Characterization of Low-Barrier Hydrogen Bonds. 5. Microsolvation of Enol−Enolate. An ab Initio and DFT Investigation

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Hartree−Fock, Møller−Plesset, and DFT calculations have been carried out using the 6-31+G-(d,p) basis set to study the effect of microsolvation on the strength of a representative low-barrier hydrogen bond. In the gas phase, the hydrogen bond formed between vinyl alcohol (enol) and the corresponding oxyanion (enolate anion) is approximately 30 kcal/mol, with a calculated energy barrier for proton transfer from the enol to the enolate anion that is lower than the zero-point vibrational energy resonant in the system. When both the enol and the enolate anion are microsolvated, by one water molecule each, the resulting hydrogen bond is actually increased in strength slightly. When the microsolvation is asymmetrical, however, so as to cause a mismatch in the pK_a values of the hydrogen-bond donor and hydrogen-bond acceptor, the resulting H-bond is weakened by approximately 4 kcal/mol. These results suggest that small amounts of interstitial water in enzyme active sites may not preclude the existence or importance of low-barrier hydrogen bonds in such biological catalysts.

Introduction

There has been a great deal of debate recently concerning whether or not low-barrier hydrogen bonds (LBHBs) are important in the chemistry of enzyme catalysis.[1−22] There is considerable evidence that a LBHB may be important during the reaction catalyzed by Δ^5-3-keto-steroid isomerase,[1] although this has recently been challenged.[2] Additional experimental evidence in favor of LBHBs being important during enzyme catalysis has been presented by Gerlt et al. in a very recent review.[3] Recent computational and gas-phase experimental work[4] has also shown that LBHBs (also known as Speakman[5]−Hadzi[6] hydrogen bonds) can readily exist in the gas phase.[4] On the other hand, condensed-phase work has shown that for the most part LBHBs do not survive in protic or very polar solvents. More recent studies in several aprotic solvents have shown quite convincingly, however, that LBHBs can form in solution, but their stabilities are highly solvent dependent.[7−10]

Whether or not SSHBs can exist in the condensed phase is of great significance to their purported importance in enzyme catalysis. It has been suggested by several researchers, most notably Kreevoy,[11] Cleland,[11a,12] and Gerlt,[13] that most of the energy required during a typical enzyme catalytic event can be provided via the formation of one short-strong, or possibly low-barrier, hydrogen bond (see below for definitions) involving either the transition state or an energetically similar reactive intermediate.[11−14] The formation of an LBHB can, in principle, supply 10−15 kcal/mol of catalytic energy per enzymatic cycle.[15,16] This is more than enough energy to account for most of the catalysis observed during many enzymatic processes. This hypothesis has been rebutted by several researchers, including Kluger,[17a] Guthrie,[17] Warshel,[18] and others.[9,10,19]

It seems prudent at this point to discuss briefly the differences between a low-barrier hydrogen bond (LBHB)

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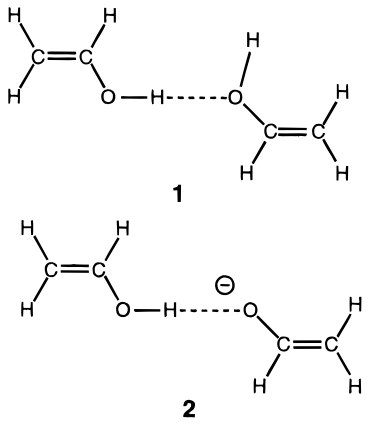
and a short-strong hydrogen bond (SSHB), since there is often confusion concerning the use of these terms. Quite simply, a short-strong hydrogen bond is any hydrogen bond that is significantly stronger (and shorter) than a traditional hydrogen bond. As discussed by Emsley in his excellent reviews,[15] these are usually ionic hydrogen bonds. The distance between the two heteroatoms involved in the SSHB is typically much less than the sum of the van der Waals radii of the atoms. Thus, SSHBs involving oxygen−oxygen donor/acceptor complexes are usually in the 2.4−2.5 Å range, although the very short, very strong H-bond between OH^- and water has been measured as 2.29 Å.[20] SSHBs can have potential energy surfaces with one or two discrete minima. That is, the hydrogen-bonded complex may be perfectly centrosymmetric (one minimum, single-well surface) or noncentrosymmetric, so that there is a minimum corresponding to when the hydrogen is closer to either the donor or the acceptor (double-well surface). The term SSHB says nothing about the shape or features of the potential energy surface. The term LBHB, on the other hand, does imply a great deal about the potential energy surface. LBHB implies that there are two discrete minima on the surface, very close in energy, with a very small barrier for the transfer of the proton from one heteroatom to the other. The intrinsic barrier for proton transfer in these systems is typically 1−2 kcal/mol, or less. This unique feature of LBHBs leads to very special and indicative spectral properties. These properties were studied by Speakman[5] and Hadzii,[6] among others, and have recently been reviewed by Emsley.[15] LBHBs are typically characterized by their unique IR, NMR, and fractionation factor properties. It is clear that in order to get a SSHB one needs reasonably similar acidities of the donor and acceptor; however, just how closely matched these pK_a values need to be to form a true LBHB is currently unknown, but under investigation. Thus, the two terms LBHB and SSHB are very closely linked, but really do refer to slightly different properties of hydrogen bonds.

The primary focus of our current research[22] is to investigate what happens to the strength of a LBHB as a function of varying environmental factors, specifically, in this case, the effect of microsolvation. By studying the effects of various environmental factors on the strength and symmetry of a LBHB we can begin to understand what conditions would be necessary for their existence in an enzyme.

One of the most common catalytic units available to many enzymes is the phenoxyl (phenol), present in the natural amino acid tyrosine (Tyr). The fundamental importance of the Tyr residue for catalysis has long been identified, particularly in enzymes such as the isomerases and the enolases.[1−3] It is the precise role, however, that the Tyr plays in such catalysis that is under debate.[1,2] Specifically, Mildvan and co-workers have reported experimental evidence for the formation of a LBHB during the reaction catalyzed by Δ^5-3-ketoisomerase.[1] During that reaction, the Tyr moiety goes from being weakly hydrogen bonded (via the phenolic hydrogen) to a carbonyl of the substrate to an intermediate (or transition state) where the Tyr phenolic-H is hydrogen bonded to what now resembles an enolate. The interaction between the phenolic-H and the enolate is purported to be a SSHB (or even a LBHB). A more recent article by Pollack and co-workers suggests a slightly different mechanism for this reaction—one where an external hydrogen-bond donor participates directly in the catalysis, and presumably (as suggested by the authors) disrupts the LBHB.[2] We have chosen to study the simplest Tyr and conjugated enol models: the interactions between two enols, and between an enol and an enolate anion (Chart 1). It is well-known that the strongest hydrogen bonds are formed when the proton donor and the proton acceptor have matching pK_a values.[15,16] Thus, the choice of studying the interaction between enol and enolate should represent one of the best possible situations for the formation of not only a SSHB, but a LBHB.

Chart 1



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2

The general approach was to study the interactions shown in Chart 1. After determining whether or not this system forms a true LBHB, we will go on to study the effect of specific solvent molecules on the complexes. For this study, water was chosen as the solvent (Chart 2). This will allow conclusions as to whether or not small amounts of water alone can disrupt a LBHB. It should be noted that the purpose here is not to study the effects of bulk solvent on the formation of a LBHB, but rather to investigate what effects a *small* amount of solvent (as might be found in an enzyme active site) will have on the formation, and stability, of a LBHB.

Methodology

As shown in Chart 1, we have chosen to study the interactions between either an enol molecule and another enol molecule (**1**) or an enol molecule and an enolate anion (**2**). The effect of an external hydrogen-bonding solvent molecule (water) on the strength and geometry of the LBHB was then modeled by studying the structures shown in Scheme 2. Formation of multiple hydrogen bonds was prevented by forcing the central hydrogen bond in compounds **1**−**6** to be linear. A separate study has shown that such a constraint is energetically inconsequential to the calculated hydrogen bond strength.[22e,22g]

All structures were optimized using the standard 6-31+G(d,p) basis set.[23] Calculations were carried out at several levels of theory, specifically, Hartree−Fock (HF), Møller−Plesset many-body perturbation truncated at the second order (MP2), and using density functionals (DFT).[24] The density functionals that were chosen for

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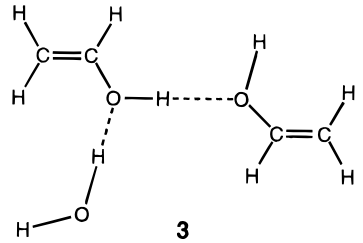
Chart 2



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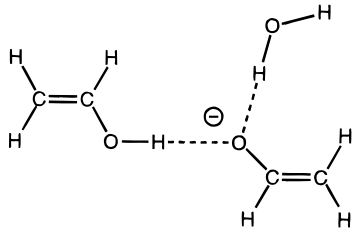

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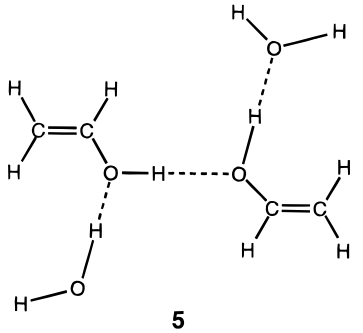

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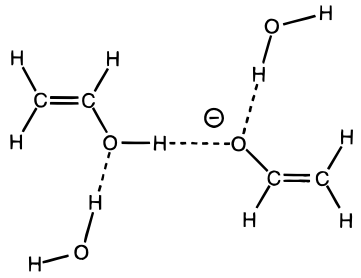

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Table 1. Calculated Energies of Interaction (E_{HB}) Using the 6-31+G(d,p) Basis Set (kcal/mol)

		E_{HB} (kcal/mol)			
no.	reaction	HF	MP2	BLYP	B3LYP
1	enol••••enol	4.4	6.1	4.5	5.0
2	enol••••enolate	25.0	30.2	29.8	30.0
3	enol••••water	3.8	5.2	3.8	4.3
4	enolate••••water	16.2	19.0	18.3	18.7
5	(H_2O)enol••••enol	5.3	7.4	5.6	6.1
6	enol••••enolate(H_2O)	22.3	26.5	24.6	25.3
7	(H_2O)enol••••enol(H_2O)	6.9	9.6	7.6	8.2
8	(H_2O)enol••••enolate(H_2O)	27.2	33.0	30.8	31.4

this study were BLYP and B3LYP. BLYP is a gradient-corrected nonlocal functional incorporating the 1988 Becke exchange functional[25] and the correlation functional of Lee−Yang−Parr (LYP).[26] B3LYP is a hybrid functional made up of Becke's exchange functional, the LYP correlation functional, and a Hartree−Fock exchange term.[27] These functionals were used as supplied in the Gaussian 94 suite of programs.[28]

Results and Discussion

Calculated total energies for all compounds studied can be found in Table 4 of the Supporting Information. Table 1 shows calculated relative energies for the many different reactions of interest to this study. Results at all four levels of theory have been included. In each case, the geometries were optimized using the 6-31+G(d,p) basis set. Inspection of Table 1 reveals that all three correlated methods (MP2, BLYP, B3LYP) give very similar interaction, or hydrogen-bonding, energies (E_{HB}). In each case, the calculated Hartree−Fock hydrogen-bond energy is slightly smaller than the corresponding correlated calculation. Since the correlated methods are generally accepted to be superior to HF, particularly for hydrogen-

Table 2. Calculated Activation Energies for Proton Transfer from Enol to Enolate Anion (kcal/mol) Using the 6-31+G(d,p) Basis Set

	HF	MP2	BLYP	B3LYP
E_A	1.93	0.01	0.01	0.00
E_A + ZPVE	−0.53	−0.42	−0.29	−0.48

Table 3. Optimized Hydrogen-Bonding Distances (Å) Using the 6-31+G(d,p) Basis Set

system	HF	MP2	BLYP	B3LYP
enol−enol (**1**)				
O- - -O	2.982	2.886	2.925	2.904
O- - -H	2.036	1.916	1.943	1.933
enol−enolate (**2**)				
O- - -O	2.525	2.418	2.444	2.422
O- - -H	1.523	1.268	1.230	1.269
H_2O−enol				
O- - -O_w	3.038	2.940	2.985	2.947
O- - -H_w	2.091	1.971	2.003	1.976
H_2O−enolate				
O- - -O_w	2.740	2.666	2.669	2.647
O- - -H_w	1.770	1.659	1.641	1.634
(H_2O)−enol−enol (**3**)				
O- - -O	2.934	2.830	2.865	2.835
O- - -H	1.986	1.857	1.880	1.861
O- - -O_w	3.009	2.898	2.941	2.909
O- - -H_w	2.062	1.928	1.957	1.936
(H_2O)−enolate−enol (**4**)				
O- - -O	2.586	2.496	2.521	2.503
O- - -H	1.601	1.448	1.450	1.453
O- - -O_w	2.773	2.701	2.727	2.703
O- - -H_w	1.808	1.706	1.716	1.705
(H_2O)−enol−enolate−(H_2O) (**6**)				
O- - -O	2.545	2.429	2.449	2.441
O- - -H	1.550	1.321	1.293	1.340
O^{Enol}- - -O_w	2.928	2.782	2.793	2.790
O^{Enol}- - -H_w	1.975	1.799	1.793	1.804
$O^{Enolate}$- - -O_w	2.780	2.725	2.754	2.724
$O^{Enolate}$- - -H_w	1.816	1.734	1.749	1.730

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bonding interactions, we will largely refer only to the correlated calculations in the discussion to follow.[23]

Table 2 contains the calculated activation energies for proton transfer from an enol molecule to an enolate anion. At each level of theory the calculated activation energy disappears after zero-point vibrational energy effects are accounted for. This results in a negative energy of activation, but simply means that there is no potential barrier for proton transfer along the reaction coordinate. This is consistent with the results of a recent quantum dynamics study on the potential energy surface for proton transfer in the $H_3O_2^-$ system.[29] That study found that even though the classical potential energy surface is that of a double-well, quantum effects result in an essentially centrosymmetric distribution of the proton, as if the real potential was single-welled. At the correlated levels of theory employed here, the difference in energy between the noncentrosymmetric hydrogen-bonded anionic complexes and the centrosymmetric hydrogen-bonded "transition states" is practically indistinguishable and further reflects the flat nature of these potential energy surfaces. In all cases, however, the centrosymmetric structures do have a "negative" frequency corresponding to motion along the reaction coordinate for proton transfer.

1. Energetics of LBHBs. Calculations at the Hartree−Fock (HF), Møller−Plesset (MP2), and density functional (DFT) levels of theory using the 6-31+G(d,p) basis set clearly show that the enol−enolate system forms a LBHB. The interaction energy, calculated as the difference between the total energy of the complex versus the infinitely separated enol and enolate pieces, is defined as the hydrogen bond energy (E_{HB}). As the second entry in Table 1 reveals, at all levels of theory the interaction energy for the enol−enolate system (**2**) is very large, ranging from 25.0 kcal/mol (HF) to 30.2 kcal/mol (MP2). At each of these levels of theory the true minima is a noncentrosymmetric complex, suggesting that the potential surface in this region resembles that of a double well, as expected for a LBHB.[15] Structures that have symmetrically positioned hydrogen bonds, representing transition states for proton transfer, are only marginally higher in energy than the true minima. The barriers for hydrogen transfer range from essentially zero to 1.9 kcal/mol. In all cases, this barrier vanishes for the true adiabatic potential energy surface, that is, when zero point vibrational energy is accounted for (Table 2). These results are in excellent agreement with a recent similar study on the formic acid−formate anion potential energy surface, which also showed a very flat potential surface at the correlated levels of theory.[22a] Interestingly, the calculated E_{HB} for enol−enolate is larger than the corresponding E_{HB} between formic acid and formate anion.[22a]

Not surprisingly, the interaction of an enol molecule with another enol molecule does not form a strong hydrogen bond (**1**). The interaction energy for this reaction (first entry, Table 1) ranges from 4.4 kcal/mol (HF) to 6.1 kcal/mol (MP2). Clearly, this is a typical weak hydrogen bond, as would be expected between a weak acid and a weak base.[15,16]

To determine the effect that a small amount of water might have on a LBHB we have reoptimized the structures of enol, enolate, and their complexes in the presence of one or two water molecules. In each case, we were only interested in complexes with one hydrogen bond to water; structures with multiple hydrogen bonds to water were not considered. As the fourth entry in Table 1 shows, enolate anion forms a very strong complex with water, ranging from 16 to 19 kcal/mol (HF, MP2). Enol, on the other hand, forms only a weak hydrogen bond (entry 3, Table 1) with a water molecule: 3.8−5.2 kcal/mol (HF, MP2). It is worth noting at this point the dramatic difference in calculated interaction energy for the enolate−water complex versus the enol−enolate anion systems. That is, while the interaction between water and enolate is calculated to be quite large (19.0 kcal/mol, MP2), it is significantly smaller than the calculated interaction between enol and enolate (30.2 kcal/mol, MP2). This considerable lowering of interaction energy as the pK_a values of the donor and acceptor are varied is also characteristic of LBHBs (and SSHBs in general). To form a true LBHB, the pK_a of the donor and the acceptor must be exactly, or nearly, matched.[15,16] Apparently, altering the pK_a from that of enol to that of water causes a decrease of 10 kcal/mol in the observed interaction energy. Conversely, no such effect is seen with the non-SSHB system. The calculated E_{HB} for the enol−enol system was 6.1 kcal/mol (MP2) and the calculated interaction energy for enol−water is 5.2 kcal/mol (MP2), a difference of only 0.9 kcal/mol. Thus, altering the pK_a of the proton donor and acceptor in a traditional weak hydrogen bond has very little energetic consequences.

The LBHB complexes reveal very interesting trends upon microsolvation. As the sixth entry in Table 1 reveals, a microsolvated enolate anion (**4**) forms a weaker hydrogen bond with enol (26.5 kcal/mol, MP2) than does a nonmicrosolvated enolate anion (30.2 kcal/mol, MP2). This difference of approximately 4 kcal/mol could be very significant. If the enol−enolate system is a true LBHB, then one would expect that the introduction of a water molecule hydrogen bonded to the enolate anion should cause a weakening of the LBHB due to a disruption in the pK_a balance between the proton donor and the proton acceptor. Thus, in effect, the pK_a of the enolate anion hydrogen bonded to water has been lowered relative to that of the non-hydrogen-bonded enolate anion. This is consistent with the idea of maximizing the strength of a SSHB when the pK_a values of the two constituents are exactly matched. Enol hydrogen bonded to water, on the other hand, does not show any dramatic differences in its interaction with another enol (entry 5, Table 1). This is to be expected since the enol−enol system (**3**) is not a SSHB.

Interestingly, when both the enolate anion and enol moieties are microsolvated, i.e., each is hydrogen bonded to a water molecule, a *stronger* LBHB is formed. Entry 8 in Table 1 shows that on average the interaction energy between microsolvated enol and monohydrated enolate anion (**6**) is 1.5 kcal/mol *larger* than in the nonmicrosolvated system (**2**). This is analogous to the results of our previous investigation of the formic acid−formate anion hydrogen-bonding surface.[22a] These are remarkable and surprising results and certainly have implications for the possible role of LBHBs in enzyme active sites. It is not clear, however, exactly why interaction with water, or presumably another hydrogen-bonding solvent, actually increases the strength of the interaction between the enol and enolate anion (or formic acid and formate anion). We believe the reduction in electrostatic repulsion between the two oxygens involved in the LBHB will eventually

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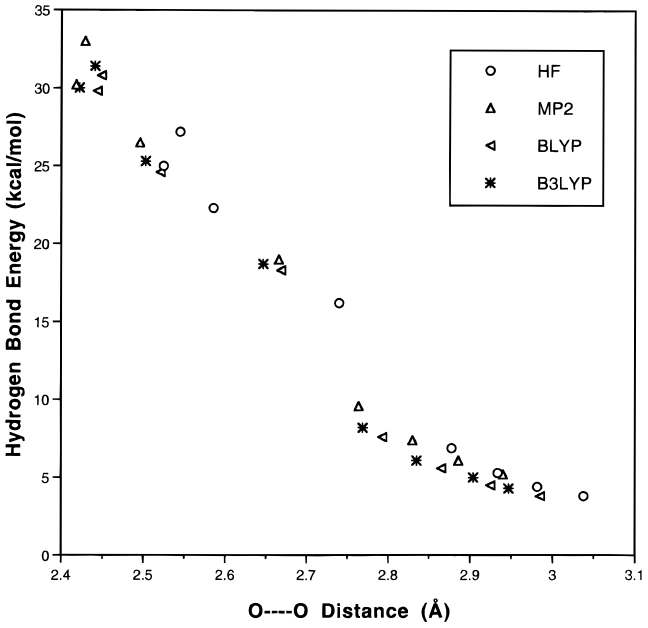

Figure 1. Plot of calculated interaction energies, E_{HB}, (kcal/mol) versus calculated oxygen−oxygen distances for the various hydrogen bonded complexes using all 4 levels of theory (HF, MP2, BLYP, B3LYP)

prove to be responsible for the net increase in E_{HB} for the dihydrated complex (**6**) versus the parent enol−enolate complex (**2**). Further investigations of this phenomenon are clearly warranted and are currently underway in our laboratory. Not surprisingly, the complex between two microsolvated enols (**5**) has about the same hydrogen bond energy (entry 7, Table 1) as the nonhydrated system (**1**). This is consistent with a weak hydrogen-bonding model for complexes **1**, **3**, and **5**.

2. Geometries of LBHBs. Table 3 contains the important hydrogen-bonding distances for all systems studied, as calculated at each level of theory. This allows for a direct comparison of how each theory handles LBHBs. Each O- - -O entry represents the distance between the oxygen atom of the proton donor and the oxygen atom of the proton acceptor. The O- - -H distance is the true hydrogen bond length, between the proton itself and the oxygen of the proton acceptor. In cases where the proton acceptor is a water molecule, the oxygen and proton of the water are represented by O_w and H_w, respectively. The complete optimized geometries of all compounds studied in this work can be found in Tables 4−15 of the Supporting Information.

Figure 1 is a graphical representation of the information in Table 3. It is a plot of calculated O- - -O distances for the hydrogen bonds in the various complexes, at all four levels of theory employed here, versus the calculated interaction energy of that complex (E_{HB}).

Table 3 reveals the dramatic difference in bonding that occurs in a low-barrier or short-strong hydrogen bond versus a traditional weak H-bond. The data clearly show that the proton involved in the LBHB of complex **2** is very nearly shared between the oxygen of the donor molecule (enol) and the acceptor molecule (enolate anion). The O−H (enol) distance is calculated (B3LYP) to be 1.153 Å, while the O- - -H (enolate anion) distance is 1.269 Å. In contrast, the O−H distance in complex **1** is 0.971 Å and the O- - -H distance is 1.933 Å. Complex **1** illustrates the localized bonding of a traditional hydrogen bond, while complex **2** aptly demonstrates the marked differences for LBHB interactions. The data for water−enolate−enol (**4**) show what happens when the pK_a of the proton donor and proton acceptor are mismatched. The geometry of complex **4** reveals a somewhat more localized proton than was found in complex **2**. The O−H distance is now 1.050 Å, while the O- - -H distance has grown to 1.453 Å. While these interaction distances are clearly still shorter than those for weak interactions, they are nonetheless significantly altered from those in the ideal LBHB, complex **2**. The manifestation of this, of course, is that the E_{HB} for complex **4** is about 4 kcal/mol weaker than for complex **2**. Thus, geometrically, the introduction of a solvent molecule (water) has caused a perturbation of the LBHB surface so that the proton is no longer "shared" between the donor and acceptor oxygens; it is now more localized. There is very little observable effect of the water molecule on the geometry of complex **3**, the interaction between two enols, as compared to complex **1**. On the other hand, as the geometry of complex **6** reveals, the proton is now even more delocalized, shared, between the donor and acceptor oxygens when complex **2** is symmetrically solvated. The O−H distance having grown to 1.101 Å, while the O- - -H distance has shortened to only 1.340 Å. This is reflected by the stronger interaction energy for this complex relative to that for either **2** or **4**. Thus, the geometries of these complexes are in excellent agreement with the conclusions reached on the basis of energetic considerations: stronger hydrogen bonds are shorter hydrogen bonds. The geometric variations observed in this study are very similar to those in our previous study of the formic acid−formate anion system.[22a] In all cases, the very short, very strong hydrogen bonds (those of enol−enolate, formic acid−formate, or their symmetricaly solvated counterparts) involve O- - -O distances of about 2.42−2.43 Å. It is thus not that surprising that the shortest known crystal structure involving an O- - -O hydrogen bond is in the HOH−OH^- system (2.29 Å), where each oxygen of the hydrogen bond is further hydrogen bonded to a solvent molecule.[20]

Figure 1 shows the relationship between the hydrogen bond distance, in this case defined as the distance between the two oxygens, and the calculated interaction energy (E_{HB}). The plot clearly reveals the nonlinear nature of this relationship. This is not unexpected, since the shorter, stronger hydrogen bonds are all ionic, while the weaker traditional hydrogen bonds are neutral. The region between approximately 2.8 and 3.1 Å represents the bonding in traditional weak hydrogen-bonded complexes. There is a somewhat abrupt jump in the calculated E_{HB} between 2.7 and 2.8 Å. This would seem to be the demarcation point between weak and moderately strong hydrogen bonding. Complexes with O- - -O distances greater than 2.75 Å must fall in the weak hydrogen-bonding category. The plot remains fairly linear in the 2.7−2.4 Å region. This is the moderately strong to strong hydrogen-bonding region. This plot agrees remarkably well with a recent solid-state study of short-strong hydrogen bonds in crystals[30] and implies that there really is not any extra, or special, stabilization associated with the formation of a LBHB (the points on the farthest left of the plot, shortest O- - -O distances). Rather, the formation of a LBHB is simply the geometric result of having nearly perfectly matched pK_a values of

(30) Gilli, P.; Bertolasi, V.; Ferretti, V.; Gilli, G. *J. Am. Chem. Soc.* **1994**, *116*, 909.

the hydrogen-bond donor and acceptor. These LBHBs are, however, very short, very strong hydrogen bonds.

3. Implications for Enzyme Catalysis. What is the exact environment in an enzyme active site? That is clearly a very important question but has never been answered definitively. If we are to ever discern whether or not LBHBs play an important role in the mechanism of enzyme catalysis we must investigate more closely what environmental factors are at work in an enzyme-active site and how that environment affects the possibility of LBHB, or SSHB, formation.

The presence of small amounts of water, or other hydrogen-bonding solvents, could very well be present in the active sites of enzymes. In this study, we have investigated what effect a small amount of water might have on the characteristics of a LBHB. As shown in Tables 1 and 3, both the geometry and energy of interaction of the low-barrier hydrogen bond formed between an enol and an enolate anion are significantly altered by the addition of one solvent molecule. This is largely due to the fact that the water molecule causes an asymmetry in the LBHB system. This causes the proton donor and proton acceptor molecules to have different pK_a values, thus disrupting the LBHB and weakening the resultant SSHB. This is further illustrated by the fact that a second water molecule, strategically placed, rebalances the pK_a values and causes the reformation of the LBHB and a very strong E_{HB}. This is in excellent agreement with recent experimental studies by Kreevoy et al., who found that the dihydrate of sodium hydrogen bis(4-nitrophenoxide) has a *shorter* (and thus presumably stronger) hydrogen bond than the nonhydrated salt.[11b] They found that the O- - -O distance for the nonhydrated salt to be approximately 2.49 Å, while that for the symmetrically dihydrated salt was 2.46 Å. This is in perfect agreement with our computational results, which predict that the hydrogen bond formed in **6** (dihydrate) is stronger than that formed in **2** (no solvent). Furthermore, our calculated O- - -O distance in the dihydrate enol−enolate complex (**6**) is 2.44−2.45 Å (depending on level of theory), within one one-hundredth of an angstrom of the reported crystal structure hydrogen bond distance. Such excellent agreement may be somewhat fortuitous but, nonetheless, lends credence to our supposition that the enol−enolate system is a suitable model for studying enzymes involving the Tyr residue. That is, inasmuch as the 4-nitrophenoxide system studied by Kreevoy and co-workers[11b] was an appropriate model for such systems, our results agree very well with theirs.

The issue of whether or not LBHBs (or SSHBs in general) play an important role in enzyme catalysis remains controversial. However, theory allows us to test out many hypotheses that would otherwise be untestable. Many aspects of this debate remain unanswered. For instance, how sensitive are LBHBs to the polarity of the environment? How sensitive are LBHBs to very small changes[31] in pK_a values? How sensitive are LBHBs to small structural changes in their geometry? How sensitive are LBHBs to macroscopic amounts of solvent? These are important questions that need to be answered to help resolve the question of whether or not LBHBs play an important role in enzyme catalysis. Our group is currently exploring the answers to these questions, for it is only through a thorough understanding of all the factors that affect low-barrier hydrogen bonds that we can hope to someday understand their precise role in nature.

Conclusions

Hartree−Fock, Moller−Plesset, and DFT calculations have been carried out using the 6-31+G(d,p) basis set to study the effect of microsolvation on the strength of a typical low-barrier hydrogen bond. For all systems studied the DFT methods gave comparable results to those at the HF and MP2 levels of theory, suggesting that DFT is a suitable model chemistry for which to study the very strong interactions present in LBHBs in the future. In the gas phase, the hydrogen bond formed between an enol molecule and an enolate anion is approximately 30 kcal/mol, with a calculated energy barrier for proton transfer from the enol to the enolate anion that is lower than the zero-point vibrational energy resonant in the system. When both the enol and enolate anion are microsolvated, by one water molecule each, the resulting hydrogen bond is actually increased in strength slightly. This suggests that LBHBs can exist in the presence of small amounts of solvent. When the microsolvation is asymmetrical, however, so as to cause a mismatch in the pK_a values of the hydrogen-bond donor and hydrogen-bond acceptor, the resulting H-bond is weakened by approximately 4 kcal/mol and is no longer a LBHB (although it is certainly still a very short, very strong H-bond). The possibility that nature may actually use solvent molecules to rebalance pK_a mis-matches in enzyme active sites is supported. The microsolvation results are in excellent agreement with a recent experimental study of microsolvated LBHBs,[11b] where the authors also concluded that small amounts of interstitial water in enzyme active sites may not preclude the existence or importance of low-barrier hydrogen bonds in such biological catalysts.

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Supporting Information Available: Tables 4−15 containing calculated total energies and geometries of all compounds studied (13 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(31) Shan, S.-O.; Herschlag, D. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14474. This study shows that the slope for a plot of ΔpK_a versus hydrogen bond strength is 0.73 in dimethyl sulfoxide. The slope of the same plot in water is only 0.05. Clearly, as a less polar environment is encountered, the sensitivity increases. This is consistent with our hypothesis that pK_a matching in the nonpolar enzyme active site will be crucial to efficient catalysis.