Characterization of Low-Barrier Hydrogen Bonds. 8. Substituent Effects on the Strength and Geometry of the Formic Acid−Formate Anion Model System. An ab Initio and DFT Investigation

Ganesh A. Kumar and Michael A. McAllister*

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

Received August 7, 1997. Revised Manuscript Received February 4, 1998

Abstract: High-level ab initio and density functional theory calculations have been used to investigate the relationship between pK_a mismatch and hydrogen bond strength in a typical low-barrier hydrogen bond system. It is found that a difference of 1 pK_a unit between the pK_a values of the two substituted formate anions vying for a proton in the substituted formic acid−formate anion complex will result in a weakening of the corresponding hydrogen bond by approximately 1.8 kcal/mol. This suggests that small differences in pK_a values (i.e. 1−2 pK_a units) of proton donors and proton acceptors in enzyme active sites should not preclude the importance or significance of LBHBs during the reactions catalyzed by many enzymes. On the other hand, larger differences in relative pK_a values (on the order of 5−6 pK_a units) should be sufficient to cause a considerable weakening of any purported SSHB that might be formed during such an enzyme reaction. It is thus concluded that, just as Gerlt and Gassman suggested in their original paper on LBHBs and enzyme catalysis,[8a] the pK_a matching within the enzyme active site of the two species involved in the LBHB is important to maximizing catalytic stabilization.

Introduction

There has been considerable debate in recent years as to whether low-barrier hydrogen bonds play an important role in enzyme catalysis.[1−23] The eventual outcome of this debate will have widespread implications for how we, as chemists and biochemists, think about enzymes, antibodies, and biological catalysis in general.

Historically, enzyme mechanism and enzyme function have been extremely closely linked. That is, a particular enzyme's function was not viewed as a result of a specific mechanism, but rather the exact mechanism that an enzyme employed was the result of years of evolution to maximize its performance

(1) (a) Garcia-Viloca, M.; Gonzalez-Lafont, A.; Lluch, J. M. *J. Am. Chem. Soc*. **1997**, *119*, 1081. (b) Garcia-Viloca, M.; Gonzalez-Lafont, A.; Lluch, J. M. *J. Phys. Chem*. **1997**, *101*, 3880. (c) Basch, H.; Stevens, W. J. J. *J. Am. Chem. Soc*. **1991**, *113*, 95. (d) Sule, P.; Nagy, A. *J. Chem. Phys*. **1996**, *104*, 8524. (e) Benedict, H.; Hoelger, C.; Aguilar-Parrila, F.; Fehlhammer, W.-P.; Wehlan, M.; Janoschek, R.; Limbach, H. H. *J. Mol. Struct. (THEOCHEM)* **1996**, *378*, 11. (f) Smirnov, S. N.; Golubev, N. S.; Denisov, G. S.; Benedict, H.; Schah-Mohammadi, P.; Limbach, H.-H. *J. Am. Chem. Soc*. **1996**, *118*, 4094. (g) Ramos, M.; Alkorta, I.; Elguero, J.; Golubev, N. S.; Denisov, G. S.; Benedict, H.; Limbach, H.-H. *J. Phys. Chem. A* **1997**, *101*, 9791.

(2) (a) Zhao, Q.; Abeygunawardana, C.; Talalay, P.; Mildvan, A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8220. (b) Zhao, Q.; Abeygunawardana, C.; Mildvan, A. S. *Biochemistry* **1997**, *36*, 3458.

(3) Wu, Z. R.; Ebrahimian, S.; Zawrotny, M. E.; Thornburg, L. D.; Perez-Alvarado, G. C.; Brothers, P.; Pollack, R. M.; Summers, M. F. *Science* **1997**, *276*, 415.

(4) (a) Shan, S.; Herschlag, D. *J. Am. Chem. Soc*. **1996**, *118*, 5515. (b) Shan, S.; Loh, S.; Herschlag, D. *Science* **1996**, *272*, 97. (c) Shan, S.; Herschlag, D. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14474.

(5) Schwartz, B.; Drueckhammer, D. G. *J. Am. Chem. Soc*. **1995**, *117*, 11902.

(6) (a) Cleland, W. W.; Kreevoy, M. M. *Science* **1994**, *264*, 1927. (b) Marimanikkuppam, S. S.; Lee, I.-S. H.; Binder, D. A.; Young, V. G.; Kreevoy, M. M. *Croat. Chem. Acta* **1996**, *69*, 1661. (c) Gerlt, J. A.; Kreevoy, M M.; Cleland, W. W.; Frey, P. A. *Chem. Biol.* **1997**, *4*, 259.

(7) Cleland, W. W. *Biochemistry* **1992**, *31*, 317.

(8) (a) Gerlt, J. A.; Gassman, P. G. *J. Am. Chem. Soc.* **1993**, *115*, 11552. (b) Gerlt, J. A.; Gassman, P. G. *Biochemistry* **1993**, *32*, 11943. (c) Gerlt, J. A.; Gassman, P. G. *J. Am. Chem. Soc*. **1992**, *114*, 5928.

(9) (a) Guthrie, J. P.; Kluger, R. *J. Am. Chem. Soc*. **1993**, *115*, 11569. (b) Guthrie, J. P. *Chem. Biol.* **1996**, *3*, 163.

(10) (a) Warshel, A.; Papazyan, A.; Kollman, P. A. *Science* **1995**, *269*, 102. (b) Warshel, A.; Papazyan, A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 13665. (c) Wesolowski, T.; Muller, R. P.; Warshel, A. *J. Phys. Chem*. **1996**, *100*, 15444.

(11) (a) Scheiner, S.; Kar, T. *J. Am. Chem. Soc.* **1995**, *117*, 6970. (b) Scheiner, S.; Yi, M. *J. Phys. Chem*. **1996**, *100*, 9235.

(12) (a) Perrin, C. L. *Science* **1994**, *266*, 1665. (b) Perrin, C. L.; Thoburn, J. D. *J. Am. Chem. Soc.* **1992**, *114*, 8559. (c) Perrin, C. L.; Nielson, J. B. *J. Am. Chem. Soc*. **1997**, *119*, 12734.

(13) (a) Frey, P. A.; Tobin, J. B.; Whitt, S. A. *Science* **1994**, *164*, 1887. (b) Frey, P. A. *Science* **1995**, *269*, 104. (c) Cassidy, C. S.; Lin, J.; Frey, P. A. *Biochemistry* **1997**, *36*, 4576. (d) Tobin, J. B.; Whitt, S. A.; Cassidy, C. S.; Frey, P. A. *Biochemistry* **1995**, *34*, 6919.

(14) Kato, Y.; Toledo, L. M.; Rebek, J., Jr. *J. Am. Chem. Soc*. **1996**, *118*, 8575.

(15) Tuckerman, M. E.; Marx, D.; Klein, M. L.; Parrinello, M. *Science* **1997**, *275*, 817.

(16) (a) Hibbert, F.; Emsley, J. *Adv. Phys. Org. Chem*. **1990**, *26*, 255. (b) Emsley, J. *Chem. Soc. Rev*. **1980**, *9*, 91.

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

(18) Jeffrey, G. A. *An Introduction to Hydrogen Bonding*; Oxford University Press: New York, 1997.

(19) Scheiner, S. *Hydrogen Bonding: A Theoretical Perspective*; Oxford University Press: New York, 1997.

(20) Wenthold, P.; Squires, R. *J. Phys. Chem*. **1995**, *99*, 2002.

(21) Laidig, J. A.; Platts, J. A. *J. Phys. Chem*. **1996**, *100*, 13455.

(22) Cunninghan, M. A.; Ho, L. L.; Nguyen, D. T.; Gillilan, R.; Bash, P. A. *Biochemistry* **1997**, *36*, 4800.

(23) (a) Pan, Y.; McAllister, M. A. *J. Am. Chem. Soc*. **1997**, *119*, 7561. (b) McAllister, M. A. *Can. J. Chem*. **1997**, *75*, 1195. (c) McAllister, M. A. *J. Mol. Struct. (THEOCHEM)* **1998**, *423*, in press. (d) Pan, Y.; McAllister, M. A. *J. Mol. Struct. (THEOCHEM)* **1998**, *423*, in press. (e) Kumar, G. A.; Pan, Y.; Smallwood, C. J.; McAllister, M. A. *J. Comput. Chem*. **1998**, *19*, in press. (f) Pan, Y.; McAllister, M. A. *J. Org. Chem*. **1997**, *62*, 8171. (g) Pan, Y.; McAllister, M. A. *J. Am. Chem. Soc*. **1998**, *120*, 166. (h) Smallwood, C. J.; McAllister, M. A. *J. Am. Chem. Soc.* **1997**, *119*, 11277. (i) Kumar, G. A.; McAllister, M. A. *J. Am. Chem. Soc*. Submitted for publication.

S0002-7863(97)02745-5 CCC: $15.00
Published on Web 03/17/1998

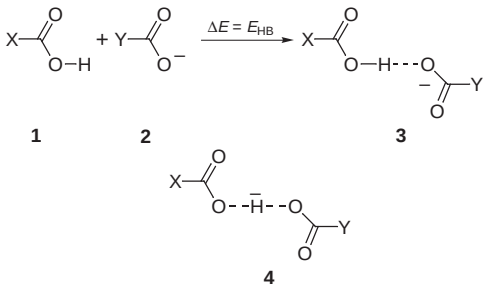
for a given function. While true, the last statement seems to somehow minimize the importance of actual enzyme mechanisms. Thus, traditionally, as is often emphasized in textbooks on general biochemistry, the specific mechanism by which an enzyme catalyzes a reaction was not as important as characterizing the exact function of that enzyme. Early explanations of how enzymes actually were able to catalyze reactions centered around shape/charge complementarity of the enzyme and the transition state, or a significant relief of strain (entropy) in the transition state, or perhaps a model where the transition state was stabilized by many more hydrogen bonds than the initial substrate.

In 1993 Gerlt and Gassman challenged these notions with their proposal that many enzyme-catalyzed reactions can be explained by the formation of *one*, short-strong hydrogen bond to the transition state of an enzyme-catalyzed reaction, as long as a similar interaction is not available in the initial substrate−enzyme complex.[8] The differential stabilization afforded by the short-strong hydrogen bond thus formed could account for up to 10−15 kcal/mol of transition state stabilization, and hence, catalysis. Their proposal, while quite general in scope, specifically called for the formation of a "special" kind of hydrogen bond. The implication was that a low-barrier hydrogen bond (LBHB), if formed, would be extraordinarily short, and strong, much more so than would be predicted on the basis of traditional models of hydrogen bonding. Subsequent papers by Cleland and Kreevoy have expounded upon the original Gerlt and Gassman hypotheses.[6,7] The "low-barrier hydrogen bond facilitated enzyme mechanism", as it has come to be known,[13] is certainly not without its detractors.[4,5,9−12] Many esteemed researchers have challenged the notion of LBHBs being important in enzyme catalysis, from both an experimental[4,5,12] and a theoretical[9−11] standpoint, although there now appears to be excellent, though limited, experimental evidence that LBHBs are involved in several enzyme systems.[2,3,6c,13]

Guthrie[9] and Drueckhammer[5] surmised that LBHBs cannot be important in enzyme catalysis since they do not exist in the condensed solution phase. This argument is perhaps no longer valid since it is now known that LBHBs, or, in a more general sense, short-strong hydrogen bonds (SSHBs), do exist in apolar-aprotic organic solvents.[4,14] Hydrogen bond energies up to 10−11 kcal/mol have been measured by Kato and co-workers in benzene solution not long ago.[14]

On the other hand, Scheiner,[11] using high-level ab initio molecular orbital calculations, was able to show that for very small model systems there does not appear to be any "special" stabilization afforded by the formation of a LBHB proper. That is, while the strongest hydrogen bond formed between two species will be when their pK_a values are matched, the hydrogen bond energy is precisely what one would predict on the basis of a linear relationship between ΔpK_a and hydrogen bond strength. Some of the early LBHB work[8] has been interpreted by others[4] as implying that this would not be the case, that is, one would expect that the formation of a proper LBHB would result in the formation of a hydrogen bond that was *stronger* than would otherwise be expected on the basis of the linear relationship stated above. It should be pointed out, however, that Gerlt and Gassman never made such claims—they suggested that an ionic hydrogen bond, if formed, would be much stronger and shorter than a neutral hydroen bond. Related to this is the recent work of Hershlag et al., who have studied the effect of changing pK_a on hydrogen bond strength in solution.[4c] They concluded that the slope of the line relating the log of the hydrogen bond strength to ΔpK_a is 0.05 in water and 0.73 in DMSO. This would suggest that in DMSO a difference in relative pK_a values of the hydrogen bond donor and acceptor of one unit would lead to a reduction in hydrogen bond strength of approximately 1.0 kcal/mol, while in water the reduction would be trivial. It is obvious from these results that as one goes from polar solvents to less polar (and aprotic) the sensitivity of the hydrogen bond strength to ΔpK_a increases dramatically. The question then is, how good a model is DMSO for an actual enzyme active site? We propose to use high-level ab initio and density functional theory calculations to investigate the relationship between hydrogen bond strength and relative pK_a values of the species involved in an environment more closely resembling that of an enzyme active site. Of course, due to practical limitations we can only study very simple model systems at the current time. However, we believe that these simple model systems contain a great deal of information concerning the fundamental interactions at play during an enzymatic reaction.

Scheme 1



We[23] have recently become very interested in the hypothesis that LBHBs, or SSHBs, may be involved in the reactions catalyzed by several enzymes. We have focused on characterizing all aspects of LBHBs and how the actual environment of an enzyme active site might affect their strength, and hence, ability, to catalyze elementary reactions in enzymes. This work is an extension of those previous studies,[23] and is designed to specifically investigate how sensitive the strength of an LBHB (or SSHB) is to small changes in the pK_a values of the acid and base involved in the hydrogen bond.

Methodology

We have studied a series of complexes between substituted formic acids and formate anions (Scheme 1) using ab initio and density functional theory (DFT) methods, as contained in the Gaussian 94 series of programs.[24] Full geometry optimizations for all complexes and reactants were performed at the Hartree−Fock (HF), Møller−Plesset (MP2), and two DFT levels of theory, using the standard split valence 6-31+G(d,p) basis set.[25] For the DFT calculations[26] both the pure BLYP[27,28] and the hybrid B3LYP[28,29] functionals were used. These

(24) *Gaussian 94* (Revision C.1), Frisch, M. J.; Trucks, G. W.; Schlegal, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T. A.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart; J. P. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A.; Gaussian, Inc.: Pittsburgh, PA, 1995.

(25) Hehre, W. J.; Radom, L.; Schleyer, P. v. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; Wiley-Interscience: New York, 1986; and references therein.

(26) (a) Kohn, W.; Becke, A. D.; Parr, R. G. *J. Phys. Chem.* **1996**, *100*, 12974. (b) Parr, R. G.; Yang, W. *Density Functional Theory of Atoms and Molecules*; Oxford University Press: New York, 1989. (c) Dreizler, R. M.; Gross, E. K. V. *Density Functional Theory*; Springer: Berlin, 1990.

(27) Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098.

(28) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.

Table 1. Calculated Proton Affinities (kcal/mol) for Substituted Formate Anions (**2**) with the 6-31+G(d,p) Basis Set

	level of theory			
substituent	HF	MP2	BLYP	B3LYP
H	356.3	349.0	345.1	348.1
CH_3	360.9	353.4	349.7	352.9
CH_2F	349.8	341.8	338.0	341.4
CHF_2	342.4	334.2	330.8	334.0
CF_3	334.8	327.3	324.1	327.1
F	336.7	328.3	324.1	328.1
OH	350.6	342.7	339.1	342.6
CN	327.7	323.4	319.7	322.0

Table 2. Calculated Hydrogen Bond (E_{HB}) Energies (kcal/mol) for Substituted Formic Acid (X)−Substituted Formate Anion (Y) Complexes (**3**) with the 6-31+G(d,p) Basis Set

substituents		level of theory			
X	Y	HF	MP2	BLYP	B3LYP
H	H	22.2	26.9	26.9	27.2
CH_3	CH_3	20.3	25.1	24.4	24.9
CH_2F	CH_2F	24.4	29.2	28.1	28.8
CHF_2	CHF_2	24.6	30.6	30.0	30.3
CF_3	CF_3	26.3	31.5	30.5	31.0
F	F	26.4	30.2	29.9	30.7
OH	OH	23.2	28.1	27.5	28.1
CN	CN	26.9	31.6	30.9	31.4
H	F	18.2	20.7	19.9	21.1
H	CN	16.3	20.2	19.2	19.7
H	CH_3	23.0			
CH_3	H		24.1	23.5	23.9
H	CH_2F	21.2	25.1	24.4	25.2
H	CHF_2	19.4	23.1	22.6	23.2
H	CF_3	17.8	21.2	20.4	21.1
H	OH	21.2	25.0	24.6	25.4

have proven to be reliable functionals for the study of other LBHB properties in the past.[23] Vibrational analysis of all stationary points was affected to determine whether the structures were true equilibrium minima or transition states. Other recent computational work[1c,d] on the hydrogen diformate parent system with use of the 6-311G(d,p) basis set showed that diffuse functions on first and second row elements tended to lower the calculated hydrogen bond energy in these complexes. This effect, while reasonably small (4−5 kcal/mol), is noteworthy, but is most likely due (as pointed out by a Referee) to a reduction in basis set superposition error when using the basis set including diffuse functions.

Hydrogen bond energies (E_{HB}) were calculated as the difference in energy between the complex **3** and the corresponding constituent monomers, **1** and **2**. Intrinsic potential energy barriers (U_A) for the transfer of the proton from formic acid to formate anion within any complex (**3**) were calculated as the difference in energy between **3** and the corresponding transition state **4**.

The following substituents were studied: X = Y = H, CH_3, CH_2F, CHF_2, CF_3, F, OH, and CN; X = H, Y = CH_3, CH_2F, CHF_2, CF_3, F, OH, and CN.

Results

Calculated proton affinities for each of the substituted formate anions (**2**) employed in this study are given in Table 1. Table 2 contains the calculated hydrogen bond (E_{HB}) energies for the various substituted complexes (**3**). These were simply taken as the difference in calculated internal energy between the complex (**3**) and the corresponding substituted formic acid (**1**) and formate anion (**2**). Table 3 shows the calculated intrinsic energy barrier (U_A) for proton transfer in selected disubstituted complexes (**3**, X = Y). U_A is the difference in energy between **3** and **4** for a given set of substituents. Table 4 contains the important optimized geometric parameters for various mono- and disubstituted complexes, **3**. Specifically, for each level of theory we report both the O···H and O···O intermolecular hydrogen bonding distances. Table 5 contains the calculated proton affinities for selected substituted formate anions and their aqueous pK_a values. All proton affinities were calculated as the change in internal energy between the substituted formate anion and the corresponding protonated molecule.

Table 3. Calculated Classical Energy Barriers (U_A, kcal/mol) for Proton Transfer from Substituted Formic Acid (X) to Substituted Formate Anion (Y) with the 6-31+G(d,p) Basis Set

substituents		level of theory			
X	Y	HF	MP2	BLYP	B3LYP
H	H	1.44	0.00	0.01	0.02
CH_3	CH_3	1.52	0.00	0.01	0.02
CH_2F	CH_2F	1.24	0.00	0.00	0.00
CHF_2	CHF_2	1.20	0.00	0.01	0.01
CF_3	CF_3	1.19	0.00	0.03	0.05
F	F	1.24	0.00	0.04	0.01
OH	OH	1.06	0.00	0.00	0.01
CN	CN	1.27	0.00	0.15	0.15

Figure 1 shows the relationship between the difference in calculated proton affinities between a formate anion (**2**, Y = H) and a substituted formate anion (**2**, Y ≠ H) and the strength of the hydrogen bond in the resulting complex (**3**, X ≠ Y). This was done at all four levels of theory used in this study, and the plot shows the results for each level of theory. Figure 2 is a similar relationship, but for the complexes (**3**) where X = Y. All ΔPA values are relative to those for X = Y = H, which corresponds to ΔPA = 0 on the figure. Thus, in this plot, the ΔPA within each complex is exactly 0, since X = Y; however, there is a ΔPA between X = Y = H and X = Y = CF_3 (for instance), which we take to be the same as the difference in calculated proton affinities for the corresponding substituted formate anions (**2**, Y = H and Y = CF_3, respectively). To simplify, what this plot is intended to show is what happens as you make the formic acid a stronger acid, while still matching its pK_a (or PA) with its conjugate base, i.e., making sure X = Y in **3**. Figure 3 is a simple pictorial representation of what happens to the calculated E_{HB} as you change substituents within any family of compounds. Thus, one should follow the lines within any one particular substituent group, i.e., (CN, CN) to (H, CN) to (H, H) shows what happens energetically as you change one of the substituents from CN to H, and then change the second one from CN to H. The calculated E_{HB} values are plotted against the sum of the calculated proton affinities for the corresponding formate anions (**2**). Figure 4 is a plot of calculated O···O interatomic distances versus calculated hydrogen bond strength for the various substituted complexes (**3**) of this study, at the four different levels of theory employed herein. And finally, Figure 5 is a plot of calculated proton affinities versus pK_a values of a few substituted formate anions for which there is accurate aqueous phase pK_a data available. These data will eventually allow us to relate differences in aqueous pK_a values to differences in hydrogen bond strength within enzyme active sites.

Discussion

The pioneering work of J. C. Speakman[30a] and D. Hadzii[30b] on the discovery and characterization of LBHBs must be

(29) (a) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648. (b) Becke, A. D. *J. Chem. Phys.* **1996**, *104*, 1040. (c) Becke, A. D. *In Modern Electronic Structure Theory*; Yarkony, D. R., Ed.; World Scientific: Singapore, 1995.

(30) (a) Speakman, J. C. *J. Chem. Soc.* **1949**, 3357. (b) Hadzi, D. *Pure Appl. Chem.* **1965**, *11*, 435.

Table 4. Calculated Hydrogen Bond Distances (Å) for Substituted Formic Acid (X)−Substituted Formate Anion (Y) Complexes (**3**) with the 6-31+G(d,p) Basis Set

substituents		level of theory							
		HF		MP2		BLYP		B3LYP	
X	Y	O···H	O···O	O···H	O···O	O···H	O···O	O···H	O···O
H	H	1.504	2.521	1.258	2.427	1.230	2.460	1.262	2.434
CH_3	CH_3	1.509	2.524	1.216	2.421	1.234	2.457	1.218	2.426
CH_2F	CH_2F	1.483	2.503	1.220	2.415	1.239	2.450	1.223	2.420
CHF_2	CHF_2	1.478	2.499	1.211	2.416	1.229	2.451	1.213	2.420
CF_3	CF_3	1.476	2.497	1.208	2.413	1.226	2.449	1.211	2.419
F	F	1.475	2.494	1.243	2.410	1.223	2.441	1.208	2.412
OH	OH	1.465	2.487	1.205	2.407	1.223	2.443	1.208	2.413
CN	CN	1.485	2.503	1.218	2.416	1.236	2.451	1.221	2.421
H	F	1.600	2.593	1.476	2.523	1.452	2.530	1.449	2.509
H	CN	1.652	2.637	1.512	2.550	1.500	2.564	1.494	2.541
H	CH_2F	1.531	2.539	1.379	2.468	1.353	2.483	1.356	2.460
H	CHF_2	1.578	2.576	1.439	2.501	1.419	2.514	1.412	2.499
H	CF_3	1.615	2.606	1.485	2.531	1.467	2.542	1.460	2.517
H	OH	1.531	2.538	1.376	2.463	1.346	2.477	1.346	2.454

Table 5. Calculated Proton Affinities (kcal/mol, 6-31+(d,p)) and Aqueous pK_a Values for Selected Substituted Formate Anions

substituent	level of theory				
	HF	MP2	BLYP	B3LYP	pK_a
H	356.3	349.0	345.1	348.1	3.77
CH_3	360.9	353.4	349.7	352.9	4.76
CH_2F	349.8	341.8	338.0	341.4	2.66
CF_3	334.8	327.3	324.1	327.1	0.23
CH_2Cl	347.2	340.8	334.6	338.7	2.86
$CHCl_2$	339.6	333.6	327.7	331.6	1.26
CCl_3	333.3	327.9	320.8	325.1	0.64
CH_2CN	341.5	335.1	330.3	333.6	2.46

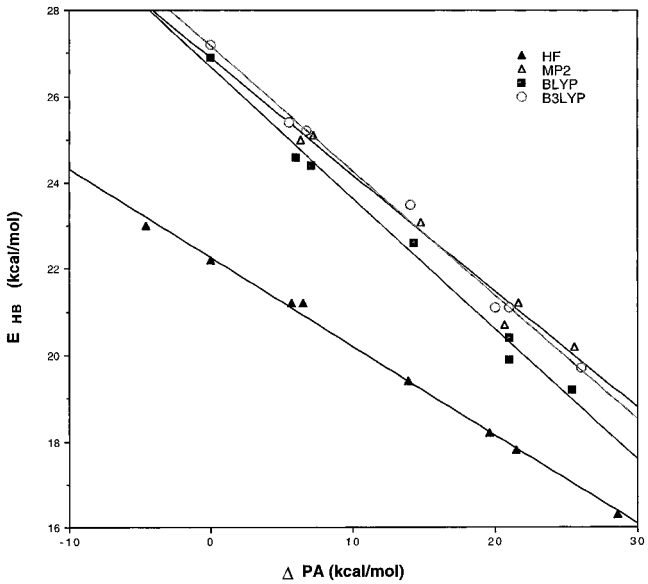


Figure 1. Calculated hydrogen bond energies (E_{HB}, kcal/mol) for complexes of formic acid and substituted formate anions (**3**) at various levels of theory, versus the calculated difference in proton affinities for formate anion and the substituted anion (**2**).

recognized, and as such, LBHBs are often referred to as "Speakman−Hadzii" hydrogen bonds.[6c] While the early work of Speakman and Hadzii concentrated primarily on the unique spectral properties of these compounds, our present discussion will focus largely on the effect of substituents on the strength and geometry of LBHBs, and what consequences that may have on their ability to provide catalytic stabilization to enzymes.

Asymmetric LBHB Complexes. Figure 1 shows the excellent correlation between changes in calculated proton affinities

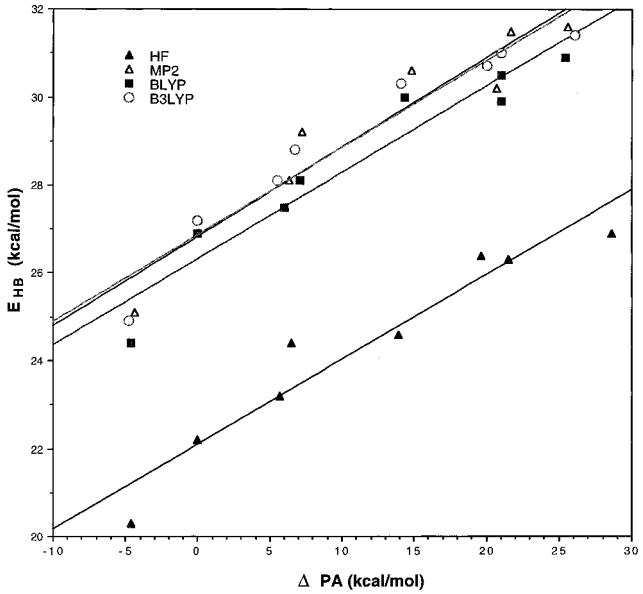


Figure 2. Calculated hydrogen bond energies (E_{HB}, kcal/mol) for complexes of substituted formic acid and substituted formate anions (X = Y) at the Hartree−Fock, Møller−Plesset, BLYP, and B3LYP levels of theory versus the difference in calculated proton affinities of formate anion and the corresponding substituted formate anion, using the 6-31+G(d,p) basis set.

of substituted formate anions and the strength of the corresponding SSHB complex (**3**, X ≠ Y) they form with formic acid. At all four levels of theory (HF, MP2, BLYP, B3LYP) the correlations are excellent when the 6-31+G(d,p) basis set is used: $r = 0.998$, 0.993, 0.995, and 0.997 for HF, MP2, BLYP, and B3LYP, respectively. This is further evidence that there really is not any special stabilization afforded by the formation of a true LBHB. Had that been the case, one would have expected a sudden increase in the calculated E_{HB} for the situation when X = Y = H, since that is the only case when a true LBHB is formed between formic acid and a substituted formate anion. The wide range of proton affinities studied (almost 30 kcal/mol) allows for a meaningful analysis of the relationship between "pK_a" (i.e., proton affinity) mismatch and the strength of the hydrogen bond in complex **3**. The slopes of the three lines corresponding to calculations at correlated levels of theory (MP2, BLYP, B3LYP) are very similar, and give an average value of 0.287. This means that a proton affinity difference between the two bases vying for the proton in **3** of 10 kcal/mol

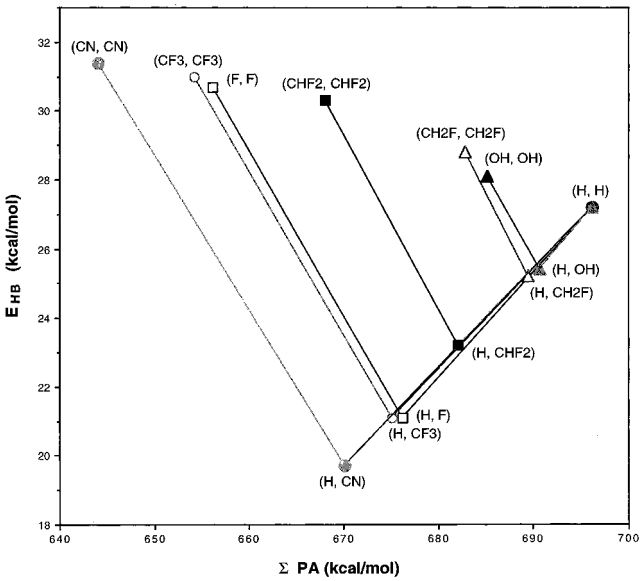


Figure 3. Calculated hydrogen bond energies (E_{HB}, kcal/mol) for complexes of substituted formic acid and substituted formate anion versus the calculated total proton affinity of the substituted anions, at the B3LYP/6-31+G(d,p) level of theory.

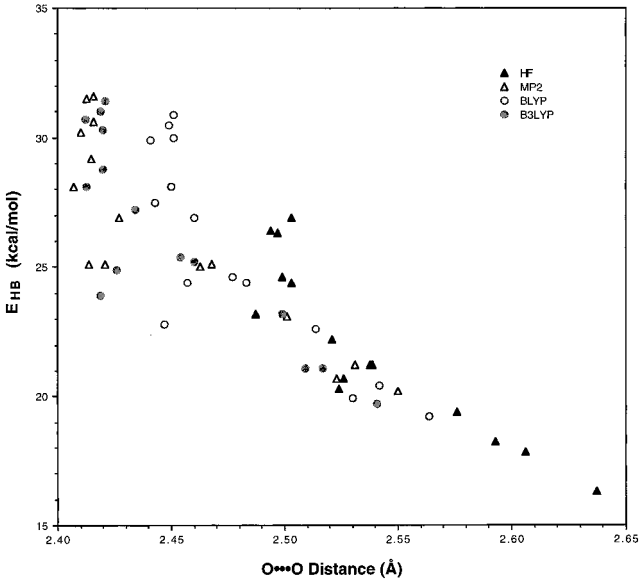


Figure 4. Calculated hydrogen bond energies (E_{HB}, kcal/mol) at various levels of theory as a function of O- - -O distance (Å) in the substituted formic acid−substituted formate anion complexes, using the 6-31+G-(d,p) basis set.

would lead to a weakening of the corresponding hydrogen bond by approximately 2.87 kcal/mol. Using Figure 5 we can attempt to relate the ΔPA values to actual changes in pK_a values of representative substituted formate anions/formic acids. Figure 5 shows the relationship between calculated proton affinities of substituted formate anions (see Table 5 for data and substitutents) and the corresponding aqueous pK_a of the substituted formic acid. Figure 5 contains only data for very closely related structures, i.e., they are all substituted formate anions/formic acids. Thus, it is not surprising that the relationship was found to be fairly good: $r = 0.968$, 0.977, 0.962, and 0.967 for HF, MP2, BLYP, and B3LYP calculations, respectively. The average slope of these lines was found to be 6.14, meaning a change of one pK_a unit resulted in a proton affinity difference of 6.14 kcal/mol. Combining the results of Figure 1 and Figure 5 gives us the relationship between pK_a and E_{HB} for

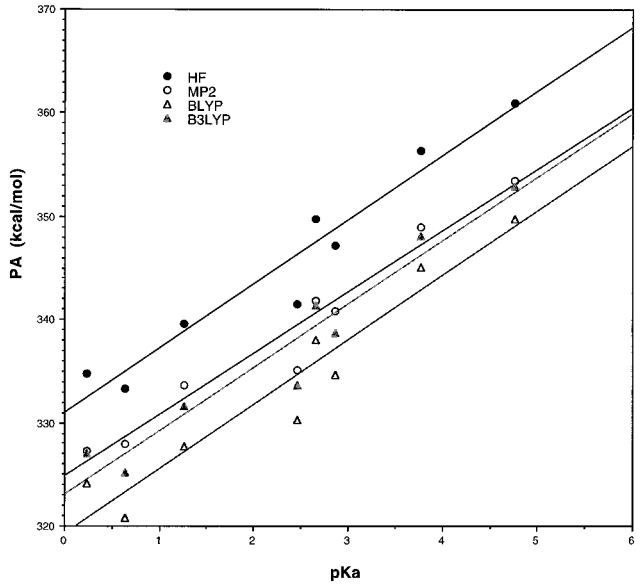
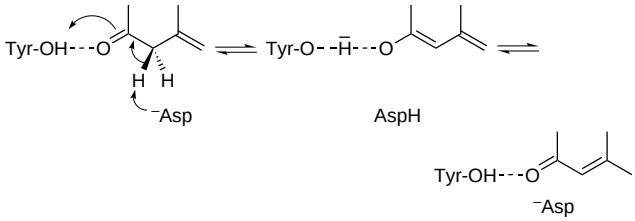


Figure 5. Calculated proton affinities for selected substituted formic acids (**1**) versus their aqueous pK_a values, using the 6-31+G(d,p) basis set.

Scheme 2



the series of substituted formic acids−formate anions of this study:

$$\text{slope}_{E_{HB}\text{ vs p}K_a} = \text{slope}_{E_{HB}\text{ vs PA}} \times \text{slope}_{\text{PA vs p}K_a}$$

$$\text{slope}_{E_{HB}\text{ vs p}K_a} = 0.287 \times 6.14$$

$$\text{slope}_{E_{HB}\text{ vs p}K_a} = 1.76$$

Thus, from this study we find that a pK_a difference of one unit between proton acceptor and the conjugate base of the proton donor of a LBHB between substituted carboxylates leads to a decrease in the corresponding LBHB strength of 1.76 kcal/mol. This certainly has implications for the proposed role of LBHBs in enzyme catalysis. For instance, a LBHB is believed to be important during the reaction catalyzed by β-ketosteroid isomerase,[2,3] as shown in Scheme 2. In this scheme (Chart 2) the reactant and product complexes both contain traditional, weak hydrogen bonds, while the intermediate (or transition state) would be strongly stabilized by the presence of a LBHB between the conjugate base of Tyrosine and the dienolate anion. While precise pK_a values for these species in the enzyme active site are not known, they are believed to be 12 and 10, respectively.[2,3] This leads to a pK_a difference of 2 units. Thus, based on our analysis here, this would lead to a less than perfect LBHB, but only by a maximum of 3.5 kcal/mol. Given the potential catalytic power of an LBHB (10−20 kcal/mol), a weakening of only 3.5 kcal/mol is perhaps not that significant. Thus, our analysis would suggest that reasonably small differences in pK_a values for the bases involved in an LBHB should not preclude them from providing significant catalytic stabilization during

an enzyme reaction. On the other hand, if the difference in pK_a values for the two bases was much more significant, say 5 pK_a units (as has been suggested for the difference in pK_a values of the His and Asp residues of Chymotrypsin),[1b] then the predicted weakening of the LBHB would be 8.8 kcal/mol. This is certainly a much more significant effect, and suggests that perhaps a short-strong hydrogen bond is not involved during the reaction catalyzed by Chymotrypsin, at least not between the His and Asp residues. We are currently investigating this particular mechanism in more detail.

The results presented here concerning the sensitivity of LBHBs to changes in pK_a matching or mismatching are somewhat in disagreement with those found by Rebek and coworkers in benzene and dichloromethane solution.[14] In that study they found reasonably strong hydrogen bonds, but did not observe a very large difference between the dicarboxylic acid (matched pK_as) and carboxylic acid−amide (mismatched pK_as) hydrogen bond energies, as one would expect if pK_a matching was important in determining the overall hydrogen bond strength. This discrepancy may be due to the effect of bulk solvation, which is not present in either our calculations or enzyme active sites. Alternatively, as we have shown in a recent paper,[23h] LBHBs are reasonably sensitive to geometrical constraints, and it is quite likely that the Kemp's triacid based template in the Rebek study was too rigid, not allowing the LBHBs to form perfectly, and thus reducing their observed bond strengths and, presumably, their sensitivity to pK_a changes.

Symmetric LBHB Complexes. Table 3 reveals that all the SSHB complexes studied here which are symmetrically substituted, i.e., X = Y, are indeed true LBHBs. That is, the classical energy barrier for proton transfer from the substituted formic acid to the substituted formate anion is essentially zero, and in all cases vanishes completely when zero-point energy effects are included in the calculation. Thus, the proton actually resonates above the intrinsic barrier for proton transfer from one base to the other, as has been seen for similar systems previously.[1a,15,23a−f]

The results in Table 2 and Figure 2 clearly show that all LBHBs do not have the same hydrogen bond strength (E_{HB}), however. Figure 2 specifically shows the relationship between calculated hydrogen bond strength of the LBHB and the calculated proton affinities of the corresponding bases involved in the LBHB. Remember, these calculations are only for symmetrically substituted systems, thus X = Y, so the two bases vying for the proton are identical within each complex **3**. What the plot in Figure 2 shows, then, is the effect of making the bases in each complex **3** weaker or stronger bases, relative to X = Y = H, the parent system. At all levels of theory, Figure 2 clearly shows that as the substituted formate anions are made more stable (i.e. they become weaker bases) the corresponding LBHB complex **3** becomes stronger, and is linearly related to the sum of the calculated proton affinities of the substituted bases involved. These results are certainly interesting in a physical sense, but probably do not have tremendous implications for enzyme catalysis. The fact that decreasing basicity leads to a stronger LBHB is most likely related to a decrease in electron repulsion between the negative charges of the oxygens involved in the LBHB.

Geometries of LBHBs. Figure 4 shows a plot of intramolecular O···O hydrogen bond distances versus calculated hydrogen bond strengths of both symmetrically and asymmetrically substituted SSHB complexes **3**, at all four levels of theory employed in this study. At each level of theory there is a good general linear relationship between E_{HB} and O···O for the asymmetrically substituted complexes (**3**, X ≠ Y). This leads to O···O distances in the 2.45−2.65 Å region, depending upon actual substitution. In every case, the longer the calculated hydrogen bond, the more weakly bound the corresponding complex **3** is. This relationship also correlates very well with calculated changes in proton affinities. Thus, the larger the calculated difference in proton affinities between the substituted formate anion and the parent unsubstituted formate anion, the longer the hydrogen bond, and the more weakly bonded the corresponding complex **3** is calculated to be. This is consistent with what we have seen in our microsolvation studies for this system,[23a] and others.[23f] The interesting feature of Figure 4 is that it clearly shows that for symmetrically substituted complexes (**3**, X = Y) a wide range of hydrogen bond strengths can be found for a given O···O distance. In other words, at each level of theory, the calculated O···O distance for the symmetrically substituted systems (**3**) are remarkably similar, regardless of actual substituent, but result in very different calculated E_{HB} values. This too is consistent with a picture of LBHBs where the hydrogen bond strength increases as the base is made weaker, thus reducing the electron−electron repulsion between the oxygen atoms of the proton donor and acceptor, since the O···O distance remains essentially constant.

Not surprisingly, as the values in Table 4 show, the proton in the various substituted complexes (**3**) is the most centrosymmetric (although never exactly so) in the symmetrically substituted cases (X = Y) and the most noncentrosymmetric, or localized, when the complexes were asymmetrically substituted. This is consistent with what we now know about LBHBs. That is, for the symmetrically substituted complexes, there is the formation of a true LBHB, such that the position of the proton is largely delocalized over the entire proton transfer reaction coordinate; while on the other hand, the asymmetrically substituted complexes lead to SSHBs which have the proton much more localized to one oxygen versus the other, and are not true LBHBs. This is clearly reflected in the calculated hydrogen bond lengths (O···H) in Table 4. When X = Y the calculated O···H distance is roughly 1.25 Å (for the correlated calculations), while the O···H distance is significantly longer for the X ≠ Y complexes.

Conclusions

This study investigates the effects of causing a mismatch in the pK_a values of the hydrogen bond donor and hydrogen bond acceptor of SSHB and LBHB complexes. This was done through the use of various substituents for the complexes (**3**) formed between a formic acid and a formate anion, a common functional group motif in enzyme active sites. An empirical relationship between the differences in pK_a values of the two bases involved in the SSHB and the resulting hydrogen bond strength is proposed. Specifically, we find that a difference in pK_a values of one unit should lead to a decreased hydrogen bond energy of approximately 1.8 kcal/mol (in the gas phase). This is compared to a similar study[4c] which showed that the predicted decrease in E_{HB} was 1.0 kcal/mol in DMSO and 0.05 kcal/mol in water. The 1.8 kcal/mol value from this gas-phase study is clearly an upper limit, while the DMSO value is most likely a lower limit to what the actual situation in an enzyme active site would be, since the environment of an enzyme active site would not be identical to either the gas phase or DMSO. However, we believe there is sufficient evidence to suggest that most enzyme active sites are generally of very low dielectric,[6c] and thus resemble more closely the gas phase than DMSO. Thus, we conclude that pK_a mismatches of 1−2 units in SSHBs are

reasonably insignificant, energetically, and should not preclude the importance of LBHBs (or SSHB in general) as a possible explanation of enzyme catalysis. On the other hand, these results also suggest that larger differences in pK_a values of the constituent bases (i.e., 5−6 pK_a units) would lead to a significant weakening of the corresponding SSHB, so much so that the formation of *one* short, strong hydrogen bond would seem unlikely to provide enough catalytic stabilization to be important during enzyme reactions.

We also find that LBHBs do not benefit from any kind of "special" stabilization, in agreement with other recent theoretical investigations.[11] An analysis of symmetrically substituted LBHBs also shows that a wide range of E_{HB} values are possible for any given O···O distance. In particular, it becomes obvious that as the constituent bases are made weaker, the corresponding E_{HB} for the complex **3** increases significantly. Thus, all LBHBs are not created equal. It is not clear if nature has ever chosen to take advantage of this particular aspect of LBHBs, but it is certainly a possibility.

As a cautionary note, and as one Referee has pointed out, the simple interactions occurring between a substituted formic acid and a substituted formate anion (as used in this study) are probably not great models for the very complex interactions occurring in a real enzyme active site. Nonetheless, we feel that these model calculations do present valuable new information and insight regarding the fundamental forces at work in LBHBs and how changes in environmental factors alter these interactions. Hopefully, a richer understanding of LBHBs will eventually lead to a more accurate description of how, or even if, LBHBs are involved in enzyme catalysis.

Acknowledgment. This research has been supported by a grant from the Texas Advanced Research Program (No. 41508), administered by the Texas Higher Education Coordinating Board, and is gratefully acknowledged.

Supporting Information Available: Tables S1−S9 containing calculated total energies and geometries of all compounds studied (12 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA972745J