Characterization of Low-Barrier Hydrogen Bonds. 1. Microsolvation Effects. An ab Initio and DFT Investigation

Yongping Pan and Michael A. McAllister*

Contribution from the Department of Chemistry, University of North Texas, Denton, Texas 76203

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Abstract: Hartree−Fock, Moller−Plesset, and DFT calculations have been carried out using the 6-31++G** basis set to study the effect of microsolvation on the strength of a typical low-barrier hydrogen bond. In the gas phase, the hydrogen bond formed between a formic acid molecule and a formate anion is approximately 25 kcal/mol, with a calculated energy barrier for proton transfer from the formic acid to the formate anion which is lower than the zero point vibrational energy resonant in the system. When both the formic acid and formate 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 spirited debate recently concerning whether or not low-barrier hydrogen bonds (LBHBs) really exist.[1−14] Recent computational and gas-phase experimental work has shown quite convincingly that LBHBs (also known as Speakman[1g]−Hadzi[1h] hydrogen bonds)[1i] can exist.[1] LBHBs, or "short-strong" hydrogen bonds as they are sometimes called, are a reality. They exist, under certain conditions. Elegant condensed phase work has shown that for the most part LBHBs do not survive in polar or protic solvents; however, there may be some remnants of LBHBs formed in apolar aprotic solvents.[2,3,7,8]

Whether or not LBHBs 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, Cleland, and Gerlt, that most of the energy required during a typical enzyme catalytic event can be provided via the formation of one LBHB involving either the transition state or an energetically similar reactive intermediate.[9−14] The formation of a LBHB can, in principle, supply 10−20 kcal/mol of catalytic energy per enzymatic cycle.[9,12] This is more than enough energy to account for most of the catalysis observed during enzymatic processes. This hypothesis has been rebutted by several researchers, including Kluger,[6b] Guthrie,[6b,c] Warshel,[4] and others.[6a,7,8]

The primary focus of this research 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 stability of a LBHB, we can begin to understand what conditions would be necessary for their existence in an enzyme.

The simplest catalytic unit available to most enzymes is the carboxylate, present in all natural amino acids, and as a side chain in aspartic (Asp) and glutamic (Glu) acids. The fundamental importance of the Asp and Glu residues for catalysis has long been identified, particularly in enzymes such as the proteases and the enolases. It is the precise role, however, that the Asp or Glu plays in such catalysis that is under debate.[13] We have chosen to study the simplest Asp and Glu models: the interactions between two formic acids, and between a formic acid and a formate 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.[12] Thus, the choice of studying the interaction between formic acid and formate should represent the best possible situation for the formation of a LBHB.

The general approach was to study the interactions shown in Chart 1. After determining whether or not this system forms a proper LBHB, we go on to study the effect of specific solvent molecules on the complexes. For this study water was chosen

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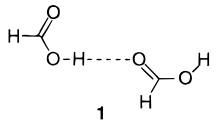
Chart 1



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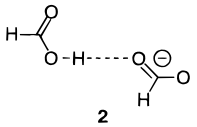

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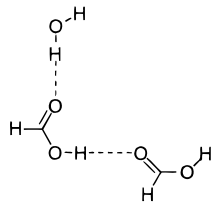
Chart 2



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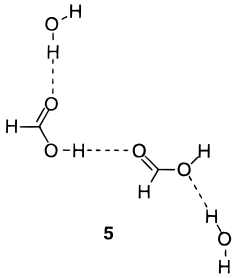

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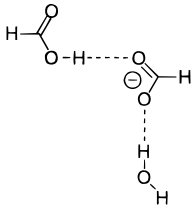

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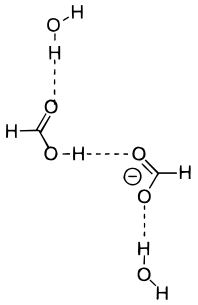

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as the solvent (Chart 2). This will allow conclusions as to whether or not small amounts of water alone can disrupt a LBHB.

The interactions of small molecules with specific solvent molecules has been of great interest recently, particularly in the development of accurate solvent force fields.[15,16] For many years researchers could not explain why parametrized solvent potentials could not reproduce the known solvation behavior of simple amines. Calculations involving Monte Carlo, molecular dynamics, continuum electrostatics, and solvent cavity methods all failed to produce accurate results.[15,16] It was only very recently that Friesner[15] and co-workers were able to solve this problem by looking at the specific interactions occurring between an amine and a solvent molecule using quantum mechanical methods that accurate solvent potentials were produced. This is only the beginning of such fundamental studies, and a great deal more work needs to be done in this area. Particularly, if one ever hoped to develop an accurate solvent potential for the study of peptides and proteins, where LBHBs may be important, then studies of this kind will be critical to the parametrization process.

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Methodology

As shown in Chart 1, we have chosen to study the interactions between either a formic acid molecule and another formic acid molecule (**1**), or between a formic acid molecule and a formate anion (**2**). It is well known that systems of this type would prefer to form multiple hydrogen bonds, usually resulting in a doubly hydrogen bonded dimer.[14] However, for the purpose of this study, we specifically did not want to study multiple hydrogen bonds of this sort. It would seem unreasonable to expect that active site residues in enzymes would have enough mobility, enough freedom of movement, to attain the necessary geometry for a fully doubly hydrogen bonded dimer. Additionally, since we are in fact using the formic acid/formate system as a model for possible interactions of other functional groups, it seemed prudent to restrict our investigation to the singly hydrogen bonded complexes, as represented in Chart 1. 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 Chart 2. Formation of multiple hydrogen bonds was prevented by forcing the central hydrogen bond in compounds **1**−**6** to be 180°. A separate study has shown that such a constraint is energetically inconsequential to the calculated hydrogen bond strength.[1f]

All structures were optimized using the standard 6-31++G** basis set.[17] Calculations were carried out at several levels of theory, specifically Hartree−Fock (HF) and Moller−Plesset many-body perturbation truncated at the second order (MP2), and using density functionals (DFT).[18] The density functionals that were chosen for this study were BLYP and B3LYP. BLYP is a gradient-corrected nonlocal functional incorporating the 1988 Becke exchange functional[19] and the correlation functional of Lee−Yang−Parr (LYP).[20] B3LYP is a hybrid functional made up of Becke's exchange functional, the LYP correlation functional, and a Hartree-Fock exchange term.[21] These functionals were used as supplied in the Gaussian 94 suite of programs.[22]

The geometry of each complex was optimized at each level of theory, with the constraint that hydrogen bonds were fixed as linear. This was necessary to prevent the energetically more favorable, but theoretically less important, multiply hydrogen bonded dimers from forming (as discussed above).

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** 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 bonding interactions,

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

	reaction	$E_{(HB)}$ (kcal/mol) HF	MP2	BLYP	B3LYP
1	$HCOO^-\cdots HOOCH$	22.2	26.9	26.8	27.2
2	$HCOOH\cdots HOOCH$	4.7	6.1	4.7	5.4
3	$HCOO^-\cdots HOH$	14.6	17.0	16.6	17.0
4	$HCOOH\cdots HOH$	4.4	5.1	4.2	4.7
5	$(H_2O)HCOO^-\cdots HOOCH$	20.1	23.4	22.9	23.4
6	$(H_2O)HCOOH\cdots HOOCH$	5.5	7.0	5.8	6.4
7	$(H_2O)HCOO^-\cdots HOOCH(H_2O)$	24.7	29.5	29.6	29.9
8	$(H_2O)HCOOH\cdots HOOCH(H_2O)$	4.7	6.1	4.6	5.5

Table 2. Calculated Activation Energies (kcal/mol) for Proton Transfer from Formic Acid to Formate Anion Using the 6-31++G** Basis Set

	HF	MP2	BLYP	B3LYP
E_A	1.40	0.01	0.02	0.00
E_A + ZPVE	−0.90	−0.02	−0.01	−0.27

we will largely refer only to the correlated calculations in the discussion to follow.[17] However, the fact that HF and correlated calculations are so similar is encouraging, and suggests that even higher level calculations may not be necessary to accurately describe these potential energy surfaces. Similarly, the excellent agreement in calculated E_{HB} from MP2 and DFT suggests that DFT methods should be reliable for the further study of LBHB systems in the future. No one had previously investigated the behavior of DFT methods in these types of systems.

Table 2 contains the calculated activation energies for proton transfer from a formic acid molecule to a formate 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.[23] 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.

1. Energetics of LBHBs. Calculations at the Hartree−Fock (HF), Moller−Plesset (MP2), and density functional (DFT) levels of theory using the 6-31++G** basis set clearly show that the formic acid/formate system forms a LBHB. The interaction energy, calculated as the difference between the total energy of the complex versus the infinitely separated carboxyl pieces, is defined as the hydrogen bond energy (E_{HB}). As the first entry in Table 1 reveals, at all levels of theory the interaction energy for the formic acid/formate system (**2**) is very large, ranging from 22.2 kcal/mol (HF) to 27.2 kcal/mol (B3LYP). At each of these levels of theory the true minimum is a nonsymmetrical complex, suggesting that the potential surface in this region is that of a double well, as expected for a LBHB.[12] Structures which have symmetrically positioned hydrogen bonds, representing transition states for proton transfer, are only marginally higher in energy than the true minimum. The barriers for hydrogen transfer range from essentially 0 to 1.4 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).

On the other hand, the interaction of a formic acid molecule with another formic acid molecule does not form a strong hydrogen bond (**1**). The interaction energy for this reaction (second entry, Table 1) ranges from 4.7 kcal/mol (HF) to 6.1 kcal/mol (MP2). Clearly, this is a typical weak hydrogen bond, as would be expected between a moderately strong acid and a weak base.[14]

To determine the effect that a small amount of water might have on a LBHB, we have reoptimized the structures of formic acid, formate, 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 third entry in Table 1 shows, formate forms a very strong complex with water, ranging from 14 to 17 kcal/mol (HF, B3LYP). Formic acid on the other hand forms only a weak hydrogen bond (entry 4, Table 1) with a water molecule: 4.4−5.1 kcal/mol (HF, MP2). It is worth noting at this point the dramatic difference in calculated interaction energy changes for the formate and water versus formic acid and water systems. That is, while the interaction between water and formate is calculated to be quite large (17.0 kcal/mol, B3LYP), it is significantly smaller than the calculated interaction between formate and formic acid (27.2 kcal/mol, B3LYP). This considerable lowering of interaction energy as the pK_a values of the donor and acceptor are varied is also characteristic of LBHBs. In order to form a true LBHB, the pK_a values of the donor and the acceptor must be exactly, or nearly, matched.[12] Altering the pK_a from that of formic acid 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-LBHB system. The calculated E_{HB} for the formic acid/formic acid system was 5.4 kcal/mol (B3LYP), and the calculated interaction energy for formic acid and water is 4.7 kcal/mol (B3LYP), a difference of only 0.7 kcal/mol. Thus, altering the pK_a values of the proton donor and acceptor in a traditional weak hydrogen bond has very little energetic consequence.

The LBHB complexes reveal very interesting trends upon microsolvation. As the fifth entry in Table 1 reveals, a microsolvated formate molecule (**4**) forms a weaker hydrogen bond with formic acid (23.4 kcal/mol, B3LYP) than does a nonmicrosolvated formate anion (27.2 kcal/mol, B3LYP). This difference of approximately 4 kcal/mol could be very significant, and will be discussed in section 3. If the formic acid/formate system is a real LBHB, then one would expect that the introduction of a water molecule hydrogen bonded to the formate 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 formate hydrogen bonded to water has been lowered relative to that of the non-hydrogen bonded formate anion (or formic acid). This is consistent with the idea of maximizing the strength of a LBHB when the pK_a values of the two constituents are exactly matched. Formic acid hydrogen bonded to water, on the other hand, does not show any dramatic differences in its interaction with another formic acid (entry 6, Table 1). This is to be expected since the formic acid/formic acid system (**3**) is not a LBHB.

Interestingly, when both the formate and formic acid moieties are microsolvated, i.e., hydrogen bonded to water, a *stronger* LBHB is formed. Entry 7 in Table 1 shows that on average the interaction energy between microsolvated formic acid and monohydrated formate anion (**6**) is 2.5 kcal/mol larger than in the nonmicrosolvated system (**2**). Why the interaction energy for this LBHB is larger than for the non-water LBHBs is an interesting question. Simplistically one might expect that partial atomic charges could explain the results; however, no clear trend between partial charges and LBHB strength can be seen from

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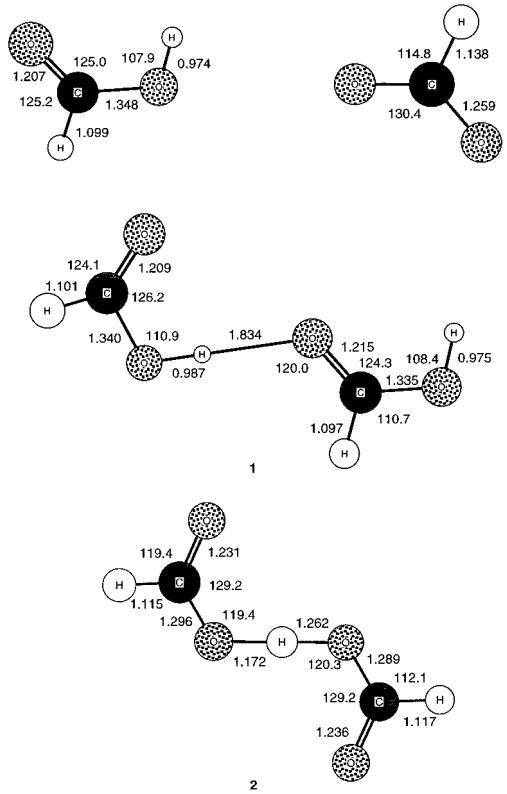

Figure 1. B3LYP/6-31++G** optimized geometries of formic acid, formate anion, the weak complex between formic acid and formic acid (**1**), and the LBHB complex between formic acid and formate anion (**2**).

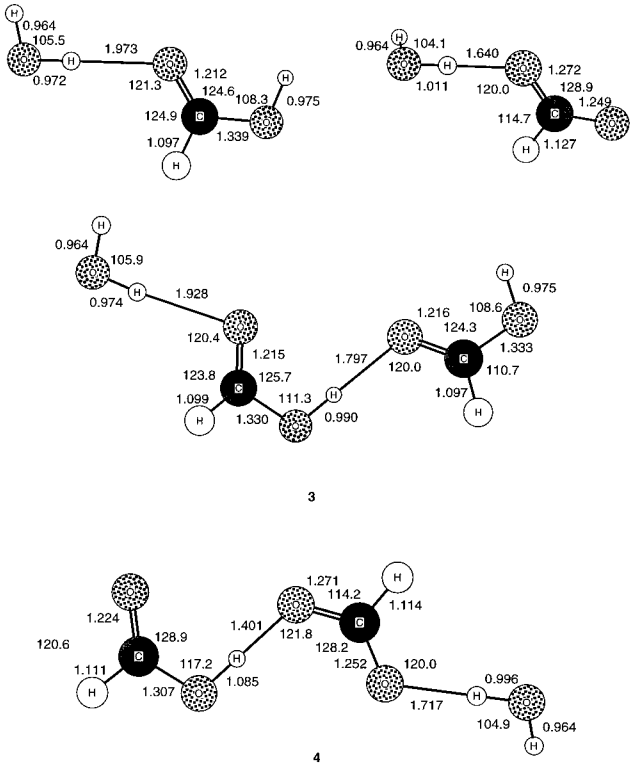

Figure 2. B3LYP/6-31++G** optimized geometries for monohydrated formic acid, monohydrated formate anion, the weak complex between a monohydrated formic acid and another formic acid (**3**), and the strongly bound complex between a monohydrated formate anion and a formic acid (**4**).

the current study, or from a similar recent study.[1d] However, this is an important aspect of low-barrier hydrogen bonding, and will demand further attention in the coming years. Qualitatively, this phenomenon can be explained: the water interacting with the formate anion makes the anion a slightly poorer proton acceptor. However, at the same time, the water interacting with the formic acid (proton donor) makes it more acidic, since the formic acid is now also acting as a lone-pair donor with the water molecule, thus removing some electron density from the acidic proton.

Not surprisingly, the complex between two microsolvated formic acids (**5**) has about the same hydrogen bond energy (entry 8, 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. Figure 1 shows the B3LYP calculated geometries of formic acid, formate anion, the weakly hydrogen bonded complex between two formic acid molecules (**1**), and the LBHB complex between a formic acid molecule and a formate anion (**2**). Figure 2 contains B3LYP geometries for microsolvated formic acid, microsolvated formate anion, the monomicrosolvated LBHB complex between water−formate and formic acid (**4**), and the weak complex between monohydrated formic acid and formic acid (**3**). Figure 3 shows the complexes formed from dimicrosolvated interactions, specifically water−formate complexed with water−formic acid (**6**) and water−formic acid complexed with water−formic acid (**5**). For the sake of both brevity and simplicity, only B3LYP calculated geometries are included here. Where significant differences in geometrical parameters between the various levels of theory were encountered, they are mentioned specifically below. The full set of optimized geometrical parameters can be found in Tables 5−14 of the Supporting Information.

Table 3 contains the important hydrogen bonding distances

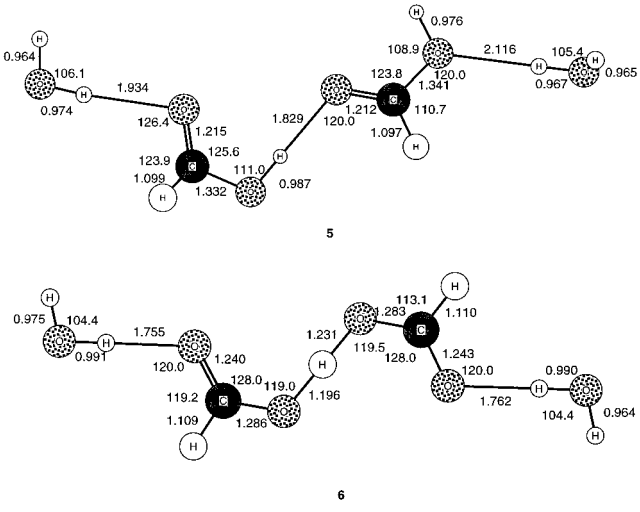

Figure 3. B3LYP/6-31++G** optimized geometries of the weak complex between two monohydrated formic acid molecules (**5**) and the strongly bound complex formed between a monohydrated formate anion and a monohydrated formic acid molecule (**6**).

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.

Figure 4 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

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

system	HF	MP2	BLYP	B3LYP
formic acid/formate (**2**)				
O···O	2.52	2.43	2.46	2.43
O···H	1.50	1.26	1.23	1.26
formic acid/formic acid (**1**)				
O···O	2.90	2.84	2.85	2.82
O···H	1.95	1.86	1.86	1.83
H_2O/formate				
O···O_w	2.74	2.67	2.67	2.65
O···H_w	1.77	1.67	1.65	1.64
H_2O/formic acid				
O···O_w	3.03	2.96	2.99	2.95
O···H_w	2.08	1.99	1.99	1.97
(H_2O)−formate/formic acid (**4**)				
O···O	2.56	2.49	2.51	2.48
O···H	1.56	1.42	1.40	1.40
O···O_w	2.79	2.73	2.73	2.71
O···H_w	1.82	1.74	1.73	1.72
(H_2O)−formic acid/formic acid (**3**)				
O···O	2.88	2.81	2.82	2.79
O···H	1.92	1.83	1.82	1.80
O···O_w	2.99	2.93	2.93	2.90
O···H_w	2.04	1.96	1.95	1.93
(H_2O)−formate/formic acid−(H_2O) (**6**)				
O···O	2.52	2.43	2.46	2.43
O···H	1.50	1.27	1.23	1.23
O^F···O_w	2.80	2.76	2.77	2.75
O^F···H_w	1.83	1.78	1.76	1.76
O^{FA}···O_w	2.88	2.79	2.77	2.75
O^{FA}···H_w	1.93	1.80	1.76	1.76

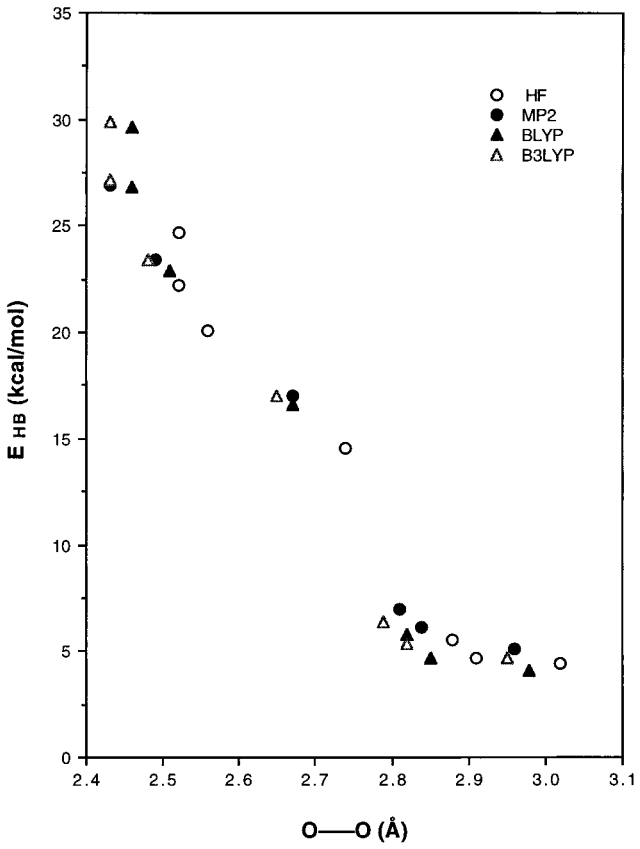

Figure 4. Calculated interaction energies, E_{HB} (kcal/mol), versus calculated oxygen−oxygen distances for the various hydrogen bonded complexes using all four levels of theory (HF, MP2, BLYP, B3LYP).

theory employed here, versus the calculated interaction energy of that complex (E_{HB}).

Figures 1−3 and Table 3 reveal the dramatic difference in bonding that occurs in a low-barrier hydrogen bond versus a traditional weak H-bond. Figure 1 clearly shows that the proton involved in the LBHB of complex **2** is very nearly shared between the oxygen of the donor molecule (formic acid) and the acceptor molecule (formate anion). The O−H (formic acid) distance is calculated (B3LYP) to be 1.172 Å, while the O···H (formate anion) distance is 1.262 Å. In contrast, the O−H distance in complex **1** is 0.987 Å, and the O···H distance is 1.834 Å. Complex **1** illustrates the localized bonding of a traditional hydrogen bond, while complex **2** aptly demonstrates the marked differences for LBHB interactions. Figure 2 shows what happens when the pK_a values of the proton donor and proton acceptor are mismatched. The geometry of complex **4** reveals a somewhat more localized proton that was found in complex **2**. The O−H distance is now 1.085 Å, while the O···H distance has grown to 1.401 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 formic acids, as compared to complex **1**. Figure 3 shows what happens when both fragments of the LBHB are now solvated. As the geometry of complex **6** reveals the proton is now even more delocalized, shared, between the donor and acceptor oxygens, the O−H distance having grown to 1.196 Å, while the O···H distance has shortened to only 1.231 Å. This is reflected by the stronger interaction energy for this complex relative to that for either **2** or **4**. Not surprisingly, the structure of **5** is very similar to that for both **1** and **3**.

Table 3 highlights the differences between the various levels of theory employed in this study. The geometries for the weakly bound complexes (**1**, **3**, **5**) are very similar at all levels of theory. There are noticeable differences, however, with the calculated geometries for the more strongly bound complexes (**2**, **4**, **6**). For each of those systems the geometries calculated using correlated methods (MP2, BLYP, B3LYP) predict structures in which the proton of the LBHB is largely delocalized between the oxygen of the donor and the oxygen of the acceptor. This is completely consistent with what is known about the potential energy surfaces of low-barrier hydrogen bonds.[1,12,14,23]

Figure 4 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 biphasic nature of this relationship. The region between approximately 2.8 and 3.1 Å represents the bonding in traditional weakly 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 2.7 Å must fall in the weak hydrogen bonding category. The plot remains fairly linear in the 2.7−2.5 Å region. This is the moderately strong to strong hydrogen bonding region. There appears to be a slight upward curvature of these plots at each level of theory as the geometries approach 2.4 Å. This would be the very strong or LBHB region. This plot agrees remarkably well with a recent solid-state study of short-strong hydrogen bonds in crystals.[24]

3. Implications for Enzyme Catalysis. What is the exact environment in an enzyme active site? That is clearly a very

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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 LBHB.

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 Figures 1−3 and Tables 1 and 3, both the geometry and energy of interaction of the low-barrier hydrogen bond formed between formic acid and formate 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. 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}. Presumably, if we had chosen to put both water molecules on the same oxygen, this would have caused a further reduction in E_{HB} for the formic acid and formate anion system. This is in excellent agreement with recent experimental studies by Kreevoy *et al.* that found that the dihydrate of sodium hydrogen bis(4-nitrophenoxide) has a *shorter* (and thus presumably stronger) hydrogen bond than the nonhydrated salt.[10b] They found 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 excellent agreement with our computational results which predict that the hydrogen bond formed in **6** (dihydrate) is stronger than that formed in **2** (no solvent).

Extending these findings to the more general cases found in actual enzymes suggests intriguing possibilities. In all the systems studied by Gerlt, Gassman, Kreevoy, and Cleland[9−11] there is always some degree of pK_a imbalance in the proposed LBHBs that are formed. For instance, they suggest that there may be a LBHB formed during the reaction catalyzed by ketosteroid isomerase.[25] During that reaction a tyrosine residue is believed to interact with the developing enolate of the steroid molecule. The pK_a of the tyrosine residue[26] is believed to be approximately 12, while that of the enolate[27] would be approximately 10. These numbers are approximations, by definition, since the exact pK_a values of these compounds *in enzyme active sites* are unknown. However, assuming they probably are not perfectly matched, a LBHB may not form. On the other hand, if a water molecule, or other hydrogen bond donor, was available to coordinate with the tyrosine moiety, thereby lowering its pK_a accordingly, perhaps a much stronger hydrogen bond could form. Perhaps that would be a LBHB. This system is still controversial, as a recent study indicates,[25c] but the best estimate thus far is that the energy of the hydrogen bond formed between the Tyr-14 and the substrate is 7.1 kcal/mol.[25b] This is certainly stronger than a traditional hydrogen bond, but is clearly at the lower limit of a Speakman−Hadzi short-strong hydrogen bond.

These issues are clearly controversial. LBHBs may or may not play an important role in enzyme catalysis. However, theory allows us to test many hypothesis that would otherwise be untestable. Many aspects of this debate remain unanswered. For instance, how sensitive are LBHBs to the polarity of their environment? How sensitive are LBHBs to very small changes[28] 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** 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 results comparable 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 a formic acid molecule and a formate anion is approximately 25 kcal/mol, with a calculated energy barrier for proton transfer from the formic acid to the formate anion which is lower than the zero point vibrational energy resonant in the system. When both the formic acid and formate 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-strong H-bond). The possibility that nature may actually use solvent molecules to balance pK_a mismatches in enzyme active sites is suggested. The microsolvation results are in excellent agreement with a recent experimental study of microsolvated LBHBs,[10b] 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 4−14 containing calculated total energies and geometries of all compounds studied (11 pages). See any current masthead page for ordering information and Internet access instructions.

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(28) 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.