Characterization of Low-Barrier Hydrogen Bonds. 7. Relationship between Strength and Geometry of Short-Strong Hydrogen Bonds. The Formic Acid−Formate Anion Model System. An ab Initio and DFT Investigation

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Abstract: High-level ab initio and density functional theory calculations have been used to investigate the dependence of the strength of a typical low-barrier hydrogen bond on geometrical distortions. In gas phase simulations, HF/6-31+G(d,p), MP2/6-31+G(d,p), and B3LYP/6-31+G(d,p) level calculations reveal that the short-strong hydrogen bond formed between a formic acid molecule and a formate anion is very sensitive to both the hydrogen bond length and the hydrogen bond angle. A 0.5 Å lengthening of the low-barrier hydrogen bond results in a weakening of that bond by over 6 kcal/mol. A 1.0 Å lengthening of the hydrogen bond results in an approximately 12 kcal/mol decrease in the calculated strength of the corresponding hydrogen bond. Similarly, an angle bending distortion of the hydrogen bond by as little as 30° can lead to a weakening of the hydrogen bond interaction by more than 5 kcal/mol. Implications for enzyme catalysis are discussed.

Introduction

There has been a great deal of interest in "short-strong" or "low-barrier" hydrogen bonds (LBHBs) in recent years.[1−17] Most of this interest has stemmed from the suggestion by

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Cleland, Kreevoy, Gerlt, and Gassman that the formation of a single short-strong, or low-barrier, hydrogen bond during an enzyme catalytic event can provide enough differential stabilization energy to account for the resulting rate enhancements typically seen in enzymatic reactions.[4−6] Briefly, their proposal involves a mechanism whereby an enzyme-bound intermediate, or transition state, is stabilized by the formation of a single LBHB. They hypothesize that such a bond, if formed, could provide 10−20 kcal/mol of stabilization energy to the enzyme complex. This would then be enough to rationalize the rate accelerations observed during many enzyme-catalyzed reactions.[4−6] This hypothesis has certainly not been without criticism. The most ardent opponents of the low-barrier hydrogen bond facilitated enzyme mechanism have been Guthrie[7] and Warshel,[8] although there have certainly been others.[2,3,9,10]

Experimental evidence for the formation of LBHBs is considerable in the gas and solid phases. Excellent reviews by Emsley[13] detail the conditions necessary for the formation of such bonds, and a detailed investigation by Gilli[14] extends these studies to the solid state. Recent studies on enzyme inhibitor complexes have produced considerable evidence for the formation of LBHBs during some enzyme-catalyzed reactions.[4c] There is, however, only limited evidence that LBHBs may be formed in the condensed phase.[2,3,10,12]

In this respect, a recent report by Rebek and co-workers described their efforts to measure the strength of a low-barrier hydrogen bond in benzene and dichloromethane solutions.[12] They chose a somewhat rigid model (substituted *m*-xylidene-diamine bis(Kemp's triacid) imide, also known as XDK) based on Kemp's triacid as a template for the formation of a LBHB. They proposed that the rigidity of the system would force the two free acids to form a stable hydrogen bond, presumably in

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the most favorable syn−syn conformation. Measuring the equilibrium for deprotonation of the diacid (**1**) leads to an estimate for the upper limit of the hydrogen bond strength in the monoanion (**2**), the species purported to involve a LBHB. Their results[12] are perhaps the best available evidence for the formation of LBHBs in nonpolar organic media. The estimated strength of the LBHB in **2** was reported to be 8.3 kcal/mol (in benzene, slightly lower in dichloromethane) higher than that of the hydrogen bond in **1**. Thus, assuming that the hydrogen bond in **1** was a traditional, weak hydrogen bond with an average HB energy of approximately 2−3 kcal/mol, the LBHB strength in **2** must be in the range of 10−11 kcal/mol. Perhaps not surprisingly, this is exactly the value recently reported for the experimentally observed LBHB in the enzyme active site of a β-ketosteroid isomerase.[1g]

(**1**)   (**2**)
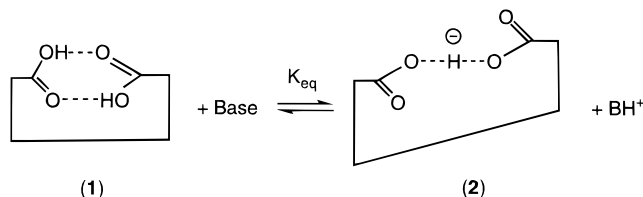
Rebek and co-workers write in their paper[12] that the Kemp's triacid system (XDK) was "rigidly locked into a conformation that enforces the formation of two intermolecular hydrogen bonds", giving compound **1**, but then later note that "there appears to be enough flexibility in the skeleton of XDK to permit independent motions of the two ends of the system", to allow adoption of conformation **2**, which minimizes electrostatic interactions of the carbonyl oxygens, while maintaining the syn−syn low-barrier hydrogen bond. This seemed a curious notion to us, that the system was both rigid and flexible at the same time. This may very well be the case, and we are not by any means questioning that; however, it led us to wonder what effect the rigidity/flexibility imposed by the template XDK may have on the strength of the resulting LBHB.

There is certainly a great deal of interest in the relationship between geometry and strength of hydrogen bonds.[18] Most of this interest is related to the importance of hydrogen bonding in protein folding. It is well-known that not all hydrogen bonds form "perfectly". That is, some hydrogen bonds are shorter than others, some are linear, some are not. The Brookhaven and Cambridge databases are full of such examples.[19] A recent theoretical paper by T. C. Bruice and co-workers[18] analyzed the potential energy surface of a typical weak intermolecular hydrogen bond. They chose to study the interactions between two amides, such as formamide and acetamide. Through the use of ab initio and semiempirical methods they were able to determine the energetic dependence of both the intermolecular hydrogen bond distance and the corresponding hydrogen bond angle. Their results showed that a lengthening of the hydrogen bond (O--H−N) by 0.5 Å led to a reduction in hydrogen bond energy of about 1 kcal/mol. A lengthening of 1.0 Å led to a decrease in the hydrogen bond strength of roughly 2 kcal/mol. Rather surprisingly they found the hydrogen bond strength to be almost insensitive to the HB angle, at least for values within ±40° of linearity. Thus, an angle deformation of up to 40° only lowered the calculated hydrogen bond energy by 0.2−0.3 kcal/mol. Thus, it would seem apparent from both experimental and theoretical studies that traditional, weak, neutral hydrogen

bonds are not very sensitive to their exact geometry. We suspected this was *not* the case for short, strong, nontraditional, ionic LBHBs.

3
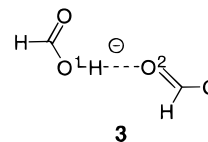
Our previous investigations[17] of LBHBs have shown that the formic acid−formate anion system (**3**) forms a very strong, very short hydrogen bond, and is indeed a true LBHB (for a detailed discussion of the differences between a LBHB and a SSHB (short, strong hydrogen bond) please see ref 17f). Those studies have shown that the hydrogen bond formed between a formic acid and a formate anion is extremely strong, with a calculated energy of interaction (E_{HB}) of approximately 27 kcal/mol (B3LYP/6-31++G(d,p)).[17a,d,e] We have also shown that small amounts of hydrogen bonding solvent molecules, present in many enzyme active sites, will *not* disrupt the strength or geometry of the LBHB formed in the formic acid−formate anion complex (**3**).[17a,f] The present study is designed to investigate what effect changes in the geometry of a LBHB (**3**) have on the corresponding strength of that hydrogen bond. This is relevant not only to the work of Kato et al.,[12] but also to the proposal that LBHBs may be involved in, or in fact responsible for, enzyme catalysis.[4−6] This work is particularly pertinent to a recent study by Cassidy and Frey, which has suggested that steric compression of a LBHB is a crucial step in the mechanism of peptide hydrolysis by serine proteases.[11c]

Methodology

Structures corresponding to the LBHB complex (**3**), formic acid, and formate anion were optimized by using the Gaussian 94 suite of programs.[20] The standard split valence basis set 6-31+G(d,p)[21] was used as provided in Gaussian 94. Geometry optimizations were accomplished by using a variety of computational methods. Ab initio calculations were performed at the Hartree−Fock (HF) and Møller− Plesset (MP2) levels of theory. Density functional theory (DFT) calculations[22] were performed with use of the BLYP and B3LYP functionals. These are gradient corrected nonlocal GGA functionals, as described elsewhere.[23−25] These methods have proven reliable in our previous investigations of these systems.[17]

The relationship between hydrogen bond length and strength for this model LBHB system was studied by systematically lengthening the distance between the hydrogen atom donor (oxygen 1) and the hydrogen bond acceptor (oxygen 2), and recalculating the hydrogen bond energy (E_{HB}) at each step. E_{HB} is calculated as the difference in energy between the complex (**3**) and the infinitely separated monomers, formic acid and formate anion. For each constrained O---O distance, the rest of

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the molecule was allowed to completely optimize. O---O values from 2.3 to 50 Å were studied.

The relationship between hydrogen bond angle and strength was investigated in a similar fashion. However, this time it was the O^1----H-O^2 angle that was frozen, while the rest of the molecule was allowed to optimize. Bond angle variations between 180° and 140° were studied. Angles less than 140° were not studied, as such small angles start to cause multiple hydrogen bonds to form. Such studies will be interesting in the future, but are not the purpose of the current investigation. Bond angle effects were studied in increments of 5°.

Cavity polarity effects were modeled by using standard quantum mechanical continuum methods,[26] as contained in Gaussian 94. Specifically, we used the SCIPCM method of Tomasi and co-workers[27] to study the effect of changing our simulations from a medium of dielectric 1.0, to a medium of dieletric 10.0, and to a medium of dielectric 78.5. In this way we hoped to determine what effect cavity polarity would have on the relationship between hydrogen bond length and hydrogen bond strength. For each O---O interatomic distance studied we obtained an optimized geometry (with the exception of the O---O distance, of course). SCIPCM single point energy calculations were then run on those geometries with use of the 6-31+G(d,p) basis set and the same level of theory the geometry was optimized at, either HF or B3LYP, to determine the total energy in a dielectric 10.0 and 78.5 medium. MP2-SCIPCM calculations were not possible due to the limitations of the SCRF method. Although SCIPCM calculations have been criticized[28,29] for being poor models for specific solvent interactions, we believe they are more than adequate for the study of cavity *polarity* effects alone, which is what this section of the present study is designed to do.

Results

Table 1 contains the calculated hydrogen bond strength (E_{HB}) in complex **3** for 30 different O---O interatomic distances, ranging from 2.3 to 50 Å. This was done at three different levels of theory: HF/6-31+G(d,p); MP2/6-31+G(d,p), and B3LYP/6-31+G(d,p). Figure 1 shows these data in the form of a plot. For the sake of clarity the plot only goes out as far as 8 Å for the O---O separation.

Table 2 contains the data from a similar study, but this time using the SCIPCM method to increase the dielectric of the medium from 1.0 to 78.5. Thus, E_{HB} values are reported at 30 different intervals of O---O from 2.3 to 50 Å, using the HF(SCIPCM)/6-31+G(d,p) and B3LYP(SCIPCM)/6-31+G(d,p) levels of theory. Figure 2 is a graphical display of the data in Table 2. It clearly shows the functional dependence of stabilization energy on the heteroatom separation (O---O). As with Figure 1, this plot is only in the 2−8 Å range, for the sake of clarity. A more limited study of $\epsilon = 10.0$ data leads to Figure 3.

Table 3 contains the results of our angle dependence study. The hydrogen bond angle was varied in increments of 5° from 180° to 140°. Energies (kcal/mol) reported in this table are relative to the global minimum for the fully optimized structure. Figure 4 is a visual representation of these data.

Discussion

It is clear from the data in Tables 1−3 that the hydrogen bond formed between formic acid and formate anion is much more sensitive to its exact geometry than is a traditional weak

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Table 1. Calculated Hydrogen Bond Strengths (E_{HB}) for the Formic Acid−Formate Anion Complex for Various O---O Distances (Å)

O^1---O^2 (Å)	E_{HB} (kcal/mol)		
	HF[a]	MP2[b]	B3LYP[c]
2.30	19.54	24.59	24.80
2.40	21.57	26.83	27.14
2.43		26.90	27.24
2.50	22.21	26.66	26.93
2.52	22.23		
2.55	22.20		
2.60	22.04	25.80	25.80
2.70	21.38	24.59	24.59
2.80	20.43	23.21	23.10
2.90	19.30	21.74	21.55
3.00	18.09	20.25	20.00
3.10	16.85	18.79	18.50
3.20	15.64	17.34	17.07
3.30	14.48	16.04	
3.40	13.38	14.80	14.49
3.50	12.37	13.65	13.36
3.60	11.44	12.59	12.31
3.70	10.58	11.63	11.36
3.80	9.80	10.75	10.50
3.90	9.08	9.96	9.72
4.00	8.43	9.23	9.00
4.50	5.92	6.44	6.28
5.00	4.29	4.65	4.54
6.00	2.44	2.64	2.60
7.00	1.49	1.62	1.61
8.00	0.96	1.05	1.05
9.00	0.63	0.71	0.71
10.00	0.44	0.50	0.50
15.00	0.09	0.11	0.12
25.00	0.00	0.01	0.01
50.00	0.00	0.00	0.00

[a] HF/6-31+G(d,p)//HF/6-31+G(d,p). [b] MP2/6-31+G(d,p)//MP2/6-31+G(d,p). [c] B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p).
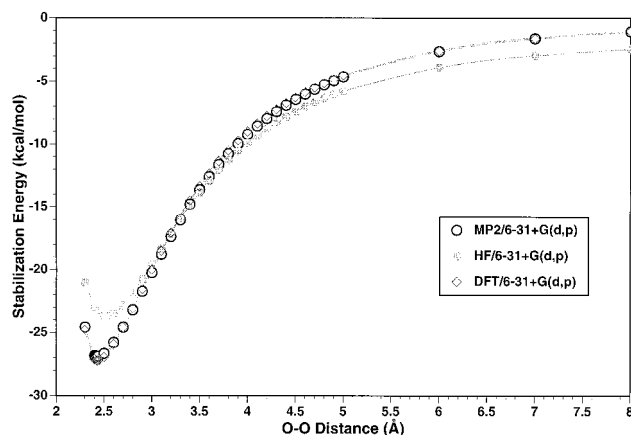
Figure 1. Calculated hydrogen bond energies (stabilization energy, kcal/mol) at various levels of theory as a function of O---O distance (Å) in **3**.

hydrogen bond.[18] Perhaps this is not surprising, but it certainly does have implications for the purported role of LBHBs in enzyme catalysis.

The data in Table 1, and the functions depicted in Figure 1, show the dependence of E_{HB} (calculated hydrogen bond energy) for formic acid−formate anion (**3**) on the internuclear separation of the hydrogen bond donor (O^1) and the hydrogen bond acceptor (O^2). As shown in Figure 1, at all three levels of theory (HF/6-31+G(d,p), MP2/6-31+G(d,p), and B3LYP/6-31+G-(d,p)) the functions are very smooth and continuous, with no singularities−or disruptions of any kind. The Hartree−Fock curve is slightly less shallow, and less steep than the MP2 and

Table 2. Calculated Hydrogen Bond Strengths (E_{HB}) for the Formic Acid−Formate Anion Complex Using SCIPCM ($\epsilon = 78.5$)

O^1---O^2 (Å)	E_{HB} (kcal/mol)		O^1---O^2 (Å)	E_{HB} (kcal/mol)	
	HF[a]	B3LYP[b]		HF[a]	B3LYP[b]
2.30	DNC	10.31	3.60	−1.04	0.24
2.40	7.33	12.53	3.70	−1.76	−0.24
2.43		12.44	3.80	−2.49	−1.79
2.50	7.51	12.42	3.90	−4.44	−1.06
2.52	7.35		4.00	−4.21	−3.15
2.60	7.04	12.27	4.50	−4.66	−5.28
2.70	6.70	11.38	5.00	−2.87	−3.53
2.80	6.38	9.37	6.00	−1.20	−1.48
2.90	5.11	8.14	7.00	−0.92	−1.11
3.00	4.47	7.16	8.00		−0.96
3.10	2.82	6.72	9.00	−0.70	−0.86
3.20	1.74	4.94	10.00	−0.63	−0.77
3.30	0.81	4.07	15.00	−0.39	−1.10
3.40	0.06	1.54	25.00	−0.17	−0.21
3.50	0.01	0.62	50.00	0.00	0.00

[a] HF/6-31+G(d,p)//HF/6-31+G(d,p). [b] B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p).
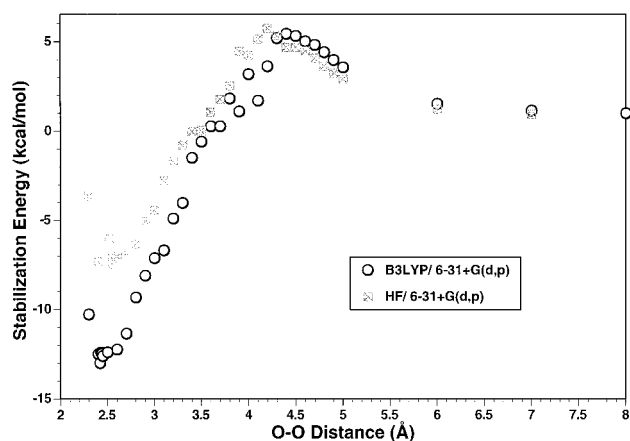
Figure 2. Calculated hydrogen bond energies (stabilization energy, kcal/mol) at various levels of SCIPCM theory, using a dielectric constant of 78.5, versus O---O distance (Å).
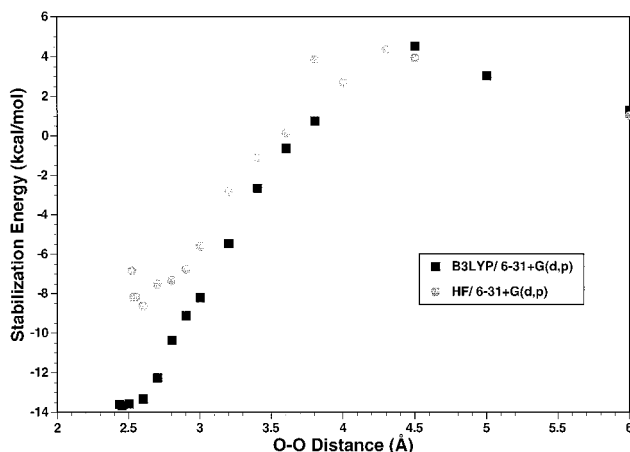
Figure 3. Calculated hydrogen bond energies (stabilization energy, kcal/mol) at various levels of SCIPCM theory, using a dielectric constant of 10.0, versus O---O distance (Å).

B3LYP generated curves, but not dramatically so. It is certainly encouraging that the MP2 and B3LYP functions are so similar. The data in Table 1 reveal that a 0.5-Å lengthening of the O^1---O^2 distance in **3** (from its equilibrium bond distance: 2.43 Å for MP2 and B3LYP and 2.52 Å for HF) reduces the effective hydrogen bond interaction by approximately 6 kcal/mol. A further lengthening of 0.5 Å causes an additional 6 kcal/mol

Table 3. Calculated Relative Energies (kcal/mol) of the Formic Acid−Formate Anion Complex for Various Hydrogen Bond Angles (O---H−O)

O^1---H−O^2 (°)	relative energy (kcal/mol)		
	HF[a]	MP2[b]	B3LYP[c]
180.0	0.04	0.00	0.00
minimum	0.00	0.00	0.00
175.0	0.03	0.14	0.16
170.0	0.29	0.61	0.62
165.0	0.79	1.37	1.37
160.0	1.52	2.39	2.40
155.0	2.42	3.62	3.67
150.0	3.40	5.01	5.11
145.0	4.63	6.50	6.66
140.0	5.88	8.05	8.31

[a] HF/6-31+G(d,p)//HF/6-31+G(d,p). [b] MP2/6-31+G(d,p)//MP2/6-31+G(d,p). [c] B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p).
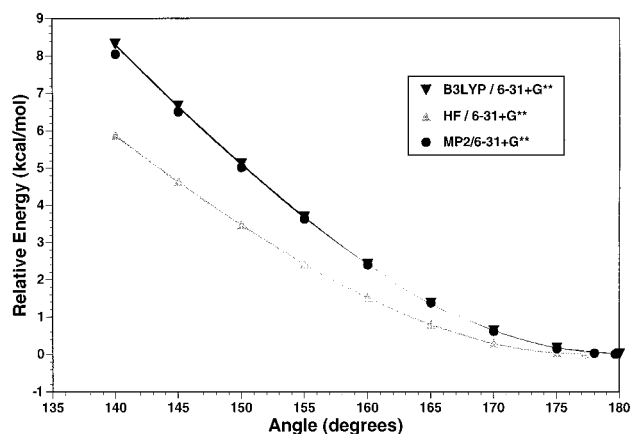
Figure 4. Effect of varying the hydrogen bond angle in the formic acid−formate anion complex. Values (relative energy, kcal/mol) are relative to the fully optimized minimum energy conformation at each level of theory.

weakening of the hydrogen bond. Inspection of Figure 1 also reveals that the functions are linear in this region, but then start falling off more slowly after a 1.0-Å lengthening of the O---O distance. The fact that the initial lengthening of the LBHB has such a linear effect on the strength of interaction is very surprising. This is most likely due to the predominance of covalent-like bonding in this region. After about a 1-Å lengthening of the LBHB the more typical exponential decay that would be expected from an electrostatically dominated hydrogen bond is observed.

Inclusion of cavity polarity effects, via the use of SCIPCM-SCRF single point energy calculations, as shown in Table 2, reveals that the relative weakening of the hydrogen bond within the polar cavities is about the same as in the ideal gas phase ($\epsilon = 1.0$). The hydrogen bond itself is much weaker, but the relative effect of stretching the bond is approximately the same. Thus, a 0.5-Å lengthening of the O---O distance causes an approximately 5-kcal/mol weakening of the hydrogen bond in dielectric 10.0 or 78.5. A further 0.5-Å lengthening of the bond causes an additional 6-kcal/mol decrease in the calculated hydrogen bond energy. Figures 2 and 3 are certainly interesting, and somewhat surprising. It suggests that in the region of O---O separations corresponding to approximately 3.7−7.0 Å the hydrogen bonded complex is less stable than the infinitely separated monomers, formic acid and formate anion. This is most likely due to a limitation of the current cavity model.

The results of our hydrogen bond angle study are shown in Figure 4 and the data are given in Table 3. These results quite clearly show that very, very small angle deformations, on the

order of $\pm 10°$, cause only minimal reductions in the corresponding calculated E_{HB} at all levels of theory. These functions, however, begin to climb rapidly after this initial flat region. Thus, an angle bend ($O^1-H\cdots O^2$) of only 30° leads to a fairly significant E_{HB} decrease of over 5 kcal/mol (MP2 and B3LYP), while a 40° bend causes a weakening of the hydrogen bond by over 8 kcal/mol (MP2 and B3LYP). These are certainly nontrivial deformations. This is in stark contrast to the recent studies of traditional weak neutral hydrogen bonds, which found that hydrogen bond angle changes as large as 40° only led to a reduction in E_{HB} of 0.2–0.3 kcal/mol.[18]

The significance of these studies is at least 2-fold. First of all, it is clear that one must be fairly careful when designing rigid templates for the study of LBHBs in solution, as described in the Rebek paper,[12] not to make the template too rigid. As discussed above, it is now apparent that SSHBs are very sensitive to even minor perturbations away from their ideal geometries. Thus, if a template is used which does not allow full relaxation and reorientation of the molecule on going from a neutral to an ionic hydrogen bond, it appears likely that the corresponding E_{HB} that is measured in solution will be too low. How much too low obviously depends on how rigid the template, and hence how deformed the hydrogen bond, is. We are certainly not trying to imply that there was anything incorrect about the values reported in the Rebek paper, but we would simply suggest that the value of 8.3 kcal/mol reported therein is actually a *lower* limit to the true LBHB energy, as opposed to an upper limit as proposed in the original paper.[12]

There are also clear implications from this study concerning the possible role of SSHBs in enzyme catalysis. That is, if LBHBs, or SSHBs in general, are to play an important role during enzyme catalysis then the enzyme active site must be either flexible enough to allow the optimum geometry for the ionic hydrogen bond to form or preoriented in the optimum geometry for the SSHB. That is, on going from a traditional neutral hydrogen bond, which is presumably present in most enzyme–substrate associated complexes, to the SSHB (or LBHB) of the transition state, there is a shortening of the hydrogen bond of approximately 0.5 Å. If the enzyme active site is not flexible enough to allow for this relaxation, then at least 6 kcal/mol (more if hydrogen bond angle deformation is involved) of the possible stabilization energy will be lost. On the other hand, if the enzyme active site is preoriented in what will eventually be the optimum geometry for the SSHB formed at the transition state, and not the optimum geometry for the original weak hydrogen bond, then maximum stabilization can occur. Of course, if enzyme active sites are completely flexible then they can attain the best possible geometry for both the initial traditional hydrogen bond and the SSHB formed at the transition state. Thus, it is only if one proposes that enzyme active sites are very rigid **and** not preoriented toward the optimum geometry for the ionic hydrogen bond that you can possibly preclude the LBHB facilitated enzyme mechanism, as proposed by Gerlt and Gassman, and Cleland and Kreevoy several years ago.[4–6] It appears most likely, however, that given the recent experimental evidence,[4c] SSHBs do play an important role in many enzyme-catalyzed reactions. This study gives a feel for how sensitive those SSHBs are to geometric distortions of their geometries, and, consequently, some small insight as to how flexible or rigid enzyme active sites might be.

Conclusions

High-level ab initio (HF/6-31+G(d,p) and MP2/6-31+G(d,p)) and density functional theory (B3LYP/6-31+G(d,p)) simulations have been used to investigate the sensitivity of a short, strong hydrogen bond (formic acid–formate anion) to changes in its geometry, away from equilibrium. We found that fairly small hydrogen bond angle deviations ($\pm 30°$) led to an over 5-kcal/mol weakening of the resulting hydrogen bond. Similarly, a 0.5-Å lengthening of the O···O distance in the SSHB led to a decrease of approximately 6 kcal/mol in the strength of the ionic hydrogen bond. These results are consistent with the low-barrier hydrogen bond facilitated mechanism for enzyme catalysis, as long as enzyme active sites are reasonably flexible, or preoriented toward the ideal geometry for the formation of an ionic, short-strong hydrogen bond.