon) ^{13}C-KIEs are typically in the range 1.002−1.011, which equates to ^{14}C-KIE values of 1.004−1.020 (eqn S1, Supporting Information),[12] and are indicative of dissociative S_N1 ($D_N{}^*A_N$) reactions that proceed via short-lived oxacarbenium ion intermediates.[24−27] For example, methyl α-D-glucopyranoside undergoes an acid-catalyzed reaction that exhibits a ^{13}C-KIE (k_{12}/k_{13}) of <1.01, which occurs via unimolecular dissociation of the protonated glycoside ($D_N{}^{\ddagger}{}^*A_N$) to give a short-lived oxacarbenium ion intermediate.[25,28] With regard to enzyme-catalyzed glycosyl transfer, typical anomeric carbon ^{13}C-KIEs are in the range of 1.002−1.018 for stepwise S_N1 reactions[25,29−31] and 1.018−1.032 for concerted S_N2[25,26,32,33] mechanisms. The utility of experimental anomeric carbon KIE values as a tool to verify or discount mechanisms has recently been applied to several synthetic reactions of carbohydrates at low temperatures.[34]

Strikingly, the reaction of α-D-glucopyranosyl fluoride with azide ion via a concerted S_N2 reaction[5] was reported to exhibit an anomeric ^{13}C-KIE of 1.085,[35] which is in the range typically associated with reactions occurring at saturated alkyl carbon

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atoms. The anomalously large anomeric ^{13}C-KIE for the aqueous S_N2 reaction of the configurationally stable α-glucopyranosyl fluoride with azide piqued our interest, and as a result we decided to solve the TS structure for the reaction using *ab initio* methods. This method requires the use of multiple experimental KIE values as constraints for the theoretical calculations. Therefore, we needed to complement the two reported KIE values for the reaction (^{13}C-1 and ^{2}H-1) with measurements for three other glucopyranosyl fluoride isotopologues (^{18}O-5, ^{2}H-2, and ^{2}H-5).

Herein we report a detailed KIE study on the S_N2 reaction of α-D-glucopyranosyl fluoride with azide ion and an *ab initio*-computed TS structure. The resultant computational TS for this S_N2 reaction is characterized by an essentially broken C−F bond, with the bond order for the concurrently forming C−N bond being small; i.e., the TS is loose. It appears that loose TS structures are a general phenomenon for concerted S_N2 reactions of glycosides, including those that are base-catalyzed,[36] spontaneous,[5] and enzyme-catalyzed.[25,37]

Recent advances in NMR spectroscopic methods augment the experimental protocols available for measuring KIEs.[38−42] We recently showed that ^{19}F NMR spectroscopy allows the simultaneous measurement of up to three KIEs (^{13}C-1, ^{2}H-1, and ^{2}H-2) during the solvolysis of α-D-glucopyranosyl fluoride in hexafluoro-2-propanol.[6] In the study presented here, we synthesized the six isotopologues of α-D-glucopyranosyl fluoride required to measure KIEs and subsequently characterized the TS for its reaction with azide ion (Supporting Information). We began the study by using the new ^{19}F NMR spectroscopy method to determine KIE values for the reaction of α-glucopyranosyl fluoride with azide ion using the same reaction conditions (0.2 M sodium succinate buffer, 2.0 M sodium azide, pH 6.0 at 50.0 °C) reported previously in the literature.[35] The data acquired while monitoring isotopologue ratios by ^{19}F NMR spectroscopy are fit to eq 1, where R is the

$$\frac{R}{R_0} = (1 - F_1)^{1/\mathrm{KIE}-1} \quad (1)$$

ratio of heavy to light isotopologues for the remaining starting material at time t ($[H]_t/[L]_t$), R_0 is the same ratio at $t = 0$, and F_1 is the fraction of reaction for the light isotopologue.[12]

We derived a value for the α-secondary deuterium KIE (α-SDKIE; k_H/k_D = 1.192) that was similar to that reported,[35] but the anomeric ^{13}C-KIE (k_{12}/k_{13}) measured using our NMR method[6,40] gave a calculated KIE value (1.024) that was worryingly different from the published value of 1.085 (Figure 1).[35]

Experimentally determined KIE values often provide the cornerstone data for the calculation of reaction TS structure. We therefore deemed it essential to investigate the origins of the discrepancy between our measured KIE and the published value,[35] which was determined using the quasi-racemate method.[43] Accordingly, we made unlabeled α-L-glucopyranosyl fluoride in order to perform independent KIE measurements on the anomeric carbon and β-secondary deuterium isotopologues using both our ^{19}F NMR spectroscopic method and the quasi-racemate method.[28,43,44] Figure 2 presents the data and the associated fit to eq 2 for a representative quasi-racemate

$$\alpha = A\, e^{-k_L t} + B\, e^{-k_L t/\mathrm{KIE}} + C \quad (2)$$

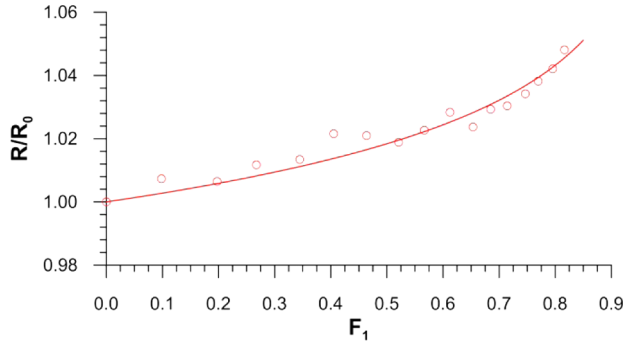

Figure 1. Typical plot of the change in integrated peak intensity ratio (R/R_0) vs F_1 for the competitive ^{13}C-KIE measurement using ∼1.5 mg of both α-D-(1-^{13}C)glucopyranosyl fluoride and α-D-glucopyranosyl fluoride with azide ion (2 M) in succinate buffer (0.2 M, pH 6.0) at 50 °C. D_2O (∼2% of the total volume) was added to facilitate signal locking. The red line is the best non-linear least-squares fit of the experimental data to eq 1.

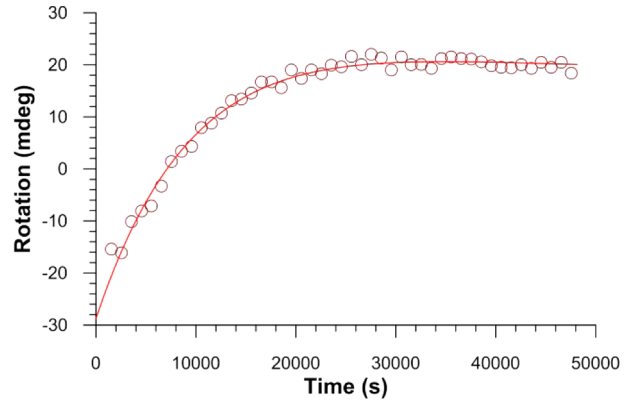

Figure 2. Plot of optical rotation (404 nM) versus time for the "quasi-racemate" reaction of both α-D-(1-^{13}C)glucopyranosyl fluoride and α-L-glucopyranosyl fluoride (∼5 mg/mL) with azide ion (2 M) in succinate buffer (0.2 M, pH 6.0) at 50 °C. Every 20th data point is shown, and the red line is the best non-linear least-squares fit of the experimental data to eq 2.

experiment in which we used concentrations of ∼5 mg/mL of both α-D-(1-^{13}C)glucopyranosyl fluoride and α-L-glucopyranosyl fluoride. For eq 2, α is the measured optical rotation, A and B are the changes in rotation for reactions that contain only a single isotopologue (α-L-glucopyranosyl fluoride for A, labeled α-D-glucopyranosyl fluoride for B), C is the final rotation, k_L is the rate constant for reaction of the light isotopologue, and KIE = k_L/k_H.

A summary of the KIE values obtained from these quasi-racemate experiments along with those we determined using ^{19}F NMR spectroscopy is given in Table 1. (All individual KIEs values are listed in Table S1, Supporting Information.)

Of note, the KIE values we determined using ^{19}F NMR spectroscopy and polarimetry are within experimental error of one another (Table 1). Also, our measured value of 1.192 for the α-SDKIE is similar to the reported value of 1.169.[35] In our polarimetry experiments, we measured a rate constant of (8.54 ± 0.28) × 10^{-5} s^{-1} that is associated with an optical rotation increase of 0.204 ± 0.004°/(mg/mL) for reactions of α-L-glucopyranosyl fluoride (4.85−5.13 mg/mL) with azide ion at a wavelength of 404.7 nm (Hg emission line) in a 1 dm path length cell. Similarly, Zhang et al. reported a rate constant of 8.3

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Table 1. Kinetic Isotope Effects for the Nucleophilic Substitution Reaction of α-D-Glucopyranosyl Fluoride with Azide Ion[a]

isotope site	^{19}F NMR-determined KIE (n)	quasi-racemate KIE (n)	KIE calcd TS_0	KIE calcd TS_1
α-D (C1)[b]	1.192 ± 0.006 (7)	n.d.[c]	1.107	1.197
β-D (C2)	1.046 ± 0.007 (3)	1.047 ± 0.010 (4)	0.965	1.041
γ-D (C5)	0.987 ± 0.004 (3)	n.d.	0.972	0.986
1-^{13}C[d]	1.024 ± 0.006 (3)	1.022 ± 0.007 (3)[e]	1.056	1.034
5-^{18}O	0.981 ± 0.003 (3)	n.d.	1.005	1.011

[a]Succinate buffer (0.2 M, pH 6.0) containing azide ion (2 M) at 50 °C (number of independent KIE determinations). [b]k_H/k_D = 1.169 ± 0.008 (ref 35, quasi-racemate). [c]n.d. = not determined. [d]k_{12}/k_{13} = 1.085 ± 0.008 (ref 35, quasi-racemate). [e]Fit to eq 2 involved constraining k_L to be within 10% of that measured in a reaction containing only α-L-glucopyranosyl fluoride.

× 10^{-5} s^{-1} that is associated with an increase in rotation of 0.600° for a 3 mg/mL reaction monitored at 404 nm (path length 1 dm).[35]

To investigate the source of the difference between measured KIEs, we reevaluated the polarimetric data reported by Zhang et al.[35] Our fit of their data to eq 2 gave a calculated KIE (k_{12}/k_{13}) of 1.025 (Figure S1, Supporting Information). On the basis of this finding, we propose that the reported large ^{13}C-KIE likely is the result of a mathematical error. Taking into consideration this reevaluated result, our suite of five KIE measurements clearly indicates that, during the reaction of α-D-glucopyranosyl fluoride with azide ion, the anomeric center is undergoing reaction via a loose (exploded) S_N2 TS, a situation that gives rise to the large α-SDKIE and small anomeric ^{13}C-KIE. Moreover, for this TS, the inverse ring ^{18}O-KIE and the β-SDKIE data (Table 1) suggest that the pyranosyl ring is stabilizing a partial positive charge.

To refine the general characteristics for this S_N2 TS structure, we calculated KIEs for the reaction of α-D-glucopyranosyl fluoride with azide ion using Gaussian 09 with the B3LYP method and the 6-31G* basis set,[45] with all calculations performed at 323.15 K (50 °C). We used a polarizable continuum model, rather than including explicit solvent water molecules, to approximate the experimental conditions. The α-D-glucopyranosyl fluoride ground-state structure was optimized starting from several $^4C_1$ chair conformations, which did not contain five-membered ring intramolecular C−F···H−O hydrogen bonds, to ensure that the structure located is a local minimum without a weak, bent[46] hydrogen bond to the anomeric fluorine atom.[47] This structural model was modified by positioning an azide ion on the β-face of the pyranose ring (a location that is opposite to the fluoride leaving group), and a C−N distance of 3.5 Å was set between the anomeric carbon and the nucleophilic nitrogen atom. Likewise, the C−F bond distance was increased to 3.5 Å. Using this initial geometry as a starting point, we located a transition-state structure (TS_0) that was consistent with a concerted S_N2 (A_ND_N) reaction. The calculated KIE values for this TS are listed in Table 1, and the TS structure is shown in Figure S2 (Supporting Information). Clearly, the calculated KIE values do not match the values determined experimentally. Thus, we independently varied both the C−N and C−F bond distances, which in TS_0 were 1.99 and 2.13 Å, respectively, by 0.1 Å increments until we obtained a matched TS structure that possessed one major imaginary frequency (Figure 3). Of note, we varied the O5−C1−N_{nuc}−N_α dihedral angle in the C−N-constrained model to verify that the spatial orientation of the azide ion did not significantly alter the calculated KIE values. Interestingly, all attempts to obtain a match between the calculated and experimental ^{18}O-KIE values were unsuccessful; that is, the KIE was normal in all calculated TS structures, including that for TS_0 (Table 1). It is possible that, if we had used explicit solvation, which is expected to change dramatically between the ground state and the transition state, instead of the polarizable continuum model, we might have obtained a better match between the calculated ^{18}O-KIE and the experimental value.

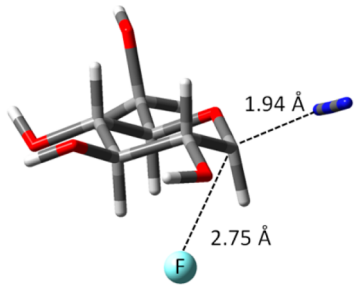

Figure 3. Transition-state model (TS_1) for the reaction of α-glucopyranosyl fluoride with azide ion.

In summary, our KIE results for the well-characterized S_N2 reaction of α-D-glucopyranosyl fluoride with azide ion are consistent with the reaction proceeding via a loose (exploded) transition state in which the bond orders to the nucleophile and the leaving group are small. We conclude that reactions of glycopyranosides in aqueous media either occur by way of such a TS or involve the formation of non-equilibrated cyclic oxacarbenium ions. Moreover, a distinguishing characteristic between these two mechanistic possibilities is the magnitude of the anomeric ^{13}C-KIEs, with k_{12}/k_{13} values of <1.016 and >1.020 being diagnostic for dissociative and concerted reactions, respectively.

■ ASSOCIATED CONTENT

Supporting Information

Complete ref 45, experimental methods, Tables S1−S4, and Figures S1 and S2. 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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