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Computational Simulation of the Lifetime of the Methoxymethyl Cation in Water. A Simple Model for a Glycosyl Cation: When Is an Intermediate an Intermediate?

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A two-dimensional free-energy surface is constructed for transfer of the methoxymethyl cation between two water molecules. These atoms are treated quantum mechanically within a box of >1000 classical solvent water molecules, and the molecular dynamics of the whole system is considered at 300 K. This provides a simple model for glycosyl transfer in water. The best surface obtained (MPWB1K/6-31+G(d,p) corrected AM1/TIP3P) contains a shallow free-energy well corresponding to an oxacarbenium ion intermediate in a stepwise mechanism. Molecular dynamics analysis at three temperatures leads to a classical estimate of the lifetime of the methoxymethyl cation in water; when quantum corrections for vibrational zero-point energy are included, the lifetime is estimated to be 1 ps. This result is in complete agreement with the best experimental estimate and suggests that computational simulation is a reliable tool for elucidation of glycosyl-transfer mechanisms in enzymes and whether these involve glycosyl cations as intermediates.

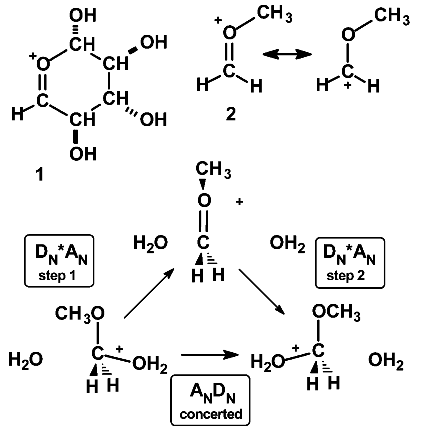
1. Introduction

Glycosides are ubiquitous in biology,[1] from the "bulk chemicals" aspect of organismal cell wall materials and biomass conversion through to the "fine chemicals" aspect of post-translational modification of proteins critical to specific cellular biological functions, and thus, the enzymes (glycosyltransferases) that control these chemical processes are immensely important.[2] In particular, glycoside hydrolases, which catalyze the transfer of a glycosyl unit to water, thereby breaking polysaccharides into smaller fragments, have both historical and topical relevance. For example, lysozyme was the first enzyme whose structure was determined by X-ray crystallography,[3] and neuraminidase is a key target for anti-influenza drug therapy.[4] However, the mechanism of action of these two glycosidases raises an old controversy: does hydrolysis proceed by way of a covalently bound glycosyl-enzyme intermediate[5] or an unbound glycosyl cation?[6]

Following Phillips[6] and Vernon,[7] it was long held that a carbocationic intermediate (an oxacarbenium ion: oxygen formally replaces carbon adjacent to the electron-deficient center) was involved, being stabilized electrostatically by a nearby negatively charged aspartate residue in the enzyme active site.[8] Experimental evidence[9] has since unambiguously demonstrated the involvement of the covalent intermediate bound to Asp52[10] (although this does not exclude the possibility of a carbocationic intermediate in hydrolysis of the covalent intermediate). Nonetheless, despite recent evidence for a covalent intermediate in bacterial neuraminidases,[11−15] it seems that an oxacarbenium intermediate (which was the basis for design of the inhibitors zanamivir[16] and oseltamivir[17]) is still considered by some to be involved in the mechanism of influenza neuraminidase.[18]

Glycoside hydrolysis mechanisms teeter on the borderline between stepwise $D_N + A_N$ (S_N1) and concerted A_ND_N (S_N2) mechanisms.[19] The key point is the lifetime of the oxacarbenium ion as a putative intermediate. Extrapolations of kinetic measurements using the azide-clock method for a range of methoxy-substituted carbenium ions in water led Amyes and Jencks[20] to conclude that the stability of the glucosyl cation **1** (see Scheme 1) was similar to that of the methoxymethyl cation **2** and to estimate the lifetime in aqueous solution as ~1 ps. In the presence of a neutral nucleophile or nucleofuge, these cations exist for longer than a typical bond vibration (0.01−0.1 ps) but shorter than the typical time scales for reorganization within an ion pair (~10 ps) or diffusional separation (~60 ps) of an ion pair; thus, neither has time to become equilibrated with dilute solutes or solvent molecules prior to their capture.[21] Consequently, substitutions involving these cations with neutral nucleophiles require preassociation of the reacting species in a $D_N{*}A_N$ mechanism or with anionic nucleophiles are enforced to be concerted.[22,23] Methoxymethyl derivatives undergo A_ND_N substitution reactions via "exploded" transition states with significant oxacarbenium ion character: there is little bond formation to the nucleophile and almost complete breaking of the bond with the leaving group.[24,25] Specific acid-catalyzed

SCHEME 1



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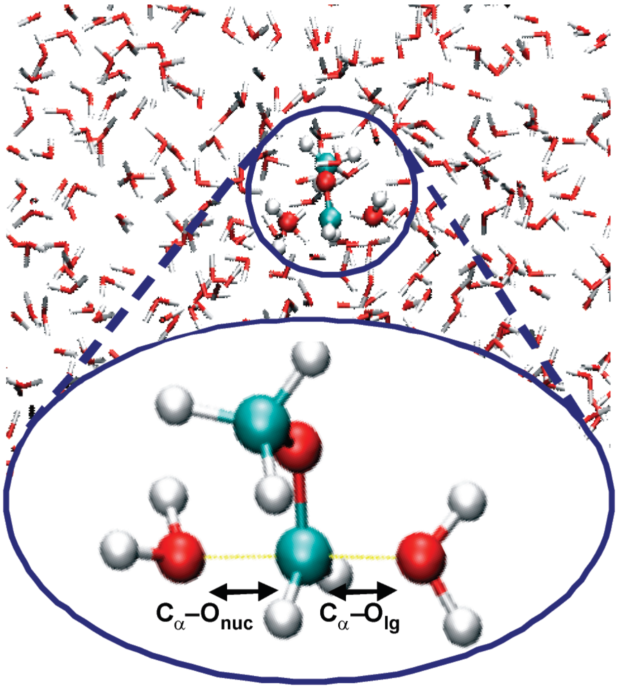

Figure 1. Hybrid quantum mechanical/molecular mechanics scheme for methoxymethyl transfer between reactive water molecules subsystem. The inset shows the 14 QM atoms treated quantum mechanically and the two distances used to describe the reaction.

hydrolysis of methoxymethanol is the simplest model for glycosyl transfer in water; Scheme 1 depicts the alternative A_ND_N and D_N*A_N mechanisms for this system.

The purpose of this paper is to obtain a theoretical estimate for the lifetime of the putative methoxymethyl cation in water to ascertain which of the mechanisms provides the better description of this prototypical reaction. We employ a combination of quantum mechanics/molecular mechanics (QM/MM) for electronic structure calculations along with molecular dynamics (MD) and statistical mechanics techniques, as successfully applied to studies of chemical reactions in condensed media.[26−28] The QM subsystem contains the methoxymethyl cation and two water molecules (nucleophile and leaving group), and the MM subsystem comprises over 1000 surrounding solvent water molecules (Figure 1). Transfer of the methoxymethyl group between the nucleofuge and nucleophile ("nucleophilic displacement by electrophile migration")[29] is described by a reaction coordinate, RC, defined as the difference between the distances of the central carbon atom to the oxygen atoms of these reacting water molecules, RC $= d(C_\alpha{-}O_{lg}) - d(C_\alpha{-}O_{nuc})$; the sum of these two distances defines a tightness coordinate, TC $= d(C_\alpha{-}O_{lg}) + d(C_\alpha{-}O_{nuc})$, describing the change from a "tight" pentacoordinate complex to a "loose" solvated oxacarbenium ion. We construct a two-dimensional potential of mean force (2D-PMF) with respect to RC and TC by averaging the atomic motions of the whole system at 300 K as governed by the QM/MM potential energy function and forces. This surface provides information about the change in free energy both for transformation of reactants (negative RC) into products (positive RC) and for variation in the tight (small TC)/loose (large TC) character of intervening species.

2. Results

At the semiempirical AM1/MM level, a path of minimum free energy can be traced on the 2D-PMF surface (Figure 2a) from the reactant free-energy minimum (left) to the product free-energy minimum (right) through a saddle point; these stationary points are found also on the corresponding potential energy surfaces (see Supporting Information). The reaction coordinate RC changes substantially (from <−1.2 to >+1.2 Å) while the tightness coordinate TC remains essentially unaltered. Using this computational model, the reaction can be described as an A_ND_N mechanism with an activation free energy of ∼7 kcal mol^{-1}. The transition-state structure (TS) on this reduced free-energy surface (and confirmed by analysis of the underlying potential energy surface) is symmetrical with $d(C_\alpha{-}O_{lg}) = d(C_\alpha{-}O_{nuc}) \approx 2.1$ Å. Displacement of the TS along the tightness coordinate toward more exploded structures has an associated free-energy penalty.

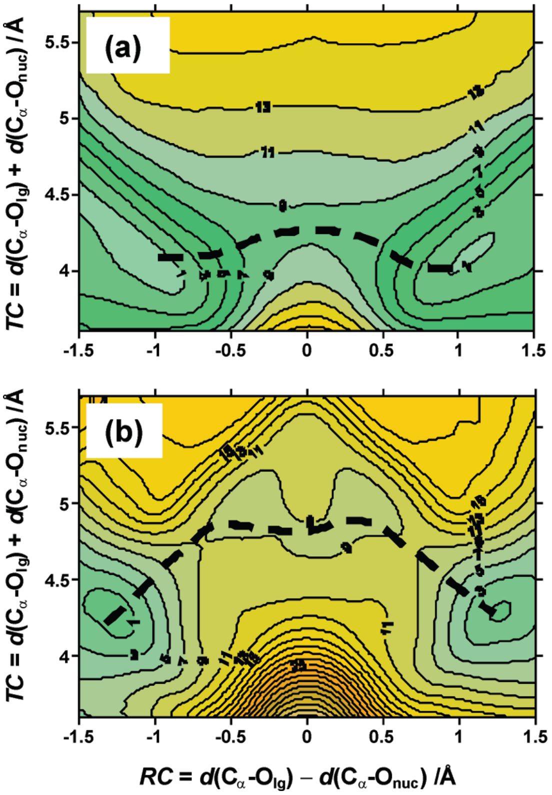

Figure 2. Two-dimensional potentials of mean force with respect to the reaction and the tightness coordinates at the AM1/MM level (top) and at the corrected MPWB1K/MM level (bottom). Free energy contour lines are spaced by 2 kcal mol^{-1}.

The free-energy surface may be improved by carrying out molecular dynamics simulations with bidimensional interpolated corrections to the QM energies and forces evaluated at the density functional MPWB1K/6-31+G(d,p) level (Figure 2b). The topography of the surface is significantly altered in that a shallow, free-energy well now appears at $d(C_\alpha{-}O_{lg}) = d(C_\alpha{-}O_{nuc}) \approx 2.7$ Å. Two equivalent saddle points are located, one at $d(C_\alpha{-}O_{lg}) = 2.1$ Å and $d(C_\alpha{-}O_{nuc}) = 2.8$ Å and the other, at $d(C_\alpha{-}O_{lg}) = 2.8$ Å and $d(C_\alpha{-}O_{nuc}) = 2.1$ Å. The free energy of activation for formation of the intermediate species is ∼9.5 kcal mol^{-1} whereas the depth of the intermediate free-energy well is only ∼0.5 kcal mol^{-1}. Using this improved computational model, the reaction can be described as a D_N*A_N mechanism involving an intermediate that is appreciably looser than the symmetric TS found at the semiempirical level of theory. The region of the free-energy surface around the intermediate is quite flat in both RC and TC; slight changes in the system (e.g., structural modification in the reacting substrate

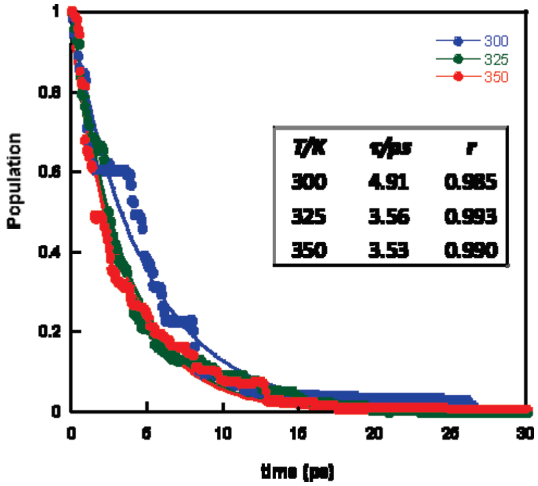

Figure 3. Lifetime of the methoxymethyl cation in water. Exponential fits (solid lines) to the simulated decay of the intermediate population as obtained from molecular dynamics trajectories at 300, 325, and 350 K.

or in the surrounding environment) could easily modify the shape of the surface and thereby the course of the most favorable reaction path.

The lifetime of the intermediate was evaluated by means of a series of molecular dynamics trajectories initiated in the shallow well on the underlying density functional corrected potential energy surface. To begin with, simulations were performed at 300, 325, and 350 K with harmonic constraints imposed upon both $d(\mathrm{C}_\alpha{-}\mathrm{O}_{\mathrm{lg}})$ and $d(\mathrm{C}_\alpha{-}\mathrm{O}_{\mathrm{nuc}})$ coordinates to allow for equilibration of the system within the region of the intermediate. For each of 200 structures, selected at 1 ps intervals along the constrained trajectory at each temperature, an unconstrained molecular dynamics trajectory was initiated with random velocities taken from the Boltzmann distribution at that temperature; NVT trajectories were followed for 30 ps. Any configuration with both $d(\mathrm{C}_\alpha{-}\mathrm{O}_{\mathrm{lg}})$ and $d(\mathrm{C}_\alpha{-}\mathrm{O}_{\mathrm{nuc}}) > 2.1$ Å was defined to be an intermediate; configurations with one of these distances <2.1 Å were considered to belong to either the reactant or the product basin. The time evolution of the intermediate population, $n(t)$, at each temperature was obtained by averaging over the set of 200 trajectories and fitting to an exponential decay function of the type $n(t) = n_0 \exp(-t/\tau)$, where τ is the lifetime. A good fit with a correlation coefficient r better than 0.98 was obtained at each of the three temperatures (Figure 3), yielding classical lifetimes of 4.91, 3.56, and 3.53 ps at 300, 325, and 350 K, respectively. An Arrhenius plot of $\ln(1/\tau)$ vs $1/T$ gave a classical activation energy of 1.4 kcal mol^{-1} ($r = 0.89$).

Although both the 2D-PMF and the dynamical analysis of the intermediate lifetime described above are based upon hybrid QM/MM potentials and, in particular, a quantum mechanical treatment of the electronic structure within the reacting system, nevertheless, the nuclear dynamics are described by classical mechanics. It is therefore necessary to apply quantum corrections to the vibrational degrees of freedom to obtain the best estimate for the lifetime of the intermediate. Harmonic frequencies computed for 10 potential-energy-minimized intermediate structures (each with a slightly different solvent configuration) and 7 potential-energy-optimized transition structures were used to obtain average values of the contribution to the activation energy for destruction of the intermediate from (a) classical vibrational energy and (b) zero-point vibrational energy; the difference between these two gives a quantum correction of −1 kcal mol^{-1}.

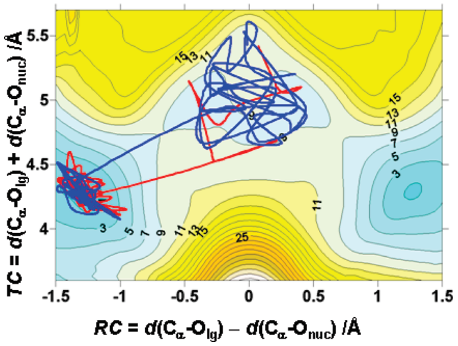

Figure 4. Individual molecular dynamics trajectories. Examples of trajectories evolving from the intermediate free-energy well to the reactant valley are shown. The blue trajectory passes through the $\mathrm{D_N{*}A_N}$ transition state, but the red trajectory follows a more concerted path.

The overall activation energy is therefore ~0.5 kcal mol^{-1}, and the lifetime of the methoxymethyl cation in water at 300 K is ~1 ps.

The 2D-PMF gives information about the behavior of the system averaged over very many separate dynamical trajectories, but the individual trajectories provide details of actual reaction events. The set of 200 trajectories considered above contained examples of two types of behavior (Figure 4). The blue trajectory evolves from the intermediate region to the reactant valley, passing through the $\mathrm{D_N{*}A_N}$ transition state. On the other hand, the red trajectory evolves toward a tighter structure and traverses the ridge above the intermediate energy minimum before descending into the reactant valley by a path along which $d(\mathrm{C}_\alpha{-}\mathrm{O}_{\mathrm{lg}})$ and $d(\mathrm{C}_\alpha{-}\mathrm{O}_{\mathrm{nuc}})$ change in a more concerted fashion. It would be easy to imagine another trajectory starting from reactants and at first evolving close to the red one but then passing directly into the product valley along an $\mathrm{A_N D_N}$ path. Thermal fluctuations of the solvent molecules would cause some reaction events to follow the stepwise route and others, the concerted route. In the intermediate state, both the nucleophile and nucleofuge water molecules possess a nearly neutral charge: the averaged Mulliken charge summed over the atoms of each of these groups is only 0.02 $|e|$. However, in each of the reactant and (identical) product states, the positive charge is largely localized on the leaving group or nucleophilic water molecule, respectively: the averaged Mulliken charge on each group is 0.56 $|e|$. Approach of a solvent molecule acting as hydrogen bond acceptor to either the nucleophile or the nucleofuge can stabilize this charge and thus determine the fate of the trajectory from the intermediate to the product/reactant valley. The radial distribution functions of solvent molecules reflect this change in the solvation shell around the nucleophilic or leaving groups (see Supporting Information).

3. Discussion

The 2D-PMF surfaces (Figure 2) are essentially symmetric about RC = 0. This is to be expected for the reacting subsystem, since it undergoes an identity reaction, but the instantaneous solvent environment is never symmetric, and symmetry is gained only by averaging over all accessible configurations. Since no symmetry constraints were imposed upon the simulations used to construct these surfaces, their symmetry suggests that the sampling of configurational space was adequate.

The AM1/MM surface does not have a free-energy minimum corresponding to the methoxymethyl cation. Note that an AM1 computational study of nucleophilic substitution by a water molecule on methoxymethyl dimethylanilinium cation in the gas phase indicated an A_ND_N mechanism with no involvement of a methoxymethyl cation as a discrete intermediate.[30] A recent assessment of average errors in reaction barrier heights calculated by a wide range of QM methods against a standard database reported[31] a mean unsigned error for AM1 of 13.0 kcal mol^{-1}, as compared with 1.7 kcal mol^{-1} for the MPWB1K/6-31+G(d,p) method, although the relative computational costs of these semiempirical and density functional methods was 1:40 000. The use of a 2D-spline interpolated MPWB1K/6-31+G(d,p) correction to the AM1 energies and gradients for the QM subsystem in the present study represents a satisfactory compromise between accuracy and expense.

The MPWB1K/6-31+G(d,p) corrected free-energy surface reveals a shallow minimum corresponding to the methoxymethyl cation coordinated symmetrically by the two reacting water molecules at a distance of ~2.7 Å and further solvated by ~1000 surrounding water molecules in a periodically repeating cell (see below). Molecular dynamics analysis of the rate of decrease in the populations of this intermediate at three temperatures leads to a classical estimate of the lifetime of the oxacarbenium ion in water; when quantum corrections for vibrational zero-point energy are included, the lifetime is estimated to be 1 ps. This result is in complete agreement with the upper-limit value published by Amyes and Jencks as a consensus of three different lines of reasoning from their measured rates of hydrolysis for other methoxyalkyl cations.[20]

A feature of the present QM/MM approach is that only two water molecules are included within the QM region, which privileges this pair as the nucleophile and leaving group over the other MM water molecules in the first solvation shell. This places a practical limit on the range of $d(C_\alpha{-}O_{lg})$ and $d(C_\alpha{-}O_{nuc})$, for which the QM/MM energy values should be considered reliable: neither distance should exceed ~4 Å, corresponding to the position of the first peak in the radial distribution function of MM waters around C_α, to ensure that the QM waters are always closest to C_α. The Car–Parrinello molecular dynamics approach avoids this limitation by treating the whole system as QM, but at the cost of restricting the size of the system.[32] A recent study of specific acid-catalyzed reaction of methanol with α-D-glucopyranose in water by this method was limited to only 57 water molecules.[33] The 1D free-energy profile with respect to $d(C_\alpha{-}O_{nuc})$ shows a single energy maximum with no energy well for a glucosyl cation intermediate, and the evolution of the geometrical and electronic structure along the dynamical trajectory seems to indicate an A_ND_N mechanism via an oxacarbenium-like TS, despite the authors' stating that they had found a two-step $D_N{*}A_N$ mechanism along an enforced reaction coordinate;[33] a two-dimensional treatment also including $d(C_\alpha{-}O_{lg})$ would be very interesting. A study of oxacarbenium ions in glycosylation reactions by a similar methodology did not include any solvent and was therefore unable to shed light on the question of the lifetimes of glycosyl cations in water.[34]

The methoxymethyl system holds an important place in the development of a physical organic understanding of borderline mechanisms. Kinetic studies of reactions of methoxymethyl substrates with dinitrophenolate[24] and *N*,*N*-dimethyaniline leaving groups[25] revealed very low sensitivity to the pK_a of the (conjugate acid of the) nucleophile, very high sensitivity to the pK_a of the nucleofuge, and α-deuterium kinetic isotope effects typical of stepwise $D_N + A_N$ reactions, despite being unambiguously bimolecular. This gave rise to the notion of the "exploded" transition state and to the idea that the mechanism of a reaction could be enforced as concerted owing to the lifetime of the putative intermediate being too short for it to have an existence as a discrete species.[22,23] If a nucleofuge is not able to diffuse away from an electrophile in solution before nucleophilic attack occurs, the reaction is either concerted A_ND_N or requires the nucleophile to be preassociated in a $D_N{*}A_N$ mechanism.

The identity nucleophilic substitution of water with a protonated alcohol in aqueous solution may happen in any glass of wine; oxygen exchange of ^{18}O-methanol with solvent ^{16}O in aqueous acid occurs by the A_ND_N mechanism[35] and has a potential energy barrier of about 27 kcal mol^{-1} at the PCM/MP2/6-31G* level.[36] The present calculations for the analogous reaction of protonated methoxymethanol in water indicate a markedly lower barrier of about 10 kcal mol^{-1}, suggesting a facile reaction. In this case, preassociation of the nucleophile with the electrophile is not a diffusive process but, instead, presumably involves rotation of a solvent molecule to present an electronic lone pair to the electrophilic carbon atom with accompanying loss of a hydrogen bond from a donor solvent molecule. In this study, the reaction coordinate RC for transfer of the methoxymethyl group between the nucleofuge and nucleophile is defined simply as the difference of distances $d(C_\alpha{-}O_{lg}) - d(C_\alpha{-}O_{nuc})$, but by analogy with the mechanism for reorientation[37] of a hydrogen bond donor water molecule (electrophile) between two hydrogen bond acceptor water molecules (nucleofuge and nucleophile), and within the range of validity for $d(C_\alpha{-}O_{lg})$ and $d(C_\alpha{-}O_{nuc}) < 4$ Å, progress along the reaction coordinate may be accompanied by changes in coordination numbers of the nucleophilic and leaving group waters and associated rotations. Whether the methoxymethyl oxacarbenium ion exists as a discrete intermediate may depend on whether these motions within the hydrogen-bonded network of the solvent are uncoupled or coupled. Recent analysis of microwave and far-infrared dielectric spectroscopy data for liquid water suggests that the relaxation time of ~1 ps at 298 K corresponds to a "single molecule rotation".[38]

Is the existence of an energy minimum for the complex of methoxymethyl cation coordinated symmetrically by the two reacting water molecules solely due to the presence of the surrounding solvent water? The AM1 energy surface with respect to the $C_\alpha{-}O_{lg}$ and $C_\alpha{-}O_{nuc}$ distances for this system in vacuo contains a minimum,[39] and closer inspection at the MPW1BK/6-31+G(d,p) level reveals a symmetric minimum with $C_\alpha{-}O$ distances of 2.33 Å. Evidently, the methoxy group R = MeO stabilizes the cationic triple complex $H_2O\cdots RCH_2^+\cdots OH_2$ just enough for this species to exist in a shallow well along the energy profile for asymmetric $C_\alpha{-}O_{lg}/C_\alpha{-}O_{nuc}$ stretching, even in the absence of solvent, in contrast to R = H or Me.[36,39] The *existence* of the methoxymethyl cation in aqueous solution is due to a stabilizing substituent effect but its *lifetime* is determined by solvent dynamcs.

Since inductive effects of α-substituents affect the rates of formation of carbocations but not their rates of reaction with water,[40] Davies, Sinnott, and Withers[2] have argued that the lifetime of a glycosyl cation in water should not be very different from that of methoxymethyl; in the presence of anionic nucleophiles or leaving groups, this lifetime would be too short for the glycosyl cation to exist as a discrete intermediate, but in the presence of neutral nucleophiles or leaving groups, it would be on the order of 1 ps. (These authors' argument overlooks the fact that Richard's observation[40] that inductive effects of α-substituents did not affect the rates of reaction with water was specific to 4-methoxylbenzyl carbocations, whereas actually, ~20% of the inductive substituent effect on the equilibrium stability of oxacarbenium ions is expressed at the transition state for addition of solvent to these cations.[20]) Notwithstanding, proposed mechanisms for enzymic glycosyl transfer would imply the intermediacy of an oxacarbenium ion in the presence of an anionic nucleophile in the enzyme active site would therefore be unreasonable. However, these authors keep an open mind as to whether, in the presence of a neutral nucleophile and water, mechanisms would involve an oxacarbenium-like exploded transition state or a transient intermediate.[2]

4. Concluding Remarks

In view of the experimental difficulty of obtaining direct information regarding very short-lived intermediates in enzyme-catalyzed reactions, computational simulation has an important complementary role in the elucidation of mechanism. Our simulations have confirmed the experimental estimate for the lifetime of the methoxymethyl cation in water, and this lends support to the related estimate of ~1 ps for the lifetime of the glucosyl cation in water.[20] However, our aim is to perform reliable computational simulations of enzyme-catalyzed glycosyl transfer reactions. The present study offers encouragement that subtle distinctions between alternative mechanisms that do or do not involve short-lived glycosyl intermediates can be made, provided an appropriate QM method is used to describe the reacting subsystem. The electrostatic environment within an enzyme active site is very different from that of water, and the issue of whether glycosyl cations exist as discrete intermediates in stepwise preassociation $D_N{*}A_N$ mechanisms or as oxacarbenium-like TSs in concerted A_ND_N mechanisms is (to quote Fersht[8]) a "question of whether the enzyme can sequester the ion". Davies, Sinnott, and Withers (quoting Wittgenstein, "whereof one cannot speak, thereof one must keep silent") concluded that this distinction is probably unknowable in many cases.[2] Our contention is that computational simulation can provide valuable insight that is not directly amenable by experiment.

5. Computational Procedures

An optimized protonated methoxymethanol molecule (11 atoms described by the semiempirical AM1 method[41]) was solvated in a preequilibrated cubic box of water molecules of 31.5 Å of side centered in the central carbon (C_α), and all water molecules closer than 2.8 Å to any non-hydrogen atom of the substrate were removed; the remaining 1030 water molecules were described by the TIP3P potential.[42] After 1 ns of NVT molecular dynamics (employing the DYNAMO library[43]) to equilibrate the system, the most suitably oriented water molecule was incorporated within the QM part as the nucleophile. This water molecule was kept close to the substrate by a harmonic constraint (with a reference distance of 2.5 Å and a force constant of 2500 kJ mol^{-1} Å^{-2}) applied to the distance between the C_α and O_{nuc} and a harmonic constraint to keep the $O_{lg}-C_\alpha-O_{nuc}$ angle close to linearity with a force constant of 2.5 kJ mol^{-1} degree^{-2}, and a further 200 ps of dynamics simulation was run to reequilibrate the system. We checked that within the range of C−O distances of interest, the harmonic constraint applied to the $O_{lg}-C_\alpha-O_{nuc}$ angle did not introduce any significant bias in the behavior of the system: the topology of the surface was identical and the relative energies were nearly identical when AM1/MM calculations were repeated without angular constraints. In addition, the lifetimes estimated with and without the linear constraint were very close. The constraint prevents the exploration of some configurations that are irrelevant for the process under study, especially in the reactant/product regions where one of the water molecules is unbonded, and then speed up the convergence of statistical magnitudes. The presence of the constraint is closely related to the fact that two water molecules are described at a different level (QM) from the rest of solvent molecules (MM). Otherwise, the constraint added was not too tight. The standard deviation of the O−C−O angle during a 10 ps simulation of the intermediate without the angular constraint was of 12°, while adding the angular constraint was of 6°.

A 2D QM/MM potential energy surface was calculated with respect to the coordinates RC and TC defined above for 14 AM1 atoms and 1029 TIP3P waters.

To improve the description offered by the AM1 Hamiltonian, the 2D Interpolated Corrections method was applied,[44,45] giving a new energy function defined as

$$E = E_{\mathrm{AM1/MM}} + S[\Delta E_{\mathrm{LL}}^{\mathrm{HL}}(\zeta_1;\zeta_2)]$$

where S denotes a two-dimensional spline function, and its argument, ΔE_{LL}^{HL}, is a correction term evaluated as the gas-phase difference between a high-level MPWB1K/6-31+G(d,p)[46,47] energy of the QM subsystem and the low-level AM1 result as a function of RC and TC. This method represents an important improvement over simply correcting single-point energies with a higher-quality QM method. The spline function allows the corrected energy and gradient to be interpolated at every step of an MD trajectory or of a geometry optimization, such that these processes employ corrected forces and consequently also yield corrected structures. Other high-level methods were also tested, obtaining similar results: the Supporting Information shows potential energy surfaces obtained at the AM1/MM level and including corrections obtained at both the MPW1BK/6-31+G(d,p) and MP2/6-31+G(d,p) levels. To account for the many possible solvent configurations of the system, the 2D-PMF was computed, which relates to the probability of finding the system at particular values of the two reaction coordinates:

$$W(\zeta_1,\zeta_2) = C' - kT\ln\int \rho(\mathbf{r}^N)\,\delta(\zeta_1(\mathbf{r}^N) - \xi_1)\,\delta(\zeta_2(\mathbf{r}^N) - \xi_2)\,\mathrm{d}\mathbf{r}^{N-2}$$

Double umbrella sampling[48] was used with harmonic constraints of 2500 kJ mol^{-1} Å^{-2} applied to the two coordinates. A total of 34 × 34 simulation windows were employed to cover the whole range of relevant values of the coordinates. The simulation in each window was started from the final configuration of the preceding window and consisted of 2 ps of equilibration, followed by 10 ps of production at the reference temperature of 300 K, using a time step of 1 fs.

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Supporting Information Available: Potential energy surface obtained at the AM1/MM level and corrected using MPWB1K/6-31+G(d,p) and MP2/6-31+G(d,p) high-level quantum corrections. Radial distribution functions of solvent oxygen atoms around O_{lg} in the intermediate and reactants state. Complete citation of reference 46. This material is available free of charge via Internet at http://pubs.acs.org.

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