A Comparison of NH-π versus Lone Pair Hydrogen Bonding Effects on Carbon Acid pK_a Shifts

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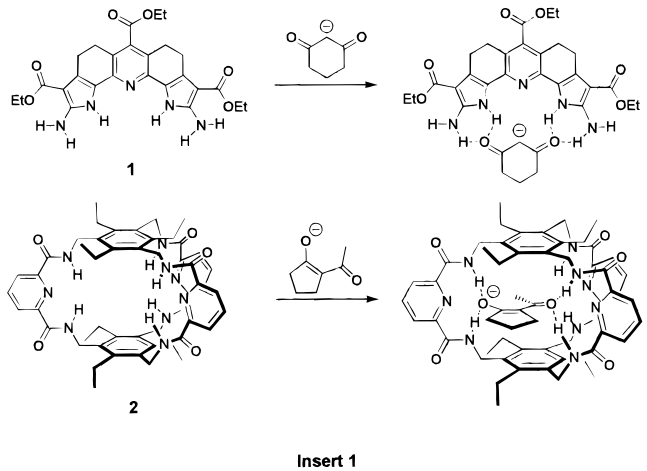
Received April 1, 1999

Hydrogen bonding plays a crucial role in stabilizing reactive intermediates in enzyme active sites.[1] For example, the acidity of carbon acids is increased when hydrogen bonds are present to stabilize the corresponding enolates.[2,3] The strength of the hydrogen bonds should depend on the hydrogen bond acceptor orbitals. Hydrogen bond donation to the π-system of an enolate rather than to the oxygen lone pairs may enhance stabilization, because this is where the formal negative charge resides. In support, our examination of the crystal structure of 4-chlorobenzoyl-CoA-dehalogenase,[4] an enzyme that catalyzes a nucleophilic substitution via a Meisenheimer intermediate, revealed that the hydrogen bonds in the oxyanion hole are oriented toward the π-system of a thioester carbonyl enolate. Thus, we set out to test whether larger pK_a shifts of carbon acids may be obtained when the hydrogen bonds are oriented toward the enolate π-system rather than the lone pair electrons.

Previously we reported that **1** binds 1,3-cyclohexanedionate in acetonitrile with four hydrogen bonds directed to the anion's lone pairs ($K_a = 1.2 \times 10^4$ M^{-1}).[5] The pK_a drop imparted to 1,3-cyclohexanedione by **1** was only 1.0 pK_a unit in acetonitrile. Given our postulate that the acceptor orbitals should be an important factor influencing the pK_a shift, we examined a new receptor capable of preferentially binding the π-system of an enolate.

Our new host design (**2**) has six convergent intracavity amide hydrogen bond donors capable of binding active methylene enolates with four hydrogen bonds. The aromatic caps are separated by a distance of 7 Å, forcing the enolates to be sandwiched parallel between the aromatic rings, as previously found for acetate.[6] Molecular modeling[7] supports encapsulation of active methylene enolates with two pairs of hydrogen bonds directed toward the π-system of the guest carbonyl groups.



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Before examining pK_a shifts, we determined which enolates were most complementary to **2**. The association constants of **2** with various enolates were determined by 300 MHz ^{1}H NMR titration experiments in 95:5 CD_3CN/CD_2Cl_2 by following the deshielding of the amide protons of **2** with increasing enolate concentration (Table 1A).[8] In cases where there was a methyl group adjacent to a carbonyl, the methyl protons were shielded relative to their chemical shift in the absence of **2**, typically on the order of 0.2 ppm. We ascribe these upfield shifts to the diamagnetic anisotropy of the aromatic rings of **2**, suggesting that these guests are positioned within the cavity.

Enolate **3** was chosen for the pK_a shift experiments because of its high affinity for **2**. A ^{1}H NMR technique was employed to probe the change in pK_a values of 2-acetylcyclopentanone in the presence of **2**. Bases were added to deprotonate 2-acetylcyclopentanone in the presence of 1 equiv of **2** and induce enolate binding. The acid−base reaction was monitored by observing the complexation-induced change in N$\underline{\text{H}}$ chemical shift of **2** (Figure 1). Although CD_3CN/CD_2Cl_2 (95:5) was used to measure the association constants, CD_2Cl_2 was used in these studies to ensure that **3** was completely encapsulated in **2** upon generation ($K_a > 10^5$ M^{-1} in CD_2Cl_2). Importantly, ^{1}H NMR titration experiments showed that 2-acetylcyclopentanone displayed minimal binding to **2** ($K_a < 10$ M^{-1}).

The bases employed to deprotonate 2-acetylcyclopentanone were 15-crown-5 sodium phenoxides of differing base strength. Each phenoxide used in the deprotonation study showed negligible binding to **2** in CD_2Cl_2 (Table 1B). Titrating a solution of 2-acetylcyclopentanone with the phenoxides in CD_2Cl_2 showed that in the absence of **2** no acid/base chemistry occurred, except with **9** which has a higher conjugate acid pK_a value in water than 2-acetylcyclopentanone. Yet in the presence of **2**, 6 equiv of 2,4,6-trichlorophenoxide, whose conjugate acid has a pK_a value nearly 1.6 units lower than that of 2-acetylcyclopentanone in water, deprotonated 40% of this diketone (Figure 1).

Next, we quantitated the pK_a shift of 2-acetylcyclopentanone promoted by the presence of **2** and compared the result to that of **1** and 1,3-cyclohexanedione. Although the deprotonation studies were conducted in CD_2Cl_2, it was necessary to determine the pK_a shift of 2-acetylcyclopentanone induced by **2** in acetonitrile so that the result could be compared to the original study using **1**.[9] By using a method employing external indicators (HI),[10] spec-

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

(2) Gerlt, J. A.; Gassman, P. G. *Biochemistry* **1993**, *32*, 11943. Gerlt, J. A.; Kreevoy, M. M.; Cleland, W. W.; Frey, P. A. *Chem. Biol.* **1997**, *4*, 259. Cleland, W. W.; Kreevoy, M. M. *Science* **1994**, *264*, 1927. Guthrie, J. P.; Kluger, R. *J. Am. Chem. Soc.* **1993**, *115*, 11569. Guthrie, J. P. *Chem. Biol.* **1996**, *3*, 163. Alagona, G.; Ghio, C.; Kollman, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 9855. Warshel, A.; Papazyan, A.; Kollman, P. A. *Science* **1995**, *269*, 102. Warshel, A.; Papazyan, A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 13665. Shan, S.; Herschlag, D. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14474. Petrounia, I. P.; Pollack, R. M. *Biochemistry* **1998**, *37*, 700.

(3) For metal-induced pK_a shifts, see: Kimura, E.; Kitamura, H.; Koike, T.; Shiro, M. *J. Am. Chem. Soc.* **1997**, *119*, 10909 and references therein.

(4) Benning, M. M.; Taylor, K. L.; Liu, R.-Q., Yang, G.; Hong, X.; Wesenberg, G.; Dunaway-Mariano, D.; Holden, H. M. *Biochemistry* **1996**, *35*, 8103.

(5) Kelly-Rowley, A. M.; Lynch, V. M., Anslyn, E. V. *J. Am. Chem. Soc.* **1995**, *117*, 3438. Compound **1** is expected to have more acidic hydrogen bond donors than **2**, and therefore would be predicted to give a larger relative pK_a shift than the cyclophane. However, this prediction is contrary to our findings.

(6) Bisson, A. P.; Lynch, V. M.; Monahan, M. C.; Anslyn, E. V. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2340.

(7) *MacroModel*, V6.0: Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caulfield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.

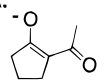
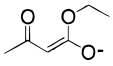
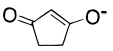
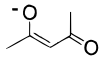
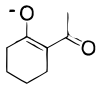
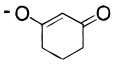
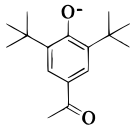
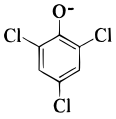
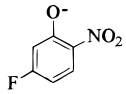
(8) Hynes, M. J. *J. Chem. Soc., Dalton Trans.* **1993**, 311. The amide proton chemical shifts followed in the ^{1}H NMR complexation studies have values below 9.3 ppm and are not indicative of low-barrier hydrogen bond formation.

(9) The determined pK_a shift of 2.9 should be a conservative estimate of the actual ΔpK_a for **3**, because CD_2Cl_2 is expected to have a broader pH scale than acetonitrile, based upon its lower dielectric constant.

(10) Higuchi, T.; Rehm, C.; Barnstein, C. *Anal. Chem.* **1956**, *28*, 1507. Safarik, L.; Stransky, Z. Titrimetric Analysis in Organic Solvents. In *Wilson and Wilson's Comprehensive Analytical Chemistry*; Svehla, G., Ed.; Elsevier Science: Amsterdam, 1986; Vol. XXII, pp 286−296. Leonard, M. A. Photometric Titrations. In *Wilson and Wilson's Comprehensive Analytical Chemistry*; Svehla, G., Ed.; Elsevier Science: Amsterdam, 1977; Vol. XIII, pp 207−270.

10.1021/ja991039a CCC: $18.00 © 1999 American Chemical Society
Published on Web 06/19/1999

Table 1. List of Sodium 15-Crown-5 (A) Enolates and (B) Phenoxides Studied as Guests with **2**, the Anion Numbers, Association Constants in (A) CD_3CN:CD_2Cl_2 (95:5) and (B) CD_2Cl_2, Aqueous pK_a[13] and Determined Nonaqueous pK_a Values of Conjugate Acids

Anion	Number	K_{assoc}(M^{-1})	pK_a^{Water}	pK_a^{MeCN}
A.				
	**3**	3060 +/- 180	7.8	25.4
	**4**	1500 +/- 65	10.6	
	**5**	1360 +/- 200		
	**6**	1060 +/- 134	9.0	
	**7**	100 +/- 10	10.1	
	**8**	95 +/- 10	5.25	
B.				
	**9**	negligible	8.05	
	**10**	negligible	6.23	21.2
	**11**	negligible	6.07	

trophotometric titrations were carried out on 2-acetylcyclopentanone and 2,4,6-trichlorophenol in anhydrous acetonitrile. Plots of [HI]/[I^-] versus the volume of Bu_4NOH (0.1N in 10:1 isopropyl alcohol/methyl alcohol) titrant allowed the calculation of pK_a values using determined pK_a values of the indicators.[11] A pK_a value of 25.4 was found for 2-acetylcyclopentanone using thymol blue sodium salt as the indicator.[12] Similarly, a pK_a value of 21.2 was determined for 2,4,6-trichlorophenol with α-naphtholbenzein. Using the extent of deprotonation of 2-acetylcyclopentanone induced by 2,4,6-trichlorophenoxide, along with the phenol's pK_a, allowed us to calculate the pK_a of the active methylene structure as 22.5 in the presence of **2**. This is 2.9 pK_a units lower than in the absence of **2**. Compound **1** only gave a 1.0 unit pK_a shift to 1,3-cyclohexanedione.

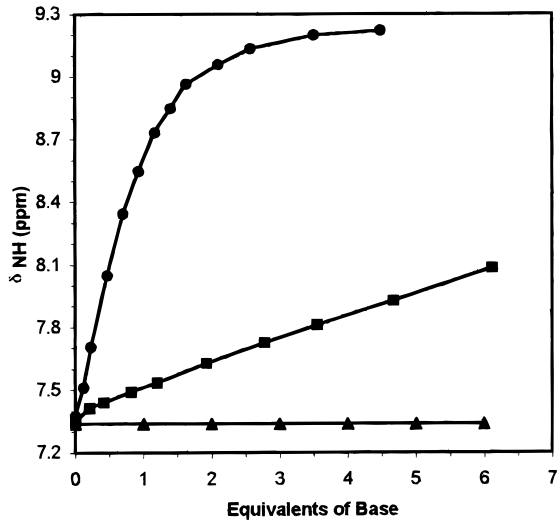


Figure 1. Change in the amide ^{1}H chemical shift of **2** with increasing base concentration in the deprotonation of 2-acetylcyclopentanone. (●) is with phenoxide **9**, (■) with **10**, and (▲) with **11**. Counterions were 15-crown-5 sodium salts, and the concentrations of **2** and **3** were 1.8×10^{-2} M in CD_2Cl_2 at 23 °C.

Although our analysis is based upon a comparison between only two receptors and their respective active methylene compounds, we suggest that NH-π hydrogen bonding has a greater effect on carbon acidity than hydrogen bonding to the lone pair electrons. The important factor for increasing carbon acidity is the differential binding of the conjugate acid and the enolate by the receptor. When a carbon acid is converted to the enolate, there is a significantly smaller increase in charge density on the oxygen lone pairs relative to the change that occurs in the π-system. Therefore, to better stabilize an enolate relative to its conjugate acid, it is more effective to employ a receptor that targets the π-system rather than the oxygen lone pairs. Enzymes may exploit this principle to increase the carbon acidities of their substrates in order to assist in deprotonation by weak general bases positioned in their active sites. Moreover, the same principle may be used to stabilize enolate-like intermediates in other reactions as suggested by the examination of 4-chlorobenzoyl-CoA-dehalogenase. In summary, the hydrogen bond acceptor orbitals are another factor to consider when seeking to understand carbon acid pK_a shifts induced by enzymes.

Acknowledgment. We are grateful for financial support from the National Institutes of Health and the Welch Foundation. We also thank Professor John Gerlt from the Department of Biochemistry at the University of Illinois at Urbana-Champaign for bringing the structure of 4-chlorobenzoyl-CoA-dehalogenase to our attention.

Supporting Information Available: Experimental procedure and plots for nonaqueous titrations in acetonitrile, and a figure of the active site of 4-chlorobenzoyl-CoA dehalogenase showing the orientation of the hydrogen bonds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Kolthoff, I. M.; Chantooni, M. K.; Bhowmik, S. *Anal. Chem.* **1967**, *39*, 315. Kolthoff, I. M.; Bhowmik, S.; Chantooni, M. K. *Proc. Nat. Acad. Sci. U.S.A.* **1966**, *56*, 1370.

(12) In acetonitrile and methylene chloride, 2-acetylcyclopentanone is approximately 40% enolized by ^{1}H NMR examination. Thus, the deprotonation studies, as well as the pK_a determinations, were performed on a mixture of enol and keto forms.

(13) Serjeant, E. P.; Dempsey, B. *Ionization Constants of Organic Acids in Aqueous Solutions*; Pergamon: Oxford, 1979.