Enthalpic Studies of Complex Formation between Carboxylic Acids and 1-Alkylimidazoles

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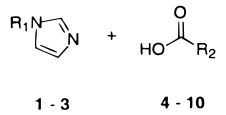
Abstract: The nature of the hydrogen bonding in complexes of alkylimidazoles and substituted carboxylic acids has been studied as a model of the hydrogen-bonding interaction of the proton bridging $N^{\delta 1}$ of His 57 and the β carboxyl group of Asp 102 in the active site of chymotrypsin. The interaction has been postulated to be a low barrier hydrogen bond (LBHB) in the enzyme and also in the model complexes which have a small ΔpK_a. In the present study, enthalpies of complex formation, $-\Delta H_{formation}$, between alkylimidazoles (1-methyl, 1-*n*-butyl-, and 1-*tert*-butylimidazole) and a series of carboxylic acids were determined by adiabatic solution calorimetry in chloroform. In FTIR studies presented here, the concentration of LBHB present in these complexes was determined. For complexation between dichloropropionic acid and alkylimidazoles for which the ΔpK_a is small in chloroform, the $-\Delta H_{formation}$ values varied from 12 to 15 kcal/mol. Thus in enzymes, where ΔG is similar to ΔH, $\Delta G_{formation}$ can be as high as −12 to −15 kcal/mol for LBHBs. If a weak hydrogen bond in the initial E·substrate complex with a $\Delta G_{formation}$ of ≤ -5 kcal/mol is converted to a low barrier hydrogen bond in the transition state, there will be 7−10 kcal/mol of energy available to lower the activation barrier and accelerate the reaction by 5−7 orders of magnitude.

Introduction

It is generally believed that hydrogen bonds increase the rate of enzyme catalysis by stabilizing the transition state more than the ground state of the chemical reaction in the active site. The importance of these hydrogen bond interactions in catalysis has been shown by X-ray crystallography, kinetics, and site-directed mutagenesis studies. It was recently hypothesized that for some enzymes short, strong hydrogen bonds or low-barrier hydrogen bonds (LBHB) with more covalent character than ordinary hydrogen bonds stabilize the transition state and greatly enhance the rate of catalysis.[1−3] This hypothesis is now being tested in potential LBHB systems by examining the physical characteristics that distinguish LBHBs. These characteristics include low fractionation factors, deuterium isotope effects on NMR shifts and IR stretching frequencies, and downfield proton NMR chemical shifts.[4]

In an enzymatic example, the proton bridging $N^{\delta 1}$ of His 57 and the β carboxyl group of Asp 102 in the active site of chymotrypsin has been identified as an LBHB based on its low-field chemical shift (18.3−19 ppm) in the absence[5,6] and in the presence of transition state analogues,[7,8] low fractionation factor (0.40),[9] and elevated basicity of $N^{\epsilon 2}$ in His 57 of the transition state analogue enzyme complex.[7,8] Complexes of alkylimidazoles (**1**−**3**) and carboxylic acids (**4**−**10**) were utilized as models to study the strength of this hydrogen bonding interaction.[10]



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R_1 = CH_3 (**1**), $CH_2CH_2CH_2CH_3$ (**2**), $C(CH_3)_3$ (**3**)

R_2 = CF_3 (**4**), $CHCl_2$ (**5**), $CHCl_2CH_3$ (**6**), CH_2Cl (**7**), CH_2OCH_3 (**8**), CH_2Ph (**9**), and CH_3 (**10**)

Proton chemical shift and antisymmetric carbonyl stretch values were observed for 1:1 methylimidazole/acid complexes as the pK_a of the carboxylic acid (**4**−**10**) was varied. The NMR studies showed a correlation between the chemical shift of the proton and the differences between the aqueous pK_a's of the acids and **1**. The maximum chemical shift occurred with an acid of aqueous pK_a 2.2, which presumably has a similar pK_a to methylimidazole in chloroform. The FTIR studies[10,11] indicated that three types of complexes (Scheme 1) are present in solution distinguished by the value of the antisymmetric carbonyl stretch: neutral hydrogen bonded (**II**), ion-paired (**III**), and an intermediate (bond order 1.5−2) complex (**I**). Weaker acids (**7**−**10**) form neutral hydrogen-bonded complexes (**II**) with stretches similar to free acids and ethyl esters. The stronger acid (**4**) formed an ion-paired complex (**III**) with a stretching frequency similar to that of its tetrabutylammonium salt. Acids (**5** and **6**) similar in pK_a to the alkylimidazoles in chloroform form both ion-paired (**III**) and intermediate complexes (**I**), the latter of which is postulated to contain a LBHB. Deuterium labeling of the acids showed a marked isotope effect, resulting in a shift in the frequency of the antisymmetric carbonyl stretch from 1692 to 1723 cm^{-1} for the proposed LBHB complexes only.[11] The isotope effect arises from the lower zero point energies of N−D

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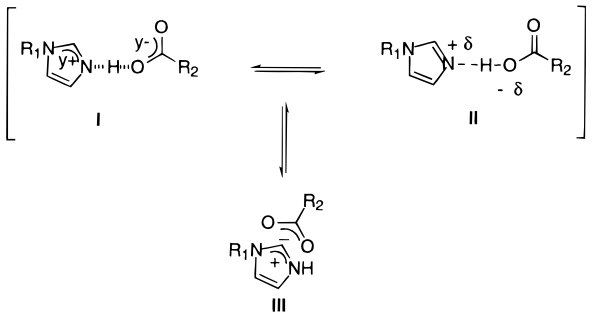
Scheme 1



and O−D bonds compared to the corresponding N−H and O−H bonds in LBHB complexes. Complexes with all of the acids contained broad bands associated with strong hydrogen bonding, Hazdi type i (2500 and 1900 cm^{-1}).[12] These bands disappeared upon deuterium labeling.

These previous studies showed that at least three species (**I**−**III**) are present in solution (Scheme 1), although **I** and **II** are simply extremes of one possible complex. A likely conformation for the ion-pair species (**III**) is one that has a face-edge interaction involving both nitrogens of the imidazole ring. Presumably also present in solution are aggregates containing more than one acid or base per complex as shown in NMR studies[13] on similar complexes of carboxylic acids and pyridine, which show complex equilibria due to concentration dependence and aggregation which can be pK_a dependent. NMR and FTIR studies[11] have been performed on the alkylimidazole/acid complexes varying the steric hindrance of the 1-alkyl group (methyl, *n*-butyl, *tert*-butyl) on alkylimidazole to show the dependence on concentration of **III**, which can have interactions between the acid group and the alkylated nitrogen. Farther downfield ^{1}H NMR chemical shifts (18.2 versus 17.9) and higher relative absorbances for the LBHB antisymmetric carbonyl stretch (1.4:1) were measured for the more sterically hindered **3** than for **1**. Alkyl substitution did not have an effect on the frequency of this carbonyl stretch.

The NMR and FTIR studies above substantiate the presence of a putative LBHB species in alkylimidazole complexes with **5** or **6** in chloroform. The values for chemical shift and antisymmetric carbonyl stretches observed for these complexes have been shown to correlate with hydrogen bond strength.[4] In the present study, adiabatic solution calorimetry was employed to measure the strength of the hydrogen bonds formed in these alkylimidazole/acid complexes. $-\Delta H_{formation}$ was determined for 1-methyl, 1-*n*-butyl, and 1-*tert*-butylimidazole with each of the following acids: trifluoroacetic (**4**), dichloroacetic (**5**), dichloropropionic (**6**), chloroacetic (**7**), methoxyacetic (**8**), phenylacetic (**9**), and acetic (**10**).

Experimental Section

Materials. All chemicals were from Aldrich (Milwaukee, WI) except *tert*-butylimidazole, which was prepared according to the literature.[14] Liquids were fractionally distilled and handled under N_2(g). Chloroacetic acid and phenylacetic acid were recrystallized from chloroform and dried under vacuum in the presence of P_2O_5 for 2 days. All solvents used were anhydrous.

FTIR Spectra. FTIR spectra were acquired on a Nicolet 5PC Fourier transform infrared spectrometer at 4 cm^{-1} resolution with use of CsF_2 window material. Samples were dissolved in chloroform-*d* and were prepared under anhydrous conditions. Overlapping peaks in the carbonyl region were deconvoluted by using the computer program Peakfit (Jandel Scientific Software). Absorbances determined with this program were used in calculations to determine relative concentrations of LBHB and ion pair in solution.

Calorimetric Measurements. Adiabatic solution calorimetry was performed with a Parr Calorimetric Precision Thermometer Model 1674 (Moline, IL). Dilute concentrations of acids were used to limit self-aggregation and higher-order aggregate formation. Excess base was used to ensure complete complexation of acid. Lower concentrations of base resulted in smaller temperature changes. No concentration dependence was shown over the small range (0.02−0.05 M) of acid concentrations used. Stock solutions of a series of 2 M acid and 5 M alkylimidazole were prepared in chloroform for duplicate or triplicate runs. In a typical run, an acid (0.02−0.05 M) in chloroform (100 mL) was stirred under N_2(g) in an insulated reaction cell fitted with a temperature probe, calibration resistor, and an immersed glass tube with a very thin end, containing alkylimidazole (∼7× excess). When the temperature reached equilibrium, the sample was introduced by breaking the sample tube. The temperature change was monitored. The heat capacity of the solution was determined electrically by sending a known current through the immersed resistor and monitoring the temperature change. The heat capacity, C_p, was calculated from the following equation where I is the current applied, R_h is the resistance, t is the time the current was applied, and ΔT is the temperature change. The

$$C_p = I^2 R_h t/\Delta T$$

enthalpy, ΔH, was calculated by the following equation

$$\Delta H = -C_p \Delta T/n$$

where ΔT is the temperature change of the solution on addition of alkylimidazole or alkylamine and n is the number of moles of acid. This value was corrected by subtracting the heat of dilution of the alkylimidazole or alkylamine in the solvent alone under identical conditions.

Results

Figure 1 shows the carbonyl stretch region of FTIR spectra of complexes between 1-methylimidazole (0.35 M) and acids (0.05 M) **4**, **6**, and **10** in chloroform-*d* under the conditions of the calorimetry experiments. In Figure 1b, both **I** (1700 cm^{-1}) and **III** (1647 cm^{-1}) antisymmetric carbonyl stretches are observed for **6** at this low concentration of acid. Under these conditions the LBHB species is present in a larger relative ratio (2.2:1) to the ion pair than at a higher concentration of complex.[10,11] As shown in Figure 1a, **10**, a weak acid, has only one carbonyl stetch at 1709 cm^{-1} with a value consistent with little proton transfer (**II**). Figure 1c shows that for strong acid **4** only the ion-pair species **III** at 1673 cm^{-1} is present. The FTIR of complexes between 1-*tert*-butylimidazole and the substituted acids at lower concentration more closely resemble those seen previously at higher concentration[10,11] because formation of alternate species was sterically hindered due to the bulk of the *tert*-butyl group.

The heat produced, $-\Delta H_{formation}$, from the addition of excess 1-alkylimidazoles **1**−**3** or nonylamine (**11**, 7× excess) to a series of substituted carboxylic acids **4**−**10** (0.02−0.05 M) was measured by adiabatic solution calorimetry as described in the Experimental Section. Dilute concentrations of acids were used to limit self-aggregation and higher-order aggregate formation.

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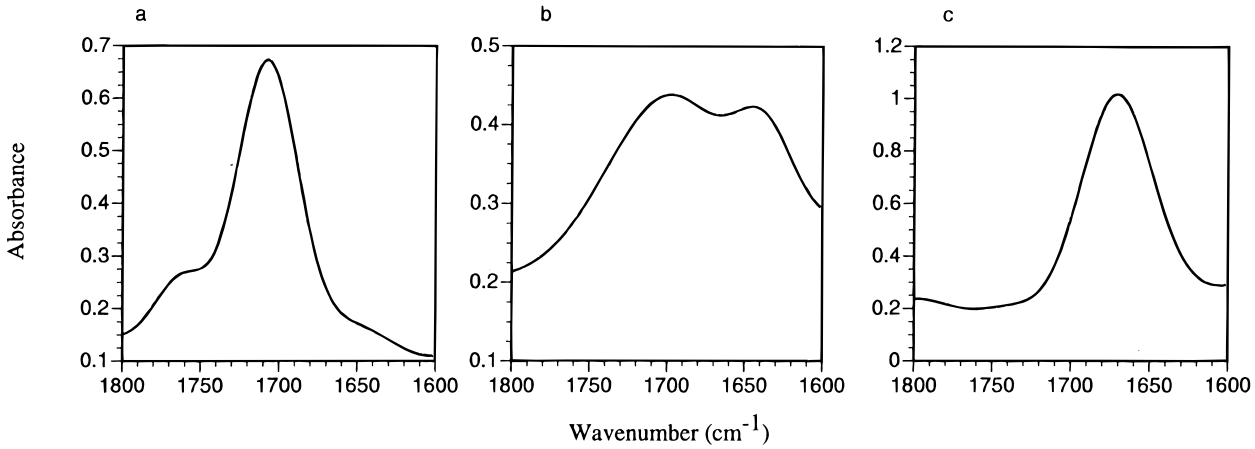

Figure 1. The carbonyl stretch region of FTIR spectra of complexes between **1** (0.35 M) and (a) acetic acid (**10**), (b) dichloropropionic acid (**6**), and (c) trifluoroacetic acid (**4**) (0.05 M) in $CDCl_3$ at room temperature. Only **6** shows both LBHB (**I**) at 1700 cm^{-1} and ion pair (**III**) at 1647 cm^{-1}. Acid **10** shows only hydrogen bond (**II**) at 1709 cm^{-1} and acid **4** shows only ion pair (**III**) at 1673 cm^{-1}. Assignments of hydrogen bond or ion pair were made by comparing the carbonyl stretch values to those of the free acids alone and those of their tetrabutylammonium salts, respectively.[10,11] In both cases, as the acid pK_a increases the carbonyl stretch value decreases to a smaller wavenumber.

Table 1. Enthalpies of Complex Formation, $-\Delta H_{formation}$ (kcal/mol) between Alkylimidazoles **1**−**3** and Substituted Carboxylic Acids **4**−**10** Determined by Adiabatic Solution Calorimetry in $CHCl_3$ at Room Temperature[a]

acids (0.02−0.05 M)	pK_a aq	imidazole (7× excess) 1-Me (**1**)	imidazole (7× excess) *n*-Bu (**2**)	imidazole (7× excess) *t*-Bu (**3**)	nonylamine (7× excess) **11**
trifluoroacetic (**4**)	0.23	13.7(0.5)	13.0(0.6)	13.6(0.3)	23.8(0.6)
dichloroacetic (**5**)	1.29	13.7(0.5)	13.4(0.3)	14.7(0.1)	21.0(0.5)
dichloropropionic (**6**)	2.06	12.3(0.8)	11.5(0.2)	14.9(0.8)	20.2(0.8)
chloroacetic (**7**)	2.86	6.8(0.2)	5.9(0.5)	9.4(0.3)	16.7(0.9)
methoxyacetic (**8**)	3.53	6.5(0.3)	6.3(0.5)	7.2(0.3)	13.8(0.7)
phenylacetic (**9**)	4.31	4.6(0.4)	3.9(0.6)	5.5(0.4)	14.2(0.5)
acetic acid (**10**)	4.76	4.8(0.5)	3.9(0.6)	4.0(0.2)	13.0(0.9)

[a] The values reported are averages from triplicate experiments and the errors are $\pm\sigma$.

The enthalpies measured are the sums of the enthalpy of proton transfer, ΔH_{PT}, and hydrogen bond formation, ΔH_{HB}, and are

$$\Delta H_{formation} = \Delta H_{PT} + \Delta H_{HB} \quad (1)$$

listed in Table 1.

As shown in Table 1, the $-\Delta H_{formation}$ of complexes of methylimidazole and acids **4**−**10** becomes more exothermic as the pK_a of the acid decreases. The aqueous pK_a values (7 ± 0.15 pH unit) of the unalkylated nitrogen are similar in the three alkylimidazoles utilized. The *n*-butylimidazole and *tert*-butylimidazole $-\Delta H_{formation}$ values with acids **4** and **8**−**10** are comparable to those of methylimidazole, although the 1-*tert*-butylimidazole $-\Delta H_{formation}$ values for **5**−**7** are somewhat more exothermic. Higher $-\Delta H_{formation}$ values (5−10 kcal/mol more exothermic) are found with nonylamine (pK_a 10.13) and acids **4**−**10**, where mixing results in full proton transfer to give only ion pairs due to the large ΔpK_a between nonylamine and all acids. That only full proton transfer occurs is shown in FTIR spectra of nonylamine and the weakest acid **10**, which has only one carbonyl stretch at 1572 cm^{-1}, near the value (1575 cm^{-1})[11] of the tetrabutylammonium salt of **10**. Likewise, the complex of nonylamine and **6** has only one stretch (1642 cm^{-1}) similar to the value (1648 cm^{-1})[11] of the tetrabutylammonium salt of **6**. Thus the ΔH_{PT} contribution to $\Delta H_{formation}$ for these complexes is large. Since ΔH_{PT} is dependent on ΔpK_a, as the pK_a values of the acid decrease, the $-\Delta H_{formation}$ values increase.

Both previous[10,11] and present FTIR studies on these complexes have shown that the equilibria of **I, II,** and **III** in solution are dependent on the pK_a of the carboxylic acid in the complex and the alkyl substitution of the alkylimidazole. Thus, the $-\Delta H_{formation}$ for carboxylic acids having different pK_a's will be influenced by the relative concentrations of the three types of complexes. The relative concentrations of the complexes can be obtained from FTIR studies. In spectra of a complex of **1** and **6**, absorbance values for the antisymmetric carbonyl stretch for the proposed LBHB and ion-pair species show a 2:1 ratio. By using complexes where only one species is present, the average extinction coefficient of the ion-pair species is twice that of the weakly hydrogen bonded species. An estimate for the extinction coefficient for the LBHB species can be determined from the average of the extinction coefficients for these two species. By using this approximation the relative concentration of **I** to **III** is 2.2:1. The relative concentration of **I**:**III** in solutions of **3** and **6** is 1.4:1. Dichloroacetic acid, **5**, also shows two carbonyl stetches in FTIR spectra in 1:1.2 and 1.2:1 relative concentrations of the LBHB to the ion pair for **1** and **3**, respectively.

For complexes of **1**−**3** and acids **4** and **7**−**10** the $-\Delta H_{formation}$ represents either normal hydrogen bonding (**7**−**10**) or ion-pair complex formation (**4**). The complexes between **1** or **3** and **5** or **6** have 45−68% of LBHB, **I**, present. The other species in solution is the ion pair, **III**. The contribution from the ion pair to the $-\Delta H_{formation}$ can be estimated to be 12−15 kcal/mol. This indicates that the $-\Delta H_{formation}$ for the LBHB is of approximately the same magnitude, 12−15 kcal/mol, as for the ion pair.

Discussion

Since the time that LBHBs were proposed to mediate enzyme catalysis, the energy stabilization gained from an LBHB interaction has been a subject of debate. It was proposed that LBHBs catalyze enzyme reactions by stabilizing otherwise high

energy transition states and thereby decrease the activation barrier of the reaction.[1−3] The amount of stabilization depends on the strength of the LBHB. In the present study, we measured $-\Delta H_{\text{formation}}$ values for complexes of alkylimidazoles and carboxylic acids and determined the contribution of LBHB formation to be 12−15 kcal/mol in chloroform.

The magnitudes of the $-\Delta H_{\text{formation}}$ values measured in the present work are consistent with values for strong hydrogen bond complexes reported in the literature. In the past, calorimetry has been used to measure the $-\Delta H_{\text{formation}}$ of a number of hydrogen bonded acid/base complexes,[15] including compounds similar to those in this study such as pyridine or pyridine−oxides and substituted acetic acids.[16] The enthalpies (11−24 kcal) obtained here for nonylamine (pK_a 10.13) are consistent with the values for triethylamine (pK_a 10.7) with the series of carboxylic acids. The enthalpies (7−18 kcal/mol) for pyridine (pK_a 5) complexes[16] were consistent with the enthalpies (12−15 kcal) measured here for the alkylimidazoles and the substituted carboxylic acids. For complexes between a series of substituted pyridine or pyridine−oxides with only dichloropropionic or dichloroacetic acid, where the base was varied instead of the acid, the $-\Delta H_{\text{formation}}$ values ranged beween 8 and 25 kcal/mol. The $-\Delta H_{\text{HB}}$ values determined from IR spectra for these acids with a series of substituted pyridines and pyridine−oxides were 8−15.5 kcal/mol, the residual value being $-\Delta H_{\text{PT}}$.

To approximate the active site of an enzyme ($\epsilon \sim 4-6$), chloroform, a nonpolar aprotic solvent ($\epsilon = 4.8$), was used in these calorimetric studies. For small organic compounds, the strengths of hydrogen bonds in solution have been found to be solvent dependent. Calorimetric studies have shown that higher hydrogen bond strengths are measured for the same complexes in nonpolar rather than polar solvents including water.[17,18] In measurements of equilibrium constants, hydrogen bond strengths were found to be both higher and more sensitive to substituent changes in aprotic solvents than in water.[19] This has been attributed in part to the greater stabilization of the negatively charged species in water than in aprotic solvents and in part to lack of solvent competition in aprotic solvent. Because of their low dielectric constants, the interiors of proteins at the active site have environments more similar to a nonpolar aprotic solvent even in the presence of water.

The $\Delta G_{\text{formation}}$ of the complexes studied here, which model the His−Asp interaction in serine proteases, has a nonzero entropy term that should vary depending on the nature of the acid. In enzymatic active sites, reactions that lead to LBHB formation should have little entropy contribution due to preorganization of enzyme and substrate in the active site. In enzymes, then, $\Delta G_{\text{formation}}$ for LBHB formation will be similar to $\Delta H_{\text{formation}}$ and can be as high as −12 to −15 kcal/mol. If a weak hydrogen bond in the initial E·substrate complex with a $\Delta G_{\text{formation}}$ of ≤ -5 kcal/mol is converted to a low-barrier hydrogen bond in the transition state, there will be 7−10 kcal/mol of energy available to lower the activation barrier and accelerate the reaction by 5−7 orders of magnitude. This magnitude of rate enhancement was proposed for the LBHB in chymotrypsin, based on literature reports in which the His57−Asp102 interaction had been disrupted.[20,21]

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