H-Bonding in Alcohols Is Reflected in the Cα−H Bond Strength: Variation of C−D Vibrational Frequency and Fractionation Factor

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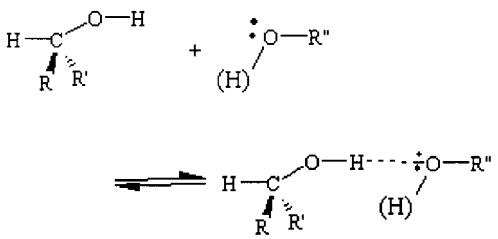
Abstract: The variation of the C−D vibrational stretching frequency in primary and secondary alcohols containing the D−C−O−H functionality has been examined for cases in which the alcohol functions as a proton donor in an H-bond. The C−D stretching frequency is a function of the H-bond enthalpy of formation determined by Hartree−Fock calculations, decreasing by approximately 5 cm^{-1} per kcal/mol. This decrease in frequency is attributed to the increase in the overlap of the O−H bonding electrons with the C−D antibonding orbital as the H-bond is strengthened. The Raman spectra of [1-D]trifluoroethanol and [1-D]trifluoroethoxide in aqueous solution serve as an example; the alcohol has two separate C−D stretches that differ by 45 cm^{-1} and deprotonation results in an average 78 cm^{-1} decrease in the C−D stretching frequency. A measured deuterium equilibrium isotope effect on the acid ionization constant of [1-D_2]trifluoroethanol of 1.13 is consistent with a decreased fractionation factor of the C-1 protons due to the decrease in the C−D stretching frequency. A model nucleoside complexed with the H-bonding residues at the active site of nucleoside hydrolase indicates that H-bond formation can explain the anomalous secondary isotope effects reported for the hydrolysis of [5′-^{3}H]inosine (Horenstein et al. *Biochemistry* **1991**, *30*, 10788−10795). The correlations of both the C−D stretching frequency and the fractionation factor with the conformation and H-bond strength with primary and secondary alcohols as donors should serve as tools for the characterization of these important interactions in biological systems.

H-bonds are one of the major intermolecular forces that provide the energetic driving force for the formation of noncovalent complexes in biological systems. The complementary donor−acceptor nature of H-bonds along with the geometric constraints of distance and angle required for the formation of H-bonds are characteristics that generate much of the specificity encountered in molecular recognition and enzyme catalysis. The H-bonds between alcohol donors and protein acceptors, shown in Scheme 1, constitute an important subset of these H-bonds.

Scheme 1



Despite considerable interest and progress in developing methods of evaluating H-bond properties, the energetic contribution of any particular H-bond in a biological system remains difficult to assess. With the advent of X-ray diffraction crystallography, it is possible to inspect a growing number of protein crystal structures for the presence of H-bonds responsible for ligand binding and/or catalysis. This method offers a satisfactory geometric description of most probable H-bonds, but it does not provide direct information regarding the energetic contribution of such interactions to the overall binding of the ligand. Some insight into the enthalpies of H-bonds involved in the protein−ligand interactions might be gained by selectively altering the structure of the ligand or the protein by site-directed mutagenesis in order to remove a specific H-bond. However, the determination of the relative strengths of H-bonds by these studies does not take into account the effects of induced alterations in structure or solvation that result from the mutations that may confound any individual measurement.[1]

Spectroscopic measurement of shifts in the X−H stretching frequency for the hydrogen-bond donor offers a straightforward method of estimating H-bond energies.[2,3] Extension of this technique to enzyme−ligand systems would require the detection of a specific X−H stretching frequency in the presence of the large background introduced by solvent water. Some special

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techniques, such as ^{18}O isotope-edited difference Raman spectroscopy,[4] might eventually be able to overcome this problem, but none have yet been successful. Hibbert and Emsley[2] summarized three NMR-based alternatives: the deshielding of the ^1H NMR resonance of the H-bonded proton,[5] the D isotope effect on the chemical shift of this proton, and the low fractionation factor of the H-bonded proton.[6] Additionally, the $^{15}N-^1H$ one-bond coupling constant, $^1J_{HN}$, can be used to characterize H-bonds with N−H donors.[7] These methods have not been applicable to O−H hydrogen bonds in enzyme complexes as the NMR experiments are frustrated by the rapid exchange of the proton of interest with solvent. Two-bond isotope effects on ^{13}C chemical shifts, $^2\Delta\ ^{13}C(OD)$, are sensitive to H-bond strength in phenols,[8−11] and solid state ^{13}C NMR shielding tensors can characterize carboxylate H-bonds,[12] but spectroscopic methods of characterizing the strength of alcohol H-bonds in aqueous solution have not been identified.

It has been previously shown that equilibrium isotope effects (EIEs) on enzyme−ligand association are capable of reflecting altered vibrational frequencies associated with H-bond formation.[13] These experimental observations indicate a potential for correlating the magnitude of measured EIEs and H-bond strength, but the small value of the ^{18}O EIE for a H-bond between oxamate and an arginine guanidinium group did not leave much hope for extension of this technique to potentially weaker interactions.

A surprising observation of a significant remote hydrogen isotope effect on the reaction catalyzed by nucleoside hydrolase attracted our attention.[14] The kinetic isotope effect of 1.051 measured with [5′-^{3}H]inosine was extraordinary, as this ^3H is four bonds removed from the site of chemical transformation. The authors attributed this large isotope effect to a decrease in the C5′−H bond order, but the structural cause for the decrease in bond order remains uncertain. We have used computational methods to examine the effects of H-bond formation by an alcohol with oxygen acceptors, as shown in Scheme 1, on the vibrational characteristics and, consequently, isotope effects of atoms not directly involved in the H-bond.

Density functional theory (DFT) and Hartree−Fock (HF) ab initio methods and semiempirical calculations were employed to investigate which isotope effects might arise upon H-bonding of an alcohol proton donor. The H-bond energy was varied by altering the acceptor, and the corresponding vibrational spectra and EIEs on H-bond formation were calculated. The results indicate that increasing the strength of the H-bond results in a dispersion of the electron density of the O−H σ-bond. This delocalization of electron density, by virtue of its increased overlap with the antibonding orbital of the αC−H, increases the length of the C−H bond and decreases its stretching frequency, ν_{C-H}. The torsion angle, H−O−αC−H, also significantly alters the vibrational frequency and the fractionation factor of the αC−H proton. These computationally predicted effects were verified by the experimentally observed secondary α-D EIE on the ionization of trifluoroethanol (TFE) and the observed decrease in C−D stretching frequency of [1-D]TFE that occurs upon ionization.

Materials and Methods

Calculated Isotope Effects. Theoretical prediction of EIEs requires the calculation of the frequencies of the normal mode vibrations of both the light isotope molecule and its substituted isotopologues.[15] EIEs were calculated for reaction 1 from two pairs of vibrational normal modes corresponding to the free alcohol (R) and hydrogen-bonded complex (P) according to the complete eq 1

$$\frac{K_H}{K_L} = \prod_i^{3n^R-6} \frac{u^R_{iH} \sinh(u^R_{iL}/2)}{u^R_{iL} \sinh(u^R_{iH}/2)} \times \prod_i^{3n^P-6} \frac{u^P_{iL} \sinh(u^P_{iH}/2)}{u^P_{iH} \sinh(u^P_{iL}/2)} \qquad (1)$$

where K_H/K_L is the EIE; n^R and n^P are the numbers of atoms in the free alcohol and the H-bonded complex, respectively; and $u_i = h\nu_i/kT$, where h and k are the Planck and Boltzman constants, respectively, T is the absolute temperature, and ν_i are the frequencies of normal vibrations. Subscripts L and H correspond to light and heavy isotopically substituted species, respectively.

Isotope effect calculations were performed with the aid of the ISOEFF program version 6 or ISOEFF98.[16] All calculated frequencies were used, but only those that exhibit shifts due to isotopic substitution contribute to isotope effects. All isotope effects were calculated for the temperature of 273 K.

Whenever practical, the geometries of the alcohol and the H-bonded complexes were determined by ab initio calculations at the Hartree−Fock level using the 6-31+G(d,p) basis set in Gaussian 94W, 98W, or 98,[17] and the vibrational frequencies of both normal and isotopically labeled molecules were subsequently calculated. No scaling factors were used to account for anharmonicity of the calculated vibrational frequencies. This method was used to calculate EIEs resulting from H-bonding between 2-propanol and methoxide, as well as for a series of H-bonded ethanol complexes. In the latter case, the H-bond donor was ethanol or its protonated form, and either ethanol, ethoxide, or formate was used as the acceptor.

Semiempirical calculations were performed using AM1[18] and PM3[19,20] Hamiltonians as implemented in Mopac, Hyperchem version 4.5 (Hypercube, Gainesville, FL), and Gaussian 98W. The semiempirical approach was primarily used to demonstrate the extension of our studies to large molecular systems for which ab initio calculations are impractical and semiempirical methods are still widely used.[21] The comparison tested whether either semiempirical method had the ability to detect the vibrational changes upon H-bond formation and conformation observed in the ab initio and DFT calculations.

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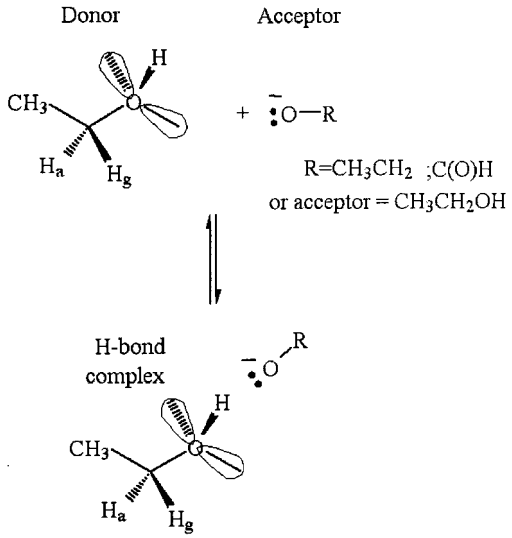
Scheme 2



The potential H-bonding of the 5′-hydroxyl of inosine at the active site of inosine−uridine nucleoside *N*-ribohydrolase (nucleoside hydrolase) was modeled at the PM3 level. The crystal structure of 1-(*p*-aminophenyl)-1,4-dideoxy-4-iminoribose bound to the active site was taken from entry 2MAS of the protein data bank.[22] The *p*-aminophenyl group was truncated to the glycosidic amino group and the ring N replaced by O in Hyperchem. Glu-166, the residue that accepts an H-bond from the 5′-hydroxyl, was truncated at the β-carbon and capped with a proton. During the optimization, the H-bond to the anti lone pair of the carboxylate was preserved without constraint. A separate optimization of formate H-bonded to the 5′-hydroxyl of adenosine was studied with an ONIOM[B3LYP/6-31+G(d,p):PM3][23] calculation in G98W. The formate ion and the 3′, 4′, and 5′ carbons along with their constituents were treated at the DFT level with the rest of the adenosine included at the semiempirical level of the mixed-mode calculation. The optimized structure included H-bonds from both the 5′ and 3′ hydroxyls to the carboxylate.

Variation of H−Bond Strength. The strength of the H-bond between 2-propanol and the acceptor was decreased by sequentially substituting fluorines for the hydrogens of the acceptor methoxide and then further reduced by using fluoroformate, formate, chloroformate (with the syn and anti electron pairs of the carboxylate), and formamide as the H-bond acceptors. The reduced proton affinity of the acceptor systematically decreased the strength of the H-bond in the complex. A second method of reducing the H-bond strength was to constrain the O−O distance at incrementally greater distances. This distance constraint generated sequentially weaker H-bonds. In a separate series to examine the effect of H-bond formation on the αC−H bonds that are either gauche or anti to the H-bond (H_g and H_a, respectively), ethanol in the gauche conformation was chosen as the donor, and ethanol, formate, and ethoxide, were used as the H-bond acceptors, as shown in Scheme 2.

Interaction energies for all complexes were obtained by subtracting the sum of heats of formation for the separately optimized substrates from the heat of formation for the optimized H-bonded complex. Although this method introduced a small systematic error because of the basis set superposition error,[24] this error will be similar for each calculation and consequently will not affect the conclusions drawn from variations in the strength of the H-bond. Stretching frequencies were identified from a set of calculated normal modes by their shifts upon isotopic substitution.

Vibrational Spectra of [1-D]-TFE and [1-D]-Trifluoroethoxide. [1-D]TFE was synthesized by reducing trifluoroacetaldehyde with $LiAlD_4$ (Aldrich) and purifying the product by simple distillation. The Raman spectra in H_2O and D_2O were acquired using 752-nm excitation from a Ti:sapphire laser (Coherent) operating at 400 mW. The Raman setup incorporates a red-intensified, back-thinned charge-coupled device detector and a Kaiser holospec single grating spectrograph (Kaiser Optical System, Inc.), as described elsewhere.[25] The trifluorethoxide spectra were obtained in 1 N NaOH and NaOD under similar conditions. IR spectra were obtained using a Hartmann & Braun MB series spectrometer, using 13 × 2 mm CaF_2 windows.

Equilibrium Isotope Effect on Ionization of [1-D_2]TFE. The equilibrium isotope effect on ionization of [1-D_2]TFE in aqueous solution was measured by monitoring the difference in chemical shift of the ^{13}C NMR resonances for C-1 of H_2 and D_2 TFE while the solution was titrated with KOH. Aqueous solutions consisting of 2.5% (v/v) TFE and 7.5% (v/v) [1-D_2]TFE in 0−1.5 M KOH were prepared. Fluorine-decoupled ^{13}C spectra of these solutions were acquired using a Varian Inova 600-MHz spectrometer operating at 150.858 MHz at 25 ± 0.5 °C. Fluorine was waltz decoupled at 564.43 MHz with a 20 000 Hz offset. The spectrometer's electronics were optimized for each sample analyzed, and spectra were obtained using a pulse width of 13 μs, an acquisition time of 1 s, and a relaxation delay of 1 s. The chemical shift differences (obtained by averaging the position of the three peaks of the triplet for the ^1H-TFE and the five peaks of the quintuplet for the D_2-TFE) were fit to eq 2 by nonlinear least-squares analysis with GraFit.[26] Y_H and Y_L were experimentally determined and are the chemical shift differences at high and low pH, respectively; f_{OH}, the fraction of [$^1H_2$]TFE that is protonated, was the experimentally varied parameter; $\Delta_{ion}\delta$ is the experimentally determined difference in chemical shift on ionization of ^1H-TFE, and ^DK is the equilibrium isotope effect on ionization and the only independent parameter.

$$\Delta\delta_{obs} = Y_H - \Delta_{ion}\delta \times f_{OH} + {}^{D}K \times f_{OH}(\Delta_{ion}\delta + Y_L - Y_H)/({}^{D}K \times f_{OH} + 1 - f_{OH}) \quad (2)$$

Results

Vibrational Spectra. Because the C−D stretching vibrations are located in a spectral region well removed from those of the other TFE groups, almost pure C−D stretching vibrations are observed with little or no coupling to other vibrational coordinates. For [1-D]TFE in H_2O, two Raman bands appear at 2217 and 2172 cm^{-1}, as shown in Figure 1. These two bands probably represent the C−D stretching fundamentals for the anti and gauche conformers around the C−O bond.[27,28] Bands at 2167 and 2097 cm^{-1} have been observed for 2-propanol-2-d_1 and are similarly assigned to conformers in which the C−D bond is either anti to the O−H bond (anti) or anti to one of the oxygen lone pairs (gauche).[29] (However, we cannot eliminate the possibility that the two bands correspond to a Fermi doublet generated by an interaction between a C−D stretching fundamental and an overtone or combination band.) Upon ionization in 1 N NaOH, an intense Raman band at 2117 cm^{-1} and a much weaker band at 2062 cm^{-1} are observed (Figure 1). For the ionized form, the situation is simplified as only one staggered conformer is possible on the basis of symmetry considerations. Thus, we assign the band at 2117 cm^{-1} to the C−D stretching fundamental and the weak band at 2062 cm^{-1} to either an

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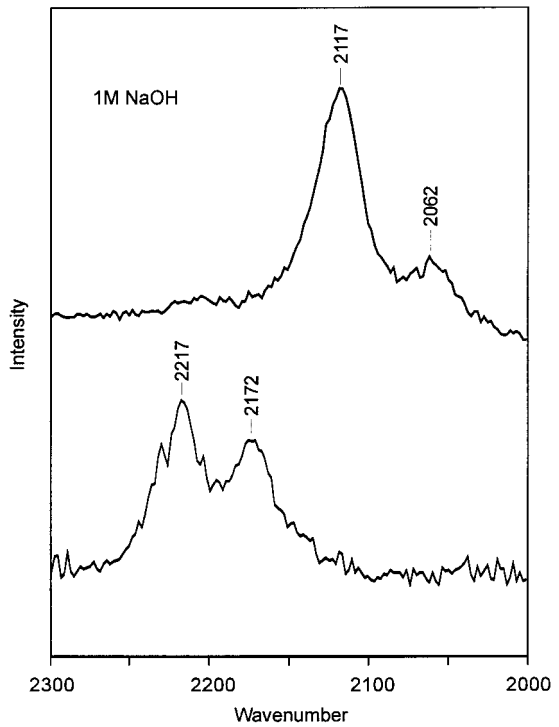

Figure 1. C−D stretching region of the Raman spectra of [1-D]-trifluoroethoxide (upper trace) and [1-D]trifluorethanol in aqueous solution. The monodeuterated molecules were examined to minimize coupling of the C−D stretching mode to other normal modes of the molecule. The two modes observed in trifluorethanol are attributed to the C−D bond being anti and gauche to the O−H σ-bond. The trifluoroethanol is completely ionized in 1 N NaOH, resulting in a single symmetrical species with a red-shifted C−D stretch.

overtone or a combination band. Using these data, we estimate the shift to lower frequency of the C−D stretching fundamental upon ionization to be between 55 and 100 cm^{-1}, which can be related to a considerable weakening of the C−D bond. Similar results were obtained in the IR spectra (data not shown), but with lower resolution.

Equilibrium Isotope Effect on Trifluoroethanol Ionization. The titration of an aqueous mixture of TFE (2.5%, v/v; triplet centered at 62.8 ppm) and [1-D_2]TFE (7.5%, v/v; quintuplet centered at 62.2 ppm) is shown in Figure 2. From this titration, it is apparent that ionization of trifluoroethanol results in a deshielding of C_α, a decrease in the one-bond coupling constants, $^1J_{HC}$ and $^1J_{DC}$, and an increase in the one-bond D isotope effect on the $^{13}C_\alpha$ chemical shift of TFE, $^1\Delta C(D_2)$. The decrease in coupling constant is consistent with a longer αC−H bond in the ionized form of TFE. The variation in $^1\Delta C(D_2)$ is a nonlinear function of the extent of ionization, as shown in Figure 3. The $^1\Delta C(D_2)$ increases from 565 to 725 ppb. This 160 ppb increase in $^1\Delta C(D_2)$ is consistent with previous correlations of $^1\Delta C$ with bond length.[30] The nonlinearity of the increase indicates that H_2-TFE ionizes preferentially, i.e., that there is a deuterium EIE (^DK_a) of 1.138 ± 0.009, or 1.067 per D substitution, determined by nonlinear least-squares analysis. Thus, both the increased $^1\Delta C$ and the EIE are consistent with the quantum chemical calculations that indicate that the C−L bond lengthens on ionization and the bond order decreases (vide infra) and with the experimentally observed decrease in ν_{C-D}.

2-Propanol−Methoxide. The H-bond between 2-propanol and methoxide was examined in detail because it is a minimal model for the interaction between a secondary alcohol H-bond donor and a hydrogen-bond acceptor in an enzyme active site. As a secondary alcohol, the orientation of the αC−H bond to the formed H-bond is easily identifiable. H-bonds between ionic and neutral species are also among the strongest, so vibrational shifts resulting from their formation would be the most prominent. The equilibrium geometries of the 2-propanol−methoxide complexes obtained from the calculations are shown in Figure 4. The most significant difference between these three structures is the O−O distance, which was predicted to be 2.676 Å by the PM3 Hamiltonian, 2.591 Å by the ab initio approach, and 2.509 Å in the DFT calculation. In the DFT calculation, the proton was transferred to the methoxide acceptor. This is a low-barrier H-bond, as there is a minimal barrier to proton transfer from 2-propanol to methoxide. In a separate set of DFT calculations, the 2-propanol O−H bond was constrained at 1.06

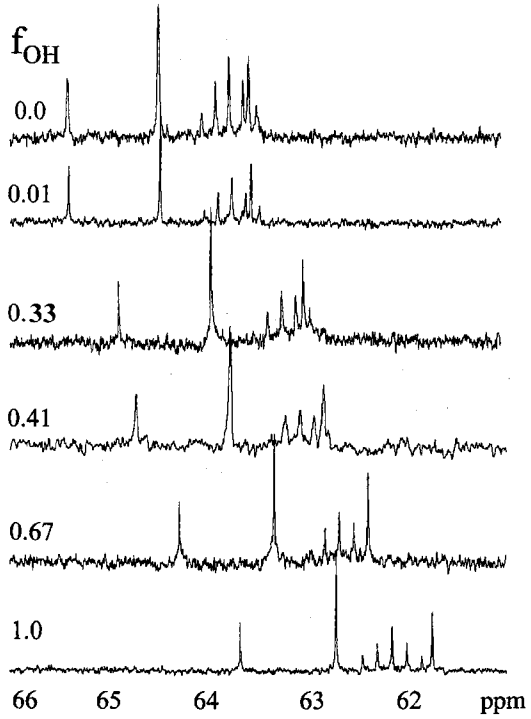

Figure 2. Fluorine-decoupled ^{13}C NMR spectra of C1 during the titration of a mixture of [1-H_2]trifluoroethanol and [1-D_2]trifluoroethanol. The H_2 species is a triplet at lower field, $^1J_{HC} \approx 150$ Hz, and the D_2 species a quintuplet, $^1J_{DC} \approx 25$ Hz. The fractional protonation of the H_2 alcohol, f_{OH}, is shown at left.

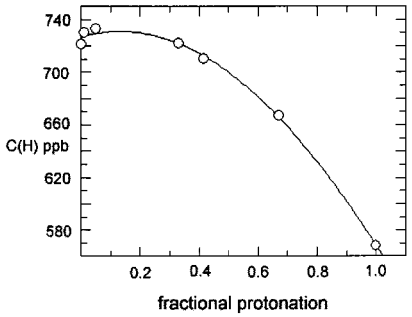

Figure 3. Equilibrium isotope effect on ionization of [1-D_2]-trifluoroethanol. The difference in the chemical shifts of [1-H_2]-trifluoroethanol and [1-D_2]trifluoroethanol, $^1\Delta C(D_2)$, in parts per billion is plotted as a function of ionization of [1-H_2]trifluoroethanol. The nonlinearity of the plot, is a result of the D EIE on ionization, which is determined by a nonlinear least-squares fit to eq 2. The solid curve is drawn with a D_2 EIE of 1.13.

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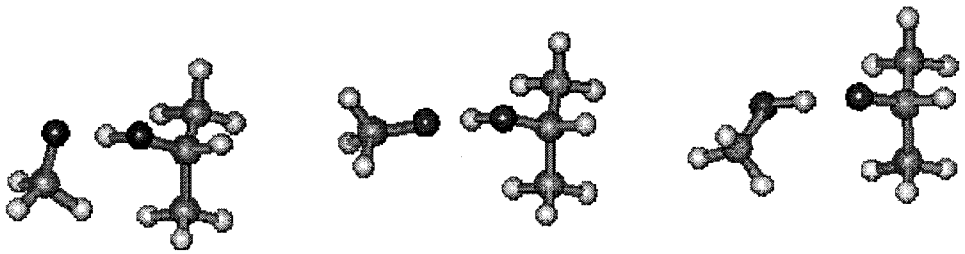

PM3		6-31+G(d,p)		6-31+G(d,p)/B3LYP	
H-C2	1.116	H-C2	1.092	H-C2	1.122
H-C2-O	103.5	H-C2-O	106.6	H-C2-O	110.9
H-C2-O-H	175.0	H-C2-O-H	178.4	H-C2-O-H	178.9
O-H	0.987	O-H	0.992	O-H	1.445
O-O	2.676	O-O	2.591	O-O	2.509

Figure 4. The optimized H-bond geometry of 2-propanol H-bonded to methoxide at the determined with semiempirical, Hartree−Fock, and DFT methods indicated. Carbon atoms are light gray spheres, oxygen atoms darker gray spheres, and H atoms smaller shaded white spheres. Parameters defining the geometry of the H-bond are given below each structure.

Table 1. Equilibrium Isotope Effects on H-Bond Formation between Isopropanol and Methoxide from Semiempirical and ab Initio Calculations

	isopropan[^{18}O]ol	[2-^{13}C]2-propanol	[2-D]2-propanol	ΔO−H stretching frequency (cm^{-1})	ΔC−D stretching frequency (cm^{-1})
AM1 anti	0.996	0.999	0.999	−291 (3500)	−14 (2230)
gauche	0.994	0.999	1.005	−332 (3503)	−7 (2204)
PM3 anti	0.988	1.001	1.030	−445 (3884)	−38 (2152)
PM3 gauche	0.991	1.001	1.026	−459 (3902)	−9 (2091)
anti	0.994	1.001	1.095	−1038 (4169)	−93 (2383)
HF 6-31+G(d,p)					
gauche	0.993	0.999	1.051	−1192 (4187)	−42 (2323)
HF 6-31+G(d,p)					
anti constrained[a] DFT	0.995	1.003	1.128	−889 (3803)	−106 (2260)
B3LYP/6-31+G(d,p)					
gauche DFT	1.014	1.008	1.23	−1870 (3837)	−153 (2202)
B3LYP/6-31+G(d,p)					
anti DFT	1.015	1.009	1.31	−1742 (3803)	−178 (2260)
B3LYP/6-31+G(d,p)					

[a] DFT optimizations resulted in a low-barrier H-bond with the proton localized nearer to the methoxide; the reported values for the anti conformation constrained the methanol O−H distance to 1.40 Å. The reported values for the gauche structure are the unconstrained values. The decrease in the O−H and αC−H stretching frequencies that occurs on complex formation and the calculated values for 2-propanol (in parentheses) are also given.

Å, which is the methanol O−H bond distance in the optimized structure shown. The calculated EIEs for the process of H-bond formation between 2-propanol and methoxide are shown in Table 1. The calculations predicted the well-known decrease in the O−H stretching frequency; however, these values are of questionable reliability because of the large anharmonicity of the O−H−O vibrational mode in strong H-bonds.[31,32] The calculated ^{18}O-2-propanol is ∼0.5% inverse. This inverse value indicates that vibrational modes other than the O−H stretching frequency are important, or this EIE would be normal. The α-D EIE calculated for [2-D]2-propanol had the surprisingly large value of 1.09 in the HF calculation. This decrease in fractionation factor for a hydrogen atom that does not change hybridization state is unanticipated,[33,34] but we now attribute it to a decrease in the C2−H(D) stretching mode, $\nu_{C-H(D)}$. ν_{C-D}, unlike ν_{C-H}, is uncoupled from other normal modes, which makes its assignment and analysis uncomplicated. The decrease in ν_{C-D} is the major source of the calculated EIEs, as indicated by the correlation between the size of the α-D EIE and the decrease in the C−D frequency. All of the computational methods appear to correctly predict a significant decrease in ν_{C-D} in the H-bonded complex, although the AM1 decrease is minimal and the PM3 decrease is roughly 30% of the decrease predicted by the ab initio methods. This correlation is evident in the comparison between model chemistries and in the comparison between the gauche and anti conformations. The larger α-D EIE is calculated in all cases for the anti conformation, correlating with the larger shift to lower wavenumber in ν_{C2-D}.

2-Propanol−Fluorine-Substituted Methoxide. By sequentially substituting one, two, or three methyl group protons of the methoxide H-bond acceptor with fluorine atoms, the H-bond was incrementally weakened. Following optimization of each of the complexes, the enthalpy of H-bond formation and the corresponding EIEs were determined as tabulated in Table 2. This intuitive way of altering H-bond energies produced satisfactory results in terms of geometric properties of the H-bond. With each added fluorine, the distance between the hydroxyl proton and oxygen acceptor increased, and the donor O−H bond was shortened. Furthermore, the stretching frequency of the 2-propanol O−H bond increased as the H-bond weakened.

Of particular interest to this study is the observation that ν_{C2-D} of 2-propanol is a clear function of the H-bond strength. The vibrational frequency of this stretching mode decreases from 2148 cm^{-1} in the complex with the weakest base, acetamide, to 2114 cm^{-1} in the methoxide−2-propanol complex with the strongest H-bond. This change in vibrational frequency correlates with an increase in the EIE calculated for [2-D]2-propanol on complex formation. The results are tabulated in

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Table 2. Calculation of the αC−D Stretching Frequency in anti Isopropanol H-Bond Complexes of Varying Strength Using the Semiempirical PM3 Hamiltonian

complex	enthalpy of interaction (kcal/mol)	αC−D frequency (cm^{-1})	O−O distance (Å)	O−H bond length (Å)	EIE on H-bond formation
2-propanol		2152		0.949	
formamide	−2.17	2148	2.781	0.9601	1.002
syn-chloroformate[a]	−6.1	2136	2.756	0.965	1.006
anti-chloroformate	−9.5	2132	2.757	0.965	1.007
anti-fluoroformate	−11.19	2127	2.723	0.9701	1.013
trifluoromethoxide	−11.86	2126	2.715	0.973	1.018
difluoromethoxide	−14.25	2120	2.702	0.977	1.022
anti-formate	−14.4	2125	2.703	0.977	1.020
fluoromethoxide	−16.64	2117	2.686	0.982	1.027
methoxide	−20.38	2114	2.676	0.988	1.029
isopropoxide		1900			

[a] The terms syn and anti refer to the two different lone pairs on the carboxylate whose position is defined relative to the other carboxylate oxygen.

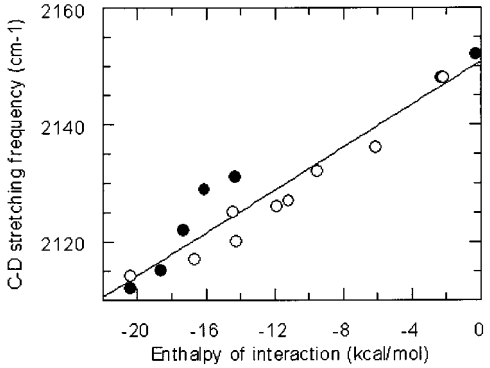


Figure 5. The stretching frequency, ν_{C-D}, of the anti αC−D bond of 2-propanol in H-bonded complexes plotted against the enthalpy of complex formation. The open circles (○) are derived from changing the chemical nature of the H-bond acceptor, and the closed circles (●) come from constraining the H-bond distance between 2-propanol and methoxide.

Table 2 and plotted versus enthalpy of H-bond formation in Figure 5.

2-Propanol−Methoxide with Varied H-Bond Lengths. It has been suggested that the donor and acceptor distance is the "fundamental" physical parameter of H-bonds.[2] Calculating interaction energies for the different separation distances yields well-known sigmoidal curves depicting the dependence of H-bond energy on donor−acceptor distance. By constraining the donor−acceptor distance, it is possible to assess how vibrational frequencies, and consequently EIEs, are a function of H-bond lengths. The drawback to this computational approach is that the optimized structure is not a stationary point. In the calculation of the vibrational frequencies, the distance constraint was removed prior to calculation of the vibrational frequencies. The results of this set of calculations are included in Figure 5. The important observations are that this alternative method of varying the H-bond strength yielded similar results, the αC−D vibrational frequency decreased as the H-bond strength decreased, and the α-D EIE on H-bond formation was normal for the strongest H-bond and decreased toward unity as the H-bond weakened.

Orientation of the H-bond to the αC−H. Two rotamers of 2-propanol, with the O−H bond either anti or gauche to the C−H bond, are shown in Scheme 3. The anti H-bonded rotamer is less favored energetically for steric reasons, as the O−H−O is gauche to both methyl groups.

Scheme 3

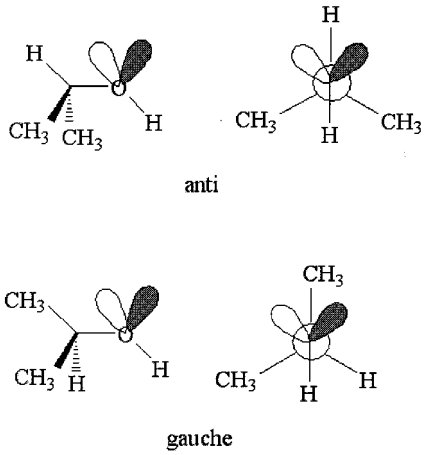


Table 3. Calculated EIEs on Formation of H-Bonded Complexes of Ethanol with Ethanol, Formate, and Ethoxide as the Acceptors at the B3LYP/6-31+G(d,p) Level

H-bond acceptor	O−O distance (Å)	interaction enthalpy (kcal/mol)	anti D EIE	anti C−D stretching frequency[a]	gauche D EIE	gauche C−D stretching frequency[a]
EtOH	2.98	−4.8	1.012	2381 (−24)	1.017	2324 (−11)
HCO_2^-	2.78	−16	1.074	2326 (−76)	1.013	2336 (−6)[1]
EtO^-	2.58	−20	1.14	2303 (−102)	1.081	2299 (−43)

[a] The C−D stretching frequency of the gauche C−D bond could not be unequivocally determined because of its strong coupling with the H-bonded O−H frequency in the complex.

The AM1, PM3, and HF 6-31+G(d,p) calculations were straightforward. As noted above, the B3LYP DFT calculations resulted in the formation of isopropoxide and methanol with a negligible activation barrier to proton transfer. To generate a structure comparable to the other model chemistries, the 2-propanol O−H bond was constrained to 1.06 Å. Both the constrained and unconstrained calculations are included in Table 1.

Ethanol H-Bonding. Encouraged by the magnitude of ab initio predictions of remote isotope effects upon H-bonding of 2-propanol and methoxide, these studies were extended to a model system in which the energy of the formed H-bond could be altered and its effect on the anti and gauche α-hydrogens monitored. H-bond properties were altered by pairing ethanol with a series of acceptors: ethanol, formate, ethoxide, and protonated ethanol. The EIEs were calculated for the donor molecule, as well as some EIEs for the acceptor molecule. The results of these ab initio calculations are included in Tables 3 and 4. EIEs calculated for the ethanol complexes show tendencies analogous to those seen for EIEs in the 2-propanol−methoxide complexes. α-D EIEs for the hydrogen-bonded ethanol are well correlated with interaction energies and H-bond lengths and are also dependent on the conformation of the H-bond. Data contained in Table 4 show that EIEs for the H-bond acceptor are smaller than those calculated for the H-bond donor.

Inosine−Nucleoside Hydrolase Complex. Because of the size of the system, a PM3 calculation was employed to model the hydrogen-bonded 5′-hydroxyl group of a nucleoside and permit a comparison of the calculated results with previously reported experiments.[14] Additionally, a [B3LYP/6-31+G(d,p): PM3] ONIOM calculation[23] of a carboxylate H-bond to the 5′ hydroxyl group of adenosine was modeled. In the calculation of the solution structure. the three rotamers about the C5′−O5′

Table 4. Calculated EIEs on Complex Formation with the Alcohol as H-bond Acceptor Using the HF 6-31+G(d,p) Basis Set

complex	theory level	C−D frequency (shift) cm^{-1}	anti αD EIE	gauche αD EIE	^{18}O EIE	^{13}C EIE_c
EtOH → EtOH	6-31+G(d,p)	a2351 (+17) g2402 (+7)	0.995	0.997	0.998	1.001
$EtOHH^+$ → EtOH	6-31+G(d,p)	a2401 (+67) g2407 (+12)	0.937	0.989	0.994	1.006
H_2O → anti lone pair of gauche 2-propanol	B3LYP	2216 (+25)	1.025	NP[a]	0.999	1.002
H_2O → gauche lone pair of gauche 2-propanol	B3LYP	2218 (+27)	NP[a]	1.017	0.998	1.002
$2H_2O$ → both lone pair of gauche 2-propanol	B3LYP	2239 (+48)	NP[a]	1.005	0.998	1.003

[a] NP is meant to indicate that this proton was not present in the structure and consequently an EIE could not be calculated.

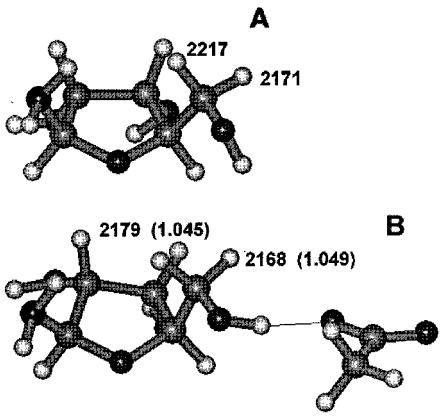


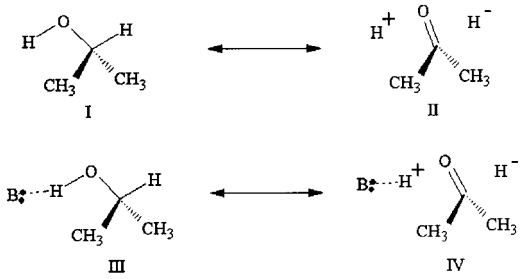
Figure 6. Structure of (A) the aminoriboside and (B) aminoriboside forming a H-bond to the anti lone pair of a carboxylate. The initial orientation of the aminoriboside and carboxylate were taken from the crystal structure of nucleoside hydrolase, 1MAS. The PM3 calculated frequency of the C−D bond stretch for each of the 5′ diastereotopic protons is indicated on the figure. The calculated D EIE on complex formation for each position is given in parentheses after the C−D stretching frequency in panel B.

were evaluated, and the +g rotamer present in the enzyme complex was found to be the most stable. Consequently, the D EIEs could be calculated between the uncomplexed and H-bonded +g rotamers. This equilibrium is shown in Figure 6.

In the ONIOM calculation, the H−O−C5′−C4′ torsion angle was optimized in the anti conformation in the uncomplexed nucleoside, which rotated to a +g rotamer when H-bonded to formate. D EIEs of 1.05 and 0.980 were calculated for the diastereotopic 5′ hydrogens. The large difference in the values of the EIEs for the diastereotopic 5′ protons is a function of their different H5′−C−O−H torsion angles in the H-bonded complex. The anti H, which changed from being anti to a lone pair when uncomplexed to being anti to the O−H σ-bond in the H-bonded complex, generated an inverse effect, whereas the gauche proton generated a larger normal effect on formation of the complex.

Solvent H-Bond Donation. Primary and secondary alcohols can both be H-bond donors and acceptors. In aqueous solution, the lone electron pairs of the alcohol will participate as H-bond acceptors. The formation of an H-bond would alter the electronic distribution and potentially have an effect on both the αC−D stretching frequency and the fractionation factor. This solvation effect was modeled by including a water molecule oriented to donate an H-bond in the structure of 2-propanol. The interactions with both the gauche and anti electron pairs were examined separately and in concert as a complex with two water molecules serving as H-bond donors. The results included in Table 4 indicate that solvent H-bond donation does have a modest effect on the stretching frequency of the αC−D bond of the acceptor, but only a minimal effect on the fractionation factor as the αD EIE on complex formation is near unity.

Scheme 4



Discussion

Equilibrium Isotope Effects on H-Bond Formation. Earlier vibrational spectroscopic results have demonstrated that having an O lone pair anti to a C−D bond reduces ν_{C-D} by ∼70 cm^{-1} in aprotic solvents.[29] Similarly, ν_{C-D} is reduced by 60 cm^{-1} when anti to the N lone pair in methylamines.[35] These are direct physical manifestations of the stereoelectronic effect. These conformation-dependent changes in stretching frequency result in EIEs on rotamer distribution. This was recognized for O- and N-containing heterocycles,[36,37] as well as for methylamines.[35]

We have correlated both the decrease in ν_{C-D} and the corresponding EIEs on H-bond formation with the alcohol acceptor H-bond strength. The most significant finding of our work is that the C−D bond stretching frequency of primary and secondary alcohols is dependent both on its participation as a donor in a H-bond and on the previously characterized dependence on the D−C−O−H torsion angle. These effects are readily apparent in Table 1, with the largest decreases in ν_{C-D} and the corresponding EIE predicted by the DFT level of theory.

A simple resonance depiction of this phenomenon is shown in Scheme 4, where the presence of the H-bond acceptor enhances the contribution of the negative hyperconjugation resonance form, **IV**.

The increased contribution of resonance form **IV** relative to that of **II** can be recognized as differences in the structure of 2-propanol alone and in the H-bonded complex. Of particular importance is the increase in the C−D bond length (∼0.01 Å),

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the increase in electron density on the D (~0.05*e*), the decrease in ν_{C-D}, the decrease in the C−O bond length (~0.02 Å), and the increase in the O−H bond length. Similar (and more complete) depictions of negative hyperconjugation have been presented.[38,39] Analysis by the Atoms-in-Molecules[40] approach indicated that, n the 2-propanol−methoxide complex, the C2-D bond order, as determined by the density Laplacian, was decreased by 7.5%.

A natural bond orbital analysis[41] indicates that the electron density in the O−H σ-bond expands on H-bond formation so that there is an increased overlap with the antibonding orbital of the C−D bond. This explanation provides a rationale for the smaller αD EIE effect of forming a gauche H-bond, i.e., the expansion of the electron density in the O−H σ-bond does not overlap as well with the C−D antibonding orbital and consequently is less effective at reducing the gauche C−D bond order.

The effects on the two C−D stretching frequencies observed in simple monodeuterated alcohols were attributed to a stereoelectronic effect.[29] Our calculations quantitatively confirm that negative hyperconjugation, arising from the antiperiplanar lone pair on the alcohol O, is responsible for the decrease of 60−70 cm^{-1} observed. The 58 cm^{-1} difference in the DFT-calculated C−D stretching frequencies of the anti and gauche conformations of 2-propanol in Table 1 matches the observed spectral change. The rotameric dependence of the C−D stretching frequency implies that incorporation of D into a primary or secondary alcohol should alter the distribution of rotational conformers. A large αD EIE of 1.04 is calculated for the isomerization reaction for going from the stiffer anti conformation to the gauche conformation at the 6-31+G(d,p) level. Anet and Kopelovich[36] reported a D EIE of 1.18 for the equatorial to axial interconversion of 5,5-dimethyl-1,3-dioxane-2-d_1. This exceptionally large conformational D EIE is the result of the interchange of two O-lone pairs anti to the C−D bond in the axial conformation for two C−C bonds that are anti in the equatorial conformation. Similar EIEs have been observed for the rotamers of trimethylamine-d_8[35] and *N*-methylpiperidine.[37] In trimethylamine-d_8, the single C−H bond enriches with an EIE of 1.10 in the C−H bond anti to the lone pair of the N where the C−H stretching frequency is decreased.[35]

Various levels of theory were included in Table 1 to determine how the level of theory affected the calculated EIEs. The magnitude of the calculated αD EIE will depend on the Hamiltonian's ability to account for the increased overlap of the O−H σ-bond electrons with the C−H antibonding orbital. The AM1 and PM3 semiempirical calculations differed in their estimation of this effect, with the PM3 method more successfully emulating the high-level ab initio and DFT calculations. Both semiempirical methods minimized the effect of H-bond formation from the gauche conformation of 2-propanol. The ab initio calculations were significantly affected by the inclusion of diffuse orbitals (data not shown). The correlation effects were less important and the HF 6-31+G(d,p) results came close to reproducing the effects observed using the B3LYP/6-31+G(d,p) level of theory. The DFT calculations modeled the 2-propanol−methoxide interaction as a low-barrier H-bond, with the proton located closer to the methoxide, but with no significant barrier to transfer. The results for this complex can be taken to suggest that there will be a large effect on the αC−H bond in a low-barrier H-bond involving an alcohol donor.

Whereas the αD EIE on association was significant, the heavy-atom isotope effects on association varied negligibly from unity. Whereas a normal ^{18}O EIE might have been anticipated on the basis of the large decrease in the O−H stretching frequency on H-bond formation, the reduced mass for this normal mode is very close to 1.0, indicating that there is very little change in frequency upon substitution with ^{18}O, thus minimizing its contribution to the ^{18}O EIE. As suggested in Scheme 4, the inverse ^{18}O EIE arises from the increase in double-bonded character of the C−O bond. Inverse ^{18}O isotope effects have been calculated previously and attributed to a new vibration that "resemble[s] an O−O stretching motion".[32] The ^{13}C EIE is near unity because the increase in the C−O frequency is offset by the decrease in ν_{C-H}.

Correlation of α-D EIEs with H-Bond Strength. The data in Table 2 and Figure 4 show the effect of the strength of the H-bond formed to anti 2-propanol using the PM3 Hamiltonian. The decrease in the C−D stretching frequency with its corresponding increase in length paralleled the increasing H-bond strength. These data are important because they indicate that the strength of the H-bond is the major cause for the observed effects. H-bond formation alone, e.g., to formamide, does not generate the observed effect. The electrostatic interaction with a negative charge, such as in chloroformate, also is insufficient to explain the H-bond correlation. Additionally, the H-bond strength was varied by constraining the H-bond O−O distance to be greater than the equilibrium distance and constraining the O−H−O bond angle to 179°. Both results are plotted in Figure 5, where linear regression indicated that the effect was a decrease in the C−D vibrational frequency of 1.8 ± 0.2 cm^{-1}/kcal/mol at the PM3 semiempirical level. The comparison of the theoretical calculations presented in Table 1 suggests that the true decrease in C−D stretching frequency upon H-bond formation will be greater. Using the two endpoints in the HF 6-31+G(d,p) level, the predicted correlation is 5.5 cm^{-1} per kcal/mol of interaction enthalpy.

H-Bonds from Ethanol. The two facets of H-bonding identified in the calculations with 2-propanol are further borne out in the HF 6-31+G(d,p) calculations of ethanol complexes, with the additional virtue that the effect on the gauche and anti α-hydrogens can be monitored in the same complex. The C−D stretching frequency for the gauche C−D bond is initially shifted by 70 cm^{-1} from the anti, and as the H-bond in the complex strengthens, both C−D stretching frequencies are shifted to lower wavenumber, with the anti one to a greater extent, so that, in the very strongly H-bonded ethanol−ethoxide complex, both C−D stretches are nearly equivalent (and in the limit of full ionization, symmetry requires that they become equivalent). Thus, at the HF 6-31+G(d,p) level of calculation, the anti and gauche C−D stretching frequencies decrease by approximately 4 and 2 cm^{-1} per kcal/mol of interaction energy, respectively.

H-Bond Donation to Alcohols. The negative hyperconjugation attributed to the lone pairs of the alcohol could be reduced either by protonation or by the alcohols acting as an H-bond acceptor. Previous calculation of an inverse βD EIE of 0.87 for the gas-phase protonation of methanol-d_3 suggests that the reduction in hyperconjugation is less than the increase that occurs on deprotonation.[39] H-bond donation will be less effective than protonation, as shown in Table 4. For weak solvent-type H-bonds, the reduction in hyperconjugation was not significantly dependent on the orientation of the H-bond, with both the gauche and anti C−H bonds being increased in frequency by 25 cm^{-1}

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in the DFT calculation with water H-bonded to 2-propanol and by an average of 12 cm^{-1} in the 6-31+G(d,p) calculation with ethanol donating a H-bond to ethanol. An important conclusion for the interpretation of isotope effects on the association of ligands with macromolecules is that the αD EIEs on formation or loss of solvation-type H-bonds are near unity. However, donation of a strong H-bond, such as that formed between protonated ethanol and ethanol, results in a 6.3% inverse αD EIE.

Experimental Validation of the Calculations. To demonstrate that the alcohol O−H bond order does significantly affect the αC−D stretching frequency and hence the fractionation factor of these hydrogens, two different observations were made on the ionization of TFE in water. The Raman spectra provide direct evidence that ionization of TFE results in the predicted decrease in ν_{C-D}. The decrease from 2217 to 2117 cm^{-1} for the anti H upon ionization of TFE is smaller than that predicted by the model gas-phase calculations. The magnitude of the shift to lower wavenumber upon ionization is suppressed by the stronger H-bonds formed between the ionized ethoxide and water and the potential for forming three rather than two solvent H-bonds. The calculations of the effect of H-bond donors on the C−D frequency in Table 4 demonstrated that both the number and strength of H-bonds donated to the alcohol O increase the αC−D frequency. The decrease in fractionation factor associated with the large decrease in ν_{C-D} upon ionization was confirmed by the observation of an αD EIE of 1.135 on ionization (1.067/deuterium). This number is significantly smaller than the value of 2 (1.26/deuterium) reported for the gas-phase ionization of methanol-d_3.[42] However, it is larger than the aqueous solution EIE upon ionization of 2-nitropropane of 1.23 (∼1.04/deuterium)[43] and KIE 1.11 (∼1.02/deuterium) for the formation of the carbanionic transition state in the decarboxylation of 2,2-[D_6]dimethylbenzoyl acetate.[44] Secondary isotope effects on ionization have also been observed for protonation of methylamines. The αD EIE of 1.5 for trimethylamine-d_9 in aqueous solution (1.047/deuterium) is[45] also less than the gas-phase observation for the protonation [1-D_2]ethyl methylamine of 1.09/deuterium.[46] Because H-bonding to the N and O lone pairs in aqueous solution will reduce the hyperconjugation in the free base, the gas-phase experiments result in significantly larger observed EIEs.

Modeling H-bonds to the 5′-Hydroxyl Ribose. On formation of an enzyme−substrate complex, the substrate has to undergo what can be thought of as a series of more specific reactions, each one of which can be characterized by an EIE. These reactions are (i) desolvation, (ii) restriction to a single conformer, and (iii) formation of the binding interactions with the active site. To test the possibility that the large $^{5'-3H}$(V/K) isotope effect observed for the hydrolysis of [5′-^{3}H]inosine catalyzed by nucleoside hydrolase could be generated by the necessary formation of a H-bond to the carboxylate of the glutamate at the active site, we calculated the EIEs on complex formation between 5′-aminoglycoside and acetate positioned as in the active site of 1MAS, shown in Figure 6 and additionally between an optimized complex of formate H-bonded to the 5′-hydroxyl of adenosine.

The desolvation of the ribose 5′-hydroxyl should have a minimal isotope effect. In the desolvation, both of the H-bonds from the O−H and to the lone pairs will have to be broken. These two processes have offsetting impacts on the C−D stretching frequency in the calculations presented, with loss of the O−H solvent H-bond resulting in an increase in frequency and loss of solvent H-bonds to the lone pairs decreasing the frequency. The effect on the fractionation factor of the 5′ protons is anticipated to be minimal.

Isotope effects on conformational selection are based on different conformations of a molecule having different fractionation factors for an isotopically substituted site. A sensitive method was devised for quantifying this phenomenon.[47] The large variation of the ν_{C-D} in the anti and gauche conformers of the 5′-hydroxyl could lead to significant EIEs if binding restricted the accessible conformations of a ligand. In nucleosides, the three staggered rotamers about the C4′−C5′ bond are all populated in solution, and in each of those rotamers, the population of the staggered rotamers about the C5′−O bond will vary.[48] In six of these rotamers, one of the 5′-hydrogens is anti to a lone pair and the other is gauche, whereas in the remaining three rotamers, both 5′-hydrogens are anti to lone pairs. In the crystallographically observed complex, only one of the 5′ C−H bonds is anti to a lone electron pair. The average number of C5′−H bonds anti to O lone pairs will necessarily decrease on complex formation, predicting an inverse EIE on the basis of conformational selection. The ability of the formation of the crystallographically observed H-bond to account for the observed normal effect was examined by two calculations. At the PM3 level, the αD EIE was calculated using the crystal structures of the riboside and carboxylate as initial structures, as shown in Figure 6. The calculated αD EIEs are larger than the secondary $^{5'-3H}$(V/K) observed in the nucleoside hydrolase reaction,[14] implying that formation of this H-bond can account for this unanticipated observation. The significant EIE generated on formation of a H-bond from the 5′-hydroxymethyl group of nucleosides to a carboxylate was further verified by an ONIOM calculation in which the H-bonded atoms were treated at the DFT level. Formation of this complex also resulted in a normal D EIE sufficiently large to account for the observed effects.

Implications of Conformation and H-Bond Dependent α-D EIEs. The existence of significant αD EIEs on conformational restriction and H-bond formation will necessarily contribute to the uncertainty in the determination of transition-state structures. For example, in work on enzyme-catalyzed dehydration reactions, large secondary $^{\alpha D}$V/K kinetic isotope effects have been attributed to rehybridization of the alcohol carbon from sp^3 to sp^2.[49,50] A second contribution to the observed effect that must be accounted for is the H−O−C−H bond in the transition state and the protonation state of the leaving group. Similarly, in the nucleoside hydrolase reaction, the observed $^{2'-3H}$V/K has been attributed to increased hyperconjugation in the transition state.[14,51] It is clear now that both the H−O−C−H torsion angle and any H-bonds formed by this hydroxyl group in the transition state will also significantly contribute to the observed $^{2'-3H}$V/K.

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The recognition that the C−D frequency of primary and secondary alcohols is a function of their conformation and of the strength of the H-bond provides a spectroscopic window into the interactions at enzyme active sites. The C−D vibrations in the 2100−2300 cm^{-1} range occur in a portion of the vibrational spectrum accessible by Raman spectroscopy in aqueous solutions with minimal background or contributions from protein functional groups. The potential of conformational variation confounding the analysis can be largely removed, as in the case of the nucleoside hydrolase, by examining the interactions in complexes that have been previously determined by crystallography.

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