Characterization of Low-Barrier Hydrogen Bonds. 6. Cavity Polarity Effects on the Formic Acid−Formate Anion Model System. 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

Received July 11, 1997. Revised Manuscript Received November 10, 1997

Abstract: High-level ab initio molecular orbital and density functional theory calculations incorporating cavity polarity effects via the use of self-consistent reaction field (SCIPCM) simulations reveal that the short, strong hydrogen bond formed between a formic acid molecule and a formate anion is significantly, but nowhere near completely, weakened by the presence of an extremely polar cavity. These results suggest that even if an enzyme active site were to present an environment as polar as aqueous water, the formation of a low-barrier, or short-strong, hydrogen bond would still be some 8 kcal/mol more favorable than the corresponding neutral, traditional, weak hydrogen bond—like the one formed between two formic acid molecules. The short, strong hydrogen bond formed between a formic acid and a formate anion is clearly much more sensitive to the effects of its environment than is a typical weak traditional hydrogen bond. However, even in the most polar of cavities, the calculated hydrogen bond energy for formic acid−formate anion is greater than 12 kcal/mol, whereas the calculated hydrogen bond energy for formic acid−formic acid is less than 4 kcal/mol. These results suggest that cavity polarity effects alone are insufficient grounds to rule out the low-barrier hydrogen bond facilitated mechanism, as proposed by Gerlt, Gassman, Cleland, and Kreevoy several years ago.

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 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 recent paper by Gilli[14] extends these studies to the solid state. Recent studies on enzyme inhibitor complexes

(1) (a) Garcia-Viloca, M.; Gonzalez-Lafont, A.; Lluch, J. M. *J. Am. Chem. Soc*. **1997**, *119*, 1081. (b) Laidig, J. A.; Platts, J. A. *J. Phys. Chem*. **1996**, *100*, 13455. (c) Scheiner, S.; Yi, M. *J. Phys. Chem*. **1996**, *100*, 9235. (d) Wesolowski, T.; Muller, R. P.; Warshel, A. *J. Phys. Chem*. **1996**, *100*, 15444. (e) Chen, J.; McAllister, M. A.; Lee, J. K.; Houk, K. N. *J. Am. Chem. Soc*. Submitted for publication. (f) Tuckerman, M. E.; Marx, D.; Klein, M. L.; Parrinello, M. *Science*, **1997**, *275*, 817. (g) Zhao, Q.; Abeygunawardana, C.; Talalay, P.; Mildvan, A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8220. (h) 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.

(2) (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.

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

(4) (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.

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

(6) (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.

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

(8) (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.

(9) Scheiner, S.; Kar, T. *J. Am. Chem. Soc*. **1995**, *117*, 6970.

(10) (a) Perrin, C. L. *Science* **1994**, *266*, 1665. (b) Perrin, C. L.; Thoburn, J. D. *J. Am. Chem. Soc*. **1992**, *114*, 8559.

(11) (a) Frey, P. A.; Tobin, J. B.; Whitt, S. A. *Science* **1994**, *164*, 1887. (b) Frey, P. A. *Science* **1995**, *269*, 104.

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

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

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

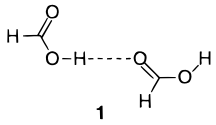
(15) Jeffrey, G. A. *An Introduction to Hydrogen Bonding*; Oxford University Press: New York, 1997.

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

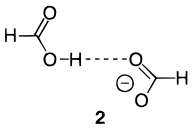
(17) (a) Pan, Y.; McAllister, M. A. *J. Am. Chem. Soc*. **1997**, *119*, 7561. (b) McAllister, M. A. *Can. J. Chem*. **1997**, *75*, 1195. (c) McAllister, M. A. *J. Mol. Struct. (THEOCHEM)* **1998**, *423*, in press. (d) Pan, Y.; McAllister, M. A. *J. Mol. Struct. (THEOCHEM)* **1998**, *423*, in press. (e) Kumar, G. A.; Pan, Y.; Smallwood, C. J.; McAllister, M. A. *J. Comput. Chem*. Submitted for publication. (f) Pan, Y.; McAllister, M. A. *J. Org. Chem*. **1997**, *62*, 8176. (g) Smallwood, C. J.; McAllister, M. A. *J. Am. Chem. Soc*. **1997**, *119*, 11277.

S0002-7863(97)02311-1 CCC: $15.00
Published on Web 01/14/1998

Chart 1



1

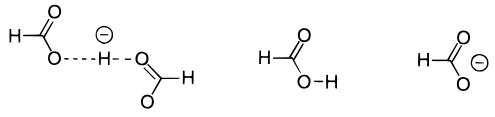


2

have produced considerable evidence for the formation of LBHBs during several enzyme-catalyzed reactions.[4c] There is, however, only limited evidence that LBHBs may be formed in the condensed phase.[2,3,10,12]

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.[11] 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_as.[13] Thus, the choice of studying the interaction between formic acid and formate anion should represent one of the best possible situations for the formation of an LBHB.

Our previous investigations[17] of LBHBs have shown that the formic acid−formate anion[17a] and enol−enolate anion[17f] systems form very strong, very short hydrogen bonds, and are indeed true LBHBs (for a detailed discussion of the differences between an LBHB and an SSHB (short, strong hydrogen bond) please see ref 17f). Those studies[17a,d,e,g] have shown that the hydrogen bond formed between **4** and **5** is extremely strong, with a calculated energy of interaction (E_{HB}) of approximately 27 kcal/mol (B3LYP/6-31++G(d,p)). The interaction between an enol and an enolate anion was even stronger (30 kcal/mol, MP2/6-31+G(d,p)).[17f] 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 (**2**). In fact, in a rather surprising result, the hydrogen bond formed when two water molecules are symmetrically placed about the complex produces a *stronger* LBHB between formic acid and formate anion![17a] This is in excellent agreement with a recent experimental result[4b] which showed that the dihydrate of 4-nitrophenoxide hydrogen bonded to 4-nitrophenol has a shorter (and presumably stronger) hydrogen bond distance than the nonhydrated crystal structure. Similarly, there has been a recent report by Zhao and co-workers of the formation of a LBHB in water.[1g] We have thus concluded that small amounts of water in enzyme active sites will not disrupt, or preclude, the existence of LBHBs being formed during enzyme-catalyzed reactions.[17a,f]



3 **4** **5**

What remains to be studied is the effect of changing the effective polarity of the surrounding cavity from that of the gas phase (standard ab initio simulations) to that of the enzyme active site. To be sure, there is considerable debate as to what the environment of an enzyme active site really is. Some researchers favor the notion that the enzyme active site is truly nonpolar,[4c] while others propose that enzyme cavities are very polar environments.[8] We propose to employ a standard Self-Consistent-Reaction-Field (SCRF) method to study the effect of changing the polarity of the cavity surrounding the formic acid−formate anion and the formic acid−formic acid complexes. In this way we hope to determine what effect a polar enzyme cavity might have on the strength of an LBHB, versus a traditional, weak hydrogen bond.

Methodology

Structures **1**, **2**, and **3** (which corresponds to the transition state for proton transfer from the formic acid to the formate anion), as well as the monomers **4** and **5**, were optimized by using the Gaussian 94 suite of programs.[18] The standard split valence basis set 6-31++G(d,p)[19] was used as provided in Gaussian 94. Geometry optimizations were accomplished with ab initio and density functional methods. Ab initio calculations were performed at the Hartree−Fock (HF) level of theory. Density Functional Theory (DFT) calculations[20] were performed with the BLYP (Becke−Lee−Yang−Parr) and B3LYP functionals. These are gradient corrected nonlocal functionals, as described elsewhere.[21−23] These methods have proven reliable in our previous investigations of these systems.[17]

Since we were primarily interested in the hydrogen bond energy of a single interaction between formic acid and formate anion, we constrained the geometry slightly to avoid multiple hydrogen bonds from forming. This was accomplished by simply forcing the hydrogen bond to be linear. The same was done for the formic acid−formic acid complex.

Cavity polarity effects were investigated by using standard SCRF methods.[24] These methods are often referred to as quantum mechanical continuum methods, and are based largely on the pioneering work of Onsager a half century ago.[25] More modern implementations of the SCRF formalism correct for some of the deficiencies of the original SCRF methods; for instance one is no longer limited to approximating the cavity (or solvent, as is often the case) as a simple dipole, more elaborate multipole expansions are available.[26] More significantly, we believe, is the work of Tomasi and co-workers in the development of reaction field methods which make it possible to define a cavity based on an isosurface of the total electron density, which is calculated

(18) *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.

(19) 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.

(20) (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.

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

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

(23) (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.

(24) Forseman, J. B.; Keith, T. A.; Wiberg, K. B.; Snoonian, J.; Frisch, M. J. *J. Phys. Chem.* **1996**, *100*, 16098.

(25) Onsager, L. *J. Am. Chem. Soc.* **1936**, *58*, 1486.

(26) (a) Rinaldi, D.; Ruiz-Lopez, M. F.; Rivail, J.-L. *J. Chem. Phys.* **1983**, *78*, 834. (b) Chipot, C.; Rinaldi, D.; Rivail, J.-L. *Chem. Phys. Lett.* **1992**, *191*, 287. (c) Rinaldi, D.; Rivail, J.-L.; Rguini, N. *J. Comput. Chem.* **1992**, *13*, 675.

Table 1. Calculated Hydrogen Bond (E_{HB}, kcal/mol) and Activation (E_A, kcal/mol) Energies for Formic Acid−Formic Acid Complexes (**1**) and Formic Acid−Formate Anion Complexes (**2**) with Use of the HF/6-31++G(d,p) Optimized Geometries

	dielectric constant (ϵ)							
	1.0	2.3	6.0	15.0	23.0	35.0	47.0	79.0
HF[a]								
E_{HB}(**1**)	4.7	3.8	3.1	2.8	2.7	2.6	2.6	2.6
E_{HB}(**2**)	22.2	13.7	9.6	8.0	7.6	7.4	7.3	7.2
E_A	1.4	1.8	2.2	2.4	2.3	2.4	2.4	2.4
BLYP[b]								
E_{HB}(**1**)	4.3	4.1	3.7	3.5	3.4	3.4	3.4	3.3
E_{HB}(**2**)	25.0	17.7	13.7	12.5	12.3	12.1	11.9	11.9
E_A	−1.6	−1.1	−1.0	−0.3	−0.1	−0.3	−0.6	−0.3
B3LYP[c]								
E_{HB}(**1**)	5.0	4.6	4.1	3.8	3.8	3.7	3.7	3.7
E_{HB}(**2**)	25.9	18.3	14.4	12.7	12.5	12.0	12.0	12.0
E_A	−1.3	−1.2	0.0	−0.2	0.1	−0.8	−0.5	1.1

[a] HF/6-31++G(d,p)//HF/6-31++G(d,p). [b] BLYP/6-31++G(d,p)//HF/6-31++G(d,p). [c] B3LYP/6-31++G(d,p)//HF/6-31++G(d,p).

quantum mechanically by using the same level of theory as applied to the rest of the molecule (solute).[27] The most common of such methods is the SCIPCM (Self-Consistent Isodensity Polarizable Continuum Method) code of Tomasi and co-workers. We have used the SCIPCM method as implemented in Gaussian 94. Although this method has been criticized recently,[28] we believe there is ample evidence for its superiority over simpler Onsager based methods.[24] Unfortunately, however, the SCIPCM method is not amenable to doing post-SCF Møller−Plesset perturbation calculations. Thus, we must rely on the DFT calculations to investigate the effects of electron correlation on the relative energies of our complexes. This has proven extremely reliable in the past, where we have shown that MP2 and several DFT methods give consistently similar results during the study of many LBHB properties.[17]

For each structure **1**−**5** we have run SCIPCM-SCRF single point energy calculations at the HF, BLYP, and B3LYP levels of theory (with the 6-31++G(d,p) basis set) using the HF/6-31++G(d,p) optimized geometry. Similarly, for structures **1**−**5** geometry optimized at the BLYP and B3LYP levels of theory, we have run single point SCIPCM-SCRF calculations (6-31++G(d,p)) at the corresponding correlated level of theory. The SCIPCM-SCRF calculations have been done for several different dielectric continuums—specifically, for $\epsilon = 2.3$, 6.0, 15.0, 23.0, 35.0, 47.0, and 79.0. The values were chosen to represent a wide range of cavity environments, and although some of the dielectric constants do match those of common solvents, there is no special significance to attribute to this.

It is fairly well established that these SCRF methods, including the PCM method used herein, do not accurately represent specific solvent interactions, such as hydrogen bonding.[24,29] That is perfectly acceptable for our purposes since we are only interested in the cavity *polarity* aspects of the enzyme active site, and not its ability or inability to hydrogen bond; that was the focus of our previous studies.[17a,e,f]

Results

Fully optimized geometries for structures **1**−**5** can be found elsewhere.[17a] Calculated hydrogen bonding energies (E_{HB}) and the calculated activation energy (E_A) for transfer of the proton from formic acid to formate anion using the HF/6-31++G(d,p) optimized geometries can be found in Table 1. This table shows the results of SCIPCM-SCRF single point energy calculations at the HF, BLYP, and B3LYP levels of theory, using the 6-31++G(d,p) basis set. E_{HB} is calculated as the difference in energy between the complex energy (either **1** or **2**) and the energy of its constituent monomers, **4** + **5** or **4** + **4**, infinitely separated. E_A is simply the difference in total energy between the complex (**2**) and the transition state (**3**). Gas-phase results are also given ($\epsilon = 1.0$).

Table 2. Calculated Hydrogen Bond (E_{HB}, kcal/mol) and Activation (E_A, kcal/mol) Energies for Formic Acid−Formic Acid Complexes (**1**) and Formic Acid−Formate Anion Complexes (**2**) with Use of Optimized Geometries from Correlated Calculations

	dielectric constant (ϵ)							
	1.0	2.3	6.0	15.0	23.0	35.0	47.0	79.0
BLYP[a]								
E_{HB}(**1**)	4.7	4.4	4.1	3.9	3.8	3.8	3.8	3.8
E_{HB}(**2**)	26.8	19.6	15.2	13.2	12.8	13.0	13.0	12.4
E_A	0.0	0.2	0.2	−0.7	−0.2	0.6	0.9	−0.2
B3LYP[b]								
E_{HB}(**1**)	5.4	4.9	4.5	4.3	4.3	4.3	4.3	4.2
E_{HB}(**2**)	27.2	18.8	14.6	13.0	12.6	12.4	12.3	12.2
E_A	0.0	−0.1	0.5	−0.3	0.1	−0.2	−0.9	−0.2

[a] BLYP/6-31++G(d,p)//BLYP/6-31++G(d,p). [b] B3LYP/6-31++G(d,p)//B3LYP/6-31++G(d,p).

Table 2 contains similar results with use of the BLYP/6-31++G(d,p) and B3LYP/6-31++G(d,p) optimized geometries of **1**−**5**. Table 2 shows the results of SCIPCM-SCRF single point energy calculations on these optimized geometries. This analysis will allow us to directly determine the relative effect of increasing the cavity polarity on the strength of a LBHB (**2**) versus a traditional hydrogen bond (**1**).

Discussion

It has been proposed that LBHBs cannot exist in enzyme active sites due to the inherent polar nature of such cavities. The argument put forth by Warshel and co-workers,[8] and Guthrie to a certain extent,[7] maintains that an ionic hydrogen bond, such as that formed in an LBHB, is necessarily destabilized by the presence of a polar cavity, relative to that of a traditional, weak, neutral hydrogen bond. So much so, they would claim, that followed to its logical conclusion, LBHBs cannot be involved in enzyme catalysis since they would in fact be *less* stable than their neutral counterparts.[8] We believe the results reported here refute this assertion. The data in Tables 1 and 2 clearly show that at all levels of theory, and for all cavity polarity values, the ionic LBHB is still significantly more stable than the traditional neutral hydrogen bond. This is also consistent with our previous study of the hydrogen maleate system,[17b] which showed that the introduction of a very polar cavity only weakened the LBHB in that system by 7 kcal/mol.

Table 2 clearly shows that there is indeed a fairly large effect on the calculated hydrogen bond energy (E_{HB}) of complex **2** as the cavity polarity is made significantly more polar. However, even in the most polar environment studied ($\epsilon = 79.0$, roughly corresponding to the dielectric constant of water), the calculated E_{HB} in complex **2** is still significantly larger than that for complex **1**. Thus, the differential stabilization afforded by the formation of an SSHB (**2**) relative to that of a traditional hydrogen bond (**1**) is on the order of 8 kcal/mol, even in a very polar environment. Of course, it is somewhat unlikely that the environment within an enzyme active site would be as polar as aqueous water, but these calculations suggest that even if that were the case, there would still be an approximately 8 kcal/

(27) (a) Miertus, S.; Scrocco, E.; Tomasi, J. *Chem. Phys.* **1981**, *55*, 117. (b) Tomasi, J.; Bonaccorsi, R.; Cammi, R.; Valle, F. J. O. *J. Mol. Struct. (THEOCHEM)* **1991**, *234*, 401. (c) Tomasi, J.; Bonnaccorsi, R. *Croat. Chem. Acta* **1992**, *65*, 29.

(28) Truong, T. N.; Stefanovich, E. V. *J. Phys. Chem.* **1995**, *99*, 14700.

(29) (a) Cramer, C. J.; Truhlar, D. G. *J. Am. Chem. Soc.* **1993**, *115*, 8810. (b) Marten, B.; Kim, K.; Cortis, C.; Friesner, R. A.; Murphy, R. B.; Ringnalda, M. N.; Sitkoff, D.; Honig, B. *J. Phys. Chem.* **1996**, *100*, 11775.

mol advantage in forming a SSHB versus a traditional weak hydrogen bond.

Table 2 also shows that the classical energy barrier for transfer of the proton from the formic acid to the formate anion is not very dependent on the polarity of the cavity. In all cases the calculated E_A remains essentially zero, indicative of a true LBHB situation. In all cases that residual barrier disappears after inclusion of zero-point vibrational energy. This, we believe, is further evidence that LBHBs are not disrupted by the presence of a polar environment. There is no doubt that SSHBs, such as that formed in **2**, do not survive in aqueous water. It would be fallicious, however, to attribute that fact to the polarity of water. It is not the polarity of bulk water itself, but rather the disorder and randomess of the multiple hydogen bonds formed between water and the ionic substrate that causes the complex to weaken. As pointed out by Perrin not too long ago,[10] it really is the entropic disorder of solvents such as water that precludes the existence of a single, stable, short-strong hydrogen bonded complex, such as **2**, and not simply the effective polarity of the environment itself. Thus, even though an enzyme active site *might* be a very polar environment, it almost certainly is not a higly disordered environment. That being the case, one must conclude that short, strong ionic hydrogen bonds can, and very likely do, offer a significant catalytic advantage over traditional, weak, neutral hydrogen bonds in enzyme active sites.

Conclusions

High-level ab initio and DFT calculations reveal that the SSHB formed between a formic acid molecule and a formate anion (**2**) is significantly, but nowhere near completely, weakened by the presence of an extremely polar cavity. Thus, even if an enzyme active site were to present an environment as polar as aqueous water, these calculations suggest that the formation of an SSHB (**2**) would still be some 8 kcal/mol more favorable than the corresponding neutral, traditional, weak hydrogen bond—like the one formed between two formic acid molecules (**1**). The SSHB formed between a formic acid and a formate anion is clearly much more sensitive to the effects of its environment than is a typical weak traditional hydrogen bond. However, even in the most polar of cavities, the calculated E_{HB} for **2** is greater than 12 kcal/mol, whereas the E_{HB} for **1** is less than 4 kcal/mol. These results suggest that cavity polarity effects alone are insufficient grounds to rule out the low-barrier hydrogen bond facilitated mechanism for enzyme catalysis, as proposed by Gerlt, Gassman, Cleland, and Kreevoy several years ago.

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.

JA972311O