resonance, lone pair repulsion, and polarizability effects. The most marked resonance effect is in imidazole, where the acidity is strengthened by 7.1 kcal/mol.

4. The Acidity of 4-Aminopyrimidine. The acidity of this compound was measured as a model for adenine and cytosine, both of which have a similar configuration of nitrogen atoms in the amine-bearing ring, and also as an approximation for guanine. The acidities of 2-aminopyridine and 4-aminopyridine were also measured for comparison.

Compared with aniline as the parent compound, the acidity of 4-aminopyridine is increased by 8.9 kcal/mol. Electronic coupling between the NH^- function and the aza substituent in the ring is not favorable, and the effect is probably electrostatic. In contrast, the acidity of 2-aminopyrimidine is increased only by 4.7 by the aza substitution, compared with aniline. The effect is probably a combination of stabilizing electrostatic effect by the electronegative substituent and destabilizing effect of lone-pair repulsion. In the deprotonated azoles, Taft et al. estimate the lone-pair repulsion between adjacent nitrogens as 8 kcal/mol.[14]

In 4-aminopyrimidine, the combined effects of 2- and 4-aza substitutions are stabilizing and the acidity is stronger by 13.7 kcal/mol compared with aniline.

In the deprotonated aminopyridines and aminopyrimidine, the aza substituents in the ring interact with the charge on the external NH^- function. It is of interest to compare these effects with aza substitution on the acidities of ring carbons in benzene.[17] In proceeding from benzene ($\Delta H°_{acid}$ = 400.7 kcal/mol) to pyridine ($\Delta H°_{acid}$ = 391.0 kcal/mol) to 1,2-diazine (382.4 kcal/mol) and 1,3-diazine (385.2 kcal/mol), the average effect of an aza substitution is increased acidity by 8–9 kcal/mol, probably mostly due again to charge–bond dipole interactions. However, in 1,4-diazine, where the lone pair of deprotonated carbon must be adjacent to a nitrogen, lone-pair repulsion destabilizes the anion and the acidity is decreased by 8–10 kcal/mol compared with the other diazines ($\Delta H°_{acid}$ = 392.6 kcal/mol). The direction and magnitude of aza substitution on ring carbon acidities is therefore similar to the effects on the external NH_2 group acidity.

In summary, the overall effect of the two aza substituents in 4-aminopyrimidine is stronger in acidity by 13.7 kcal/mol compared with the parent compound, aniline.

Conclusion

In the present work we measured the intrinsic gas-phase acidities of model compounds for the principal hydrogen donors in biological hydrogen bonds. CH_3CO-Ala-OCH_3 served as a model for peptide links that are donors for the NH·O bonds in the helix of proteins. 4-Aminopyrimidine served as a model for NH donor amino groups in adenine, cytisine, and guanine. Imidazole served as a model for the NH donor imidazole center of histidine residues in enzyme active sites.

In each of these compounds it is found that the acidity is stronger by 7–14 kcal/mol compared with the respective parent structures due to electrostatic and resonance effects. This, in turn, strengthens peptide and DNA hydrogen bonds of these NH donors by about 0.7–1.4 kcal/mol, according to the correlations found by Zeeger-Huyskens.[1] This amounts to about 20% of the total hydrogen bond energy, and with long chains of hydrogen bonds, this can have a substantial effect on the conformational stabilities of biopolymers.

Due to various molecular factors, the intrinsic acidities of the principal biological NH donors therefore fall into a narrow range between $\Delta H°_{acid}$ = 352 and 355 kcal/mol. This is just slightly less acidic than carboxylic groups, 345–350 kcal/mol. Therefore the acidities are about as strong as possible without becoming ionized at biological pH in solvent-free environments. This in turn optimizes the hydrogen bond strengths.

The principal biological hydrogen donors therefore reveal a common feature in their similar optimized intrinsic acidities. The intrinsic acidity may have been a factor in the natural selection of these molecules for their biological roles.

Models for Strong Interactions in Proteins and Enzymes. 2. Interactions of Ions with the Peptide Link and with Imidazole

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Abstract: Cluster ions provide an experimental measure of ionic interaction energies in proteins. Peptide links, modeled by the alanine derivative CH_3CO-Ala-OCH_3, are strong hydrogen donors and bond by 30 kcal/mol to anions such as $RCOO^-$ and Cl^-. The protein environment as modeled by two peptide amide groups stabilizes an anion by about 45 kcal/mol and a cation by about 50 kcal/mol. Therefore, in general, a protein backbone can stabilize a charge-separated ion pair by 90–110 kcal/mol. Applying clustering results to a specific biological system, anionic centers in the active site of serine proteases are examined. The model suggests that the aspartate carboxyl of the enzyme is solvated by four hydrogen bonds by about 65 kcal/mol, and the tetrahedral oxyanion intermediate is stabilized by hydrogen bonds to two peptide links by about 30 kcal/mol. The latter stabilization may be the major energy factor in protease catalysts, in agreement with theory. The results also suggest a new proteolytic mechanism, where the substrate's peptide link would hydrogen bond or transfer a proton to the imidazole of the enzyme. The ionic hydrogen bond strengths in a series of present clusters show a direct correlation with the intrinsic acidities of the components. This shows that the strong acidity of the peptide link increases its stabilizing power for ionic intermediates. Strong intrinsic acidities therefore emerge as an important factor in the natural selection of donor molecules in biological hydrogen bonds in ionic as well as neutral systems.

Proteins and enzymes contain ionic components and ionic reaction intermediates. These ionic components can interact strongly with polar and hydrogen-bonding groups, as well as with solvent molecules. These strong interactions may be critical to protein conformation and enzyme energetics, but because of the complexity of the system, no experimental data are available about the interaction energies.

In the peptide interior the ions are in an environment from which bulk solvent is excluded, and in this respect the ionic interactions can be modeled by gas-phase measurements. Ion–

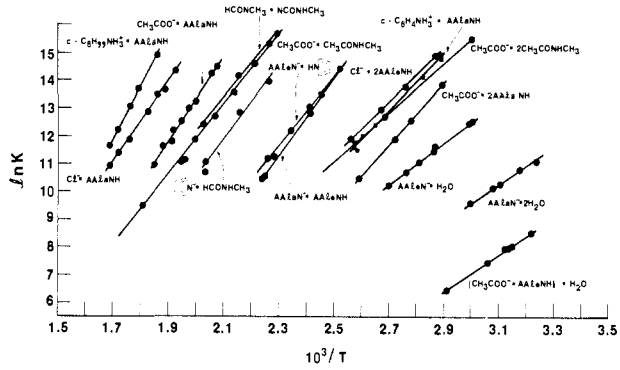

Figure 1. Van't Hoff plots for association reactions of amides and AAlaNH.

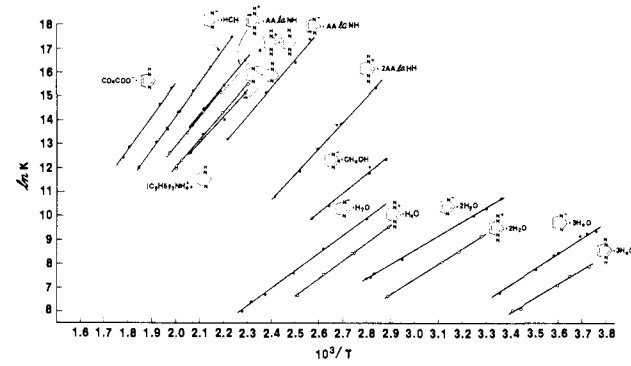

Figure 2. Van't Hoff plots for association reactions of azoles.

molecule clustering can be applied therefore to measure pairwise interactions, from which the interaction energies of the network of hydrogen bonds in a biological system may be evaluated. Indeed, gas-phase clustering studies on simple molecules indicate that ionic interactions are strong, being usually in the range 10–30 kcal/mol.[1-4] However, most gas-phase clustering studies of anions involved to date simple monofunctional organic species. It is desirable that such studies will be extended to molecules that more closely resemble protein and enzyme components.

In the present work we study ionic interactions of the peptide link and of imidazole. The preceding paper showed that both have strong intrinsic acidities.[5] Therefore, they may be ionized under some conditions and serve as anionic centers for hydrogen bonds. Also, in the neutral form, they should be strong hydrogen donors in bonding to anions. We shall therefore deal both with the bonding of deprotonated, anionic peptide links and imidazole to neutral hydrogen donors as well as the interactions of the neutral forms of these molecules with anions. Some related cationic interactions will also be studied. As in the preceding paper, the peptide link will be modeled by the dipeptide analogue alanine derivative CH_3CO-Ala-OCH_3 and the imidazole function of histidine by imidazole itself.

After presenting the results, the discussion will be divided to two main parts. The first part will discuss structural aspects of the gas-phase complexes. The second part will present some conclusions on the stabilization of ions by a peptide backbone and, as a specific example, on ionic interactions in the active sites of protease enzymes.

Experimental Section

The measurements were done on the NBS pulsed high-pressure mass spectrometer. The conditions were similar to those described in the preceding paper.[5] The concentrations of the neutral ligands were usually kept below 1% of the gas mixture. In this manner clustering studies could be done at the lowest possible temperatures, avoiding ion dissociation outside the ion source. Higher ligand concentrations were used only to study some of the higher hydration steps. Total ion source pressures were between 0.8 and 2.4 Torr.

In relation to the reactions of CH_3COO^-, it should be noted that the ion clusters strongly with CH_3COOH, which had to be used therefore in trace amounts. An attempt was made to use acetic anhydride instead, but an apparent CH_3COOH impurity still caused self-clustering at low temperatures.

For the reactions where a second AAlaNH molecule is added to the cluster, temperatures of 60–100 °C were required. The compound was at partial pressures of 10^{-3}–10^{-4} Torr in the source, and there was no indication that it condensed in the ion source under these conditions. However, attempts to examine the addition of a third molecule of AAlaNH to the ionic cluster indicated that the compound condensed on the stainless steel source below about 20 °C.

Table I. Thermochemistry[a] of Interactions of Ionized Peptide Links and Imidazole with Solvent Molecules

		$\Delta H°_D$	$\Delta S°_D$	$\Delta H°_{acid}$
1	$AAlaN^-·H_2O$	15.2	20.9	35.4
2	$(AAlaN^-·H_2O)·H_2O$	13.0	19.8	
3	$(CH_3COO^-·AAlaNH)·H_2O$	13.2	25.4	
4	$AAlaN^-·CH_3OH$	18[b]	(25)	26.2
5	$ImN^-·H_2O$	15.2	22.6	39.0
6	$(ImN^-·H_2O)·H_2O$	11.8	18.2	
7	$(ImN^-·2H_2O)·H_2O$	12.9	29.2	
8	$ImN^-·CH_3OH$	17.1	24.2	29.8
9	$ImN^-·HCN$	23.9	21.9	8.0
10	$ImNH^+·H_2O$	14.8	23.8	
11	$(ImNHH^+·H_2O)·H_2O$	12.6	23.3	
12	$(ImNHH^+·2H_2O)·H_2O$	12.2	29.8	

[a] $\Delta H°$ in kcal/mol, $\Delta S°$ in cal/(mol·K). [b] From $\Delta G°(392) = 6.8$ kcal/mol, $\Delta S°$ estimated from similar reactions.

Table II. Thermochemistry[a] of Complexes of Amides

		$\Delta H°_D$	$\Delta S°_D$	$\Delta H°_{acid}$
13	$HCONCH_3^-·HCONHCH_3$	28 ± 2	31 ± 4	0.0
14	(pyrrole H)$^-$·$HCONHCH_3$	26 ± 2	31 ± 4	3.0
15	$CH_3CONCH_3^-·CH_3CONHCH_3$	27[b]	(30)	0.0
16	(pyrrole H)$^-$·$CH_3CONHCH_3$	27[c]	(30)	3.4
17	$CH_3COO^-·CH_3CONHCH_3$	25.4	27.4	4.7
18	$(CH_3COO^-·CH_3CONHCH_3)·CH_3CONHCH_3$	17.6	21.8	

[a] Units and error estimates: $H°_D$, ±1 kcal/mol; $S°$, ±4 cal/(mol·K). [b] From $\Delta G°(497) = 12.5$ kcal/mol, $\Delta S°$ estimated from similar reactions. [c] From $\Delta G°(497) = 11.9$ kcal/mol, $\Delta S°$ estimated from similar reactions.

Results

The thermochemistry for association reactions leading to hydrogen-bonded complexes is obtained from equilibria 1.

$$A^- + BH \rightarrow A^-·BH \quad (1)$$

Temperature studies yield van't Hoff plots which are presented in Figures 1 and 2. The accuracies of temperature studies, based on the standard deviations of the slopes and intercepts of the plots and on reproducibility, are usually ±1 kcal/mol for $\Delta H°_D$ and ±2 cal/(mol·K) for $\Delta S°_D$.

The reactions in Table II involve the complexing of $HCONHCH_3$ and $CH_3CONHCH_3$ or their anions. These data in Table II were obtained incidentally in the course of proton-transfer studies and the estimated error of $\Delta H°_D$ is ±2 kcal/mol and that of $\Delta S°_D$ ±4 cal/(mol·K).

Discussion

1. Correlation between Hydrogen Bonding Energies and Acidities. The general trend observed in ionic hydrogen bonds is that for a given type of bond, the dissociation energy $\Delta H°_D$ increases as the components are better able to share the bonding proton.[3,6,7] Therefore, $\Delta H°_D$ increases as the difference between

(1) Kebarle, P. *Annu. Rev. Phys. Chem.* **1977**, *28*, 445.
(2) Meot-Ner (Mautner), M. *Acc. Chem. Res.* **1984**, *17*, 186.
(3) Meot-Ner (Mautner), M.; Sieck, L. W. *J. Am. Chem. Soc.* **1986**, *108*, 7525.
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(5) Meot-Ner (Mautner), M. *J. Am. Chem. Soc.*, preceding paper in this issue.
(6) Caldwell, G.; Rozeboom, M. D.; Kiplinger, J. P.; Bartmess, J. E. *J. Am. Chem. Soc.* **1984**, *106*, 4660.

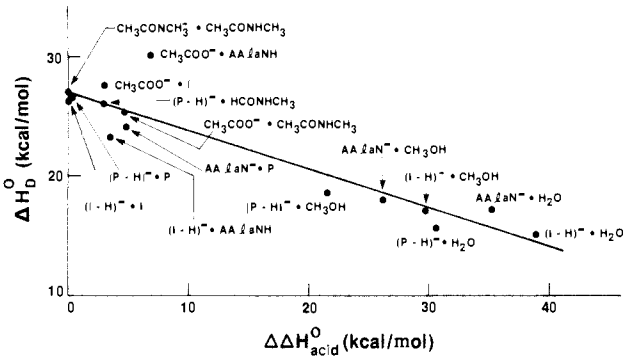


Figure 3. Correlation between ΔH°_{acid} and ΔH°_D for $N^-\cdot HN$ and $N^-\cdot HO$ complexes (I = indole, P = pyrrole, AAlaNH = $CH_3CONHCH(CH_3)COOCH_3$).

Table III. Thermochemistry[a] of Complexes Containing the Peptide Analogue AAlaNH or Imidazole (ImNH) as Hydrogen Donors and Some Complexes of These Molecules with Cations

		ΔH°_D	ΔS°_D	ΔH°_{acid}
19	$AAlaN^-\cdot AAlaNH$	28.3	40.4	0.0
20	$ImN^-\cdot AAlaNH$	23.1	24.8	3.9
21	$CH_3COO^-\cdot AAlaNH$	30.2	33.8	6.9
22	$(CH_3COO^-\cdot AAlaNH)\cdot AAlaNH$	21.2	34.1	
23	$(CH_3COO^-\cdot H_2O)\cdot AAlaNH$	27.6	38.9	
24	$Cl^-\cdot AAlaNH$	29.4	28.3	
25	$(Cl^-\cdot AAlaNH)\cdot AAlaNH$	19.8	27.0	
26	$ImN^-\cdot ImNH$	26.4	29.3	0.0
27	$CH_3COO^-\cdot ImNH$	27.7	24.4	3.4
28	$AAlaN^-\cdot$pyrrole	24.0	32.1	4.7
	Cationic Complexes			
29	$c\text{-}C_6H_{11}NH_3^+\cdot AAlaNH$	37.8	40.9	
30	$(c\text{-}C_6H_{11}NH_3^+\cdot AAlaNH)\cdot AAlaNH$	20.4	29.5	
31	$ImNH^+\cdot AAlaNH$	27.7	27.1	
32	$(ImNH^+\cdot AAlaNH)\cdot AAlaNH$	21.8	31.3	
33	$(C_2H_5)_2NH_2^+\cdot ImNH$	22.6	21.2	
34	$ImNH^+\cdot ImNH$	23.7	21.7	

[a] Units and error estimates: ΔH°, ±1 kcal/mol; ΔS°, 2 cal/(mol·K).

the acidities of the components, $\Delta\Delta H^\circ_{acid}$, decreases. An inverse linear correlation between ΔH°_D and $\Delta\Delta H^\circ_{acid}$ was observed for $O^-\cdot HO$ hydrogen bonds.[3,6,7] For $N^-\cdot HO$ and $N^-\cdot HN$ bonds, a rough correlation as shown in Figure 3 can be established from the present data.

$$\Delta H^\circ_D(N^-\cdot HB) = 26.8 \pm 2 - (0.32 \pm 0.06)\Delta\Delta H^\circ_{acid} \text{ kcal/mol} \quad (2)$$

The scatter is fairly large. More data are desirable, and it is desirable to establish separate correlations for $N^-\cdot HN$ and $N^-\cdot HO$ type bonds.

The intercept of the correlation line gives the maximum strength of the bond. This may be termed the intrinsic strength of the bond after the acidity differences have been accounted for. The present correlation line shows that the intrinsic strength of $N^-\cdot HN$ and $N^-\cdot HO$ bonds is substantial, 26.8 ± 2 kcal/mol, and similar to the $O^-\cdot HO$ bond which is 28.1 kcal/mol.[3] Interestingly, the intrinsic strength of $N^-\cdot HN$ bonds is similar to that of the analogous cationic bonds $NH^+\cdot N$ which is 24 kcal/mol.[8]

2. Solvation of Deprotonated Amide Links and Imidazole. The attachment energies of solvent molecules to deprotonated $CH_3CO\text{-}Ala\text{-}OCH_3$, denoted as $AAlaN^-$, and to deprotonated and protonated imidazole, denoted as ImN^- and $ImNHH^+$, respectively, are summarized in Table I. The acidity difference between AAlaNH or ImNH and H_2O is large, 35 and 38 kcal/mol. Correspondingly, eq 2 predicts relatively small hydrogen-bonding energies of about 15 kcal/mol, as is indeed observed.

Due to the dispersion of charge and repulsion between the solvent molecules, the attachment energy of a second H_2O molecule to both ions is smaller, and already approaches the limiting low value of $\Delta H^\circ_{vap}(H_2O)$ = 10.5 kcal/mol. A similar trend is observed in the hydration of $ImNHH^+$. The results therefore indicate that the ionic charge of the solvated ionic peptide and imidazole cores is dissipated already after solvation by two H_2O molecules. Therefore, in solvation by bulk water, the ionic charge of the solvated peptide and imidazole functions would have only limited significance.

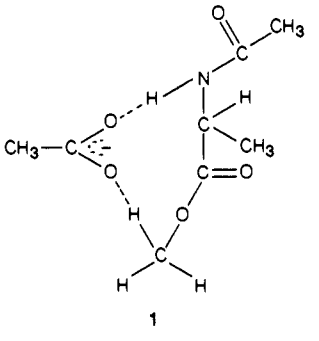
The usual trends in clustering would predict further decrease in ΔH°_D after the second solvation step especially since the first two solvent molecules attached to $ImNHH^+$ and ImN^- and occupy the immediate attachment sites to the ions, and the third H_2O molecule should go to an outer shell. However, the third attachment energy in both is similar to the second. This suggests that possibly a cyclic structure is formed in both cases, where the third water molecule is hydrogen bonded to the first two, and stabilization is obtained by multiple bonding.

The interaction energies of the $AAlaN^-$ and ImN^- with CH_3OH, HCN, and pyrrole are larger than those of H_2O, in agreement with correlation 2. Also in agreement with the correlation, the strongest bonding of these ions, 26–28 kcal/mol, is with AAlaNH and imidazole. Similar bonding strengths are also observed in $HCONCH_3^-\cdot HCONHCH_3$ and $CH_3CONCH_3^-\cdot CH_3CONHCH_3$. In all of these cases the acidity difference is zero, and the interactions exhibit the intrinsic strengths of $N^-\cdot HN$ bonds.

3. Hydrogen Bonding of the Peptide Link and Imidazole to Ions. The CH_3COO^- ion is a model for ionized carboxylate residues in proteins. Table II shows that an amide hydrogen can bond to the carboxylate strongly, by 25.4 kcal/mol. The peptide link should hydrogen bond to CH_3COO^- even more strongly, by 27 kcal/mol according to correlation 2, since it is more acidic than the amides by 8.0 kcal/mol. In fact, the observed bonding energy is even stronger than expected from this relation, 30.2 kcal/mol.

The additional strength of the $CH_3COO^-\cdot AAlaNH$ complex can be due to secondary interactions of the large ligand with the ion. For example, the methyl groups may bond to the anion by $CH\cdot O^-$ hydrogen bonds such as ion **1**. The two bonding sites in CH_3COO^- are well oriented for such interactions. In fact, we observed recently that the bonding of AAlaNH to CH_3COO^-, Cl^-, CN^-, and SH^- is stronger by 5–9 kcal/mol than would be predicted for a single hydrogen bond in these complexes.[9] Therefore the large ligand molecule can interact by multiple interactions with most ions that are likely to be in a protein environment, and such interactions are responsible for the strong bonding observed here for $CH_3COO^-\cdot AAlaNH$ and $Cl^-\cdot AAlaNH$.

In contrast, in $ImN^-\cdot AAlaNH$, the ion has only one hydrogen bonding site, since the distance and orientation of the second nitrogen in ion **2** make it unavailable for a second bond to the same neutral molecule. Correspondingly, the bonding energy is somewhat smaller, 23.1 kcal/mol.



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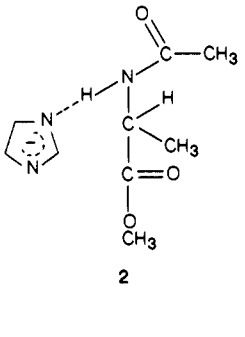

2

A similar trend is observed in the cationic complexes. AAlaNH bonds very strongly to the protonated amine group in $c\text{-}C_6H_{11}NH_3^+$, indicating multiple hydrogen bonding.[10] In contrast, with imidazoleH^+ the geometry of the hydrogen bonding protons is unfavorable, similar to the two lone electron pairs in ImN^-.

(7) Yamdagni, R.; Kebarle, P. *J. Am. Chem. Soc.* **1971**, *93*, 7139.
(8) Meot-Ner (Mautner), M. *J. Am. Chem. Soc.* **1984**, *106*, 1257.
(9) Meot-Ner (Mautner), M. *J. Am. Chem. Soc.*, submitted for publication.
(10) Meot-Ner (Mautner), M. *J. Am. Chem. Soc.* **1984**, *106*, 278.

Correspondingly, the enthalpy and entropy of the $ImNHH^+$–AAlaNH complex does not indicate multiple interactions.

4. Bonding to Several Ligand Molecules. Because of charge dispersion and ligand–ligand repulsion, the attachment energy of a second ligand molecule to a core ion, $\Delta H°_{1,2}$, is usually smaller than the bonding energy of the first solvent molecule, $\Delta H°_{0,1}$.[11] For a large variety of clusters, the decrease in the attachment energy as expressed by the ratio $\Delta H°_{1,2}/\Delta H°_{0,1}$ is a constant 0.75 ± 0.05.[23]

In the cluster $CH_3COO^-{\cdot}2CH_3CONHCH_3$, $\Delta H°_{1,2}/\Delta H°_{0,1}$ = 0.71, a value within the usual range. This is of interest because here the two ligands can attach to two different atoms in the ion. The results suggest that charge delocalization to a ligand is transmitted efficiently through the $(O–C–O)^-$ system. Regular values of $\Delta H°_{1,2}/\Delta H°_{0,1}$ = 0.70 and 0.67 are observed also in $CH_3COO^-{\cdot}2AAlaNH$ and $Cl^-{\cdot}2AAlaNH$, respectively. However, this may mask several cancelling effects in the complex systems with multiple interactions.

In fact, an effect of multiple bonding is observed in $c\text{-}C_6H_{11}NH_3^+{\cdot}2AAlaNH$, where the first ligand shows multiple interactions.[9] These can be displaced by the second ligand molecule, and the required energy shows up as a decreased value for $\Delta H°_{1,2}$. As a result, the value of $\Delta H°_{1,2}/\Delta H°_{0,1}$ = 0.54 is unusually small. In contrast, in $imidazoleH^+{\cdot}2AAlaNH$, where the first ligand molecule shows no signs of multiple interactions, $\Delta H°_{1,2}/\Delta H°_{0,1}$ = 0.78. We note that here, as in $CH_3COO^-{\cdot}2AAlaNH$, the ligand molecules attach to two separate bonding sites. Here the mutual effects of the ligands are transmitted through an $(HN–C–NH)^+$ system.

In the $CH_3COO^-{\cdot}AAlaNH{\cdot}H_2O$ cluster, the mutual interactions of a weak and a strong ligand can be observed. In adding AAlaNH to $CH_3COO^-{\cdot}H_2O$, we find $\Delta H°_D((CH_3COO^-{\cdot}H_2O){\cdot}AAlaNH/\Delta H°_D(CH_3COO^-{\cdot}AAlaNH)$ = 0.91, a small weakening effect of the first ligand on the second. In other words, solvation of a carboxylate function by a water molecule weakens only slightly, from 30.2 to 27.6 kcal/mol, the bonding of $RCOO^-$ to strongly hydrogen bonding molecules such as AAlaNH or, probably, imidazole. Subsequent solvation steps are usually even weaker, and further H_2O molecules attached to carboxylate will have even smaller effects. This is important, since solvent molecules may be present in protein interiors and may be attached to carboxylate groups that also hydrogen bond to peptide or imidazole.

5. Modeling Biological Systems by Gas-Phase Clusters. We shall also use the general observation[11] that each strong ligand decreases the interaction energy with further ligands by a factor of about 0.7. First, the merits and limitations of the gas-phase model must be considered.

The gas-phase measurements have the unique capability to measure the strengths of individual hydrogen bonds in isolation. However, real systems involve multiple interactions which result in collaborative effects. The interactions of an ion with several neutral molecules or groups can be accounted for in part by measuring large gas-phase clusters containing many ligands. With large biomolecules, we were limited to two ligands, but this still allows, fortunately, for examining the pairwise effects of multiple ligands, which is the most important term.

A given ion in a protein cavity interacts strongly with other ions as well as with neutral groups. These ion–ion interactions cannot be modeled by gas-phase clusters at this time. Therefore the present results model charge separated entities at infinite distance.

Another limitation is that vapor pressure requirements necessitate the use of volatile model compounds. For the present purposes, a volatile dipeptide or tripeptide would have been desirable. Fortunately, in the AAlaNH, i.e., $CH_3CONHCH(CH_3)COOCH_3$, model molecule the NH group is related to the carbonyl functions in the same manner as in a peptide link, and for the present purposes this is the essential feature.

Finally, the structures of bimolecules impose steric constraints on the geometries of hydrogen bonds, while the gas-phase complexes allow optimized structures. Therefore, the gas-phase interaction energies should be considered as upper limits for the strengths of ionic hydrogen bonds in biological systems.

6. Interactions of Ions with the Protein Backbone. Stabilization of a Charge-Separated Ion Pair. With the above reservations in mind, AAlaNH molecules are considered as models of a peptide backbone interacting with ionic intermediates. Two AAlaNH molecules should block interactions with further ligands and in this simulate a protein environment that envelopes the charged species.

The primary observation is that the interaction energies of anions with the model protein environment are large. Altogether, the first two AAlaNH molecules bond to CH_3COO^- by 51.4 kcal/mol and to Cl^- by a similar energy, 49.2 kcal/mol. As we noted, about 5 kcal/mol of these interactions may be due to secondary interactions that may not apply in a protein, so the total interaction energy of two peptide links with an anionic residue in optimized geometry is about 45 kcal/mol. Elsewhere, we observed that the clustering properties of CH_3COO^-, Cl^-, SH^-, and CN^- to many ligands, including AAlaNH, are similar.[9] Therefore, similar interaction energies, about 45 kcal/mol, may be expected also with many anions and anionic residues in protein interiors.

As to interaction with cations, $c\text{-}C_6H_{11}NH_3^+$ is a model for cations that have several protons for multiple bonding to a ligand. The bonding energy to two AAlaNH molecules is 56.2 kcal/mol. Where multiple interactions to a single peptide residue are sterically prohibited, such as $imidazoleH^+$, the interaction energy with two ligands is still substantial, 49.5 kcal/mol.

As we noted above, partial solvation of the anions, such as by a residual water molecule in the enzyme cavity, decreases the interaction energy with the model peptide only by a few kcal/mol. Therefore, the present energies are good models even when solvent molecules are present in the enzyme cavity.

Warshel investigated theoretically enzyme catalysis that proceeds through charge separation.[12,13] The general function of the protein environment was found to be the stabilization of the charge separated ion pair, which reduces the activation energy for charge separation. To provide a better environment than water, the required stabilization should be greater than about 70 kcal/mol for an ion pair separated by 4 Å and 140 kcal/mol for an infinitely separated pair.[13] In the present results, two peptide amide links bonding to a protonated amine or $imidazoleH^+$ give about 50 kcal/mol and to an anionic center such as CH_3COO^- or Cl^- also about 45 kcal/mol. Altogether, the protein environment as simulated by four AAlaNH molecules interacts with a charge-separated pair of ionic species by 90–110 kcal/mol. This is a substantial fraction of the 140 kcal/mol required to stabilize an infinitely separated ion pair better than water. Further factors, for example by charged groups in the enzyme or bulk effects, are required to contribute about 40 kcal/mol for the enzyme to give better solvation than water. In any event, the present results show that a few nearby polar amide links alone, without any special structural elements, can supply a large fraction of the required stabilization energy.

7. Interaction Energies at Active Enzyme Sites. Active sites of serine proteases invariably contain a triad of residues consisting of aspartate, histidine, and serine. The overall catalytic center in, for example, trypsin, includes an anionic group, the carboxylate of Asp 102 in the resting state, and an additional anionic center, the tetrahedral oxyanion, in the tetrahedral intermediate. These anionic sites interact with neighboring peptide links, an alkoxy function, and bound water molecules, and these interactions are critical to the catalytic activity of the enzyme.

We shall use clustering data to estimate some of the interaction energies between ionic and nearby hydrogen-bonding groups at the active site of trypsin. The relationship of the hydrogen bonds in the cluster model to those in the active site and in the tetrahedral intermediate is illustrated in Figure 4. The structure of the

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(12) Warshel, A. *Acc. Chem. Res.* **1981**, *14*, 284.

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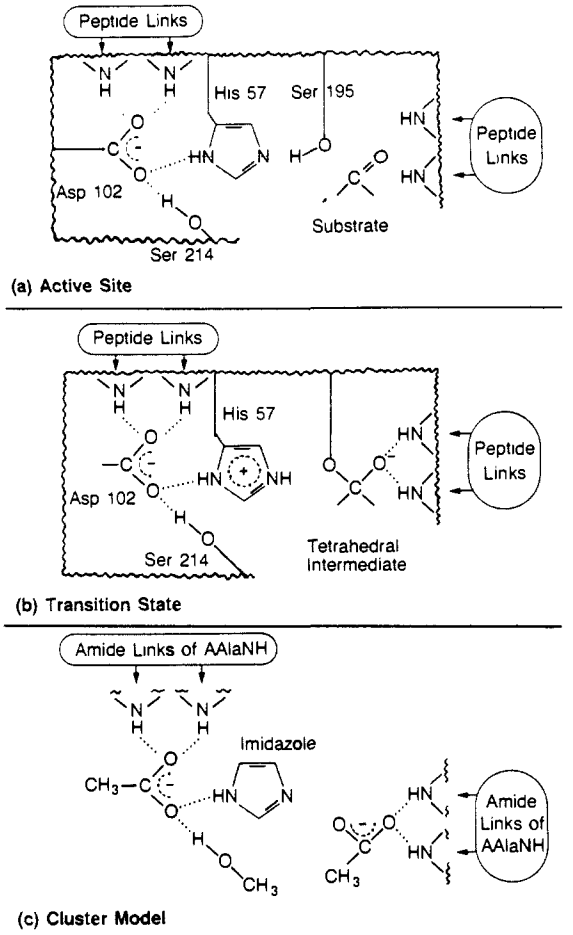

Figure 4. The relationship between the active site of trypsin (a), the tetrahedral intermediate (b), and the cluster model (c). The strengths of the hydrogen bonds to aspartate in (a) and to the tetrahedral oxyanion in (b) are evaluated from measured and estimated strengths of the hydrogen bonds in (c).

transition state is as given by Warshel and Russell.[14] Figure 4c indicates the hydrogen bonds considered but does not imply this geometry in the gas-phase clusters.

Both in the resting state and in the tetrahedral intermediate, Asp 102 is hydrogen bonded to two peptide link amide hydrogens and to the hydroxyl of Ser 214. According to the present results, two carboxylate–peptide NH hydrogen bonds contribute a stabilization energy of about 45 kcal/mol. The interaction between Asp 102 and Ser 214 can be modeled by $CH_3COO^-\cdot CH_3OH$ as 18 kcal/mol. However, the two strong amide ligands will decrease this by a factor of about $0.7 \times 0.7 = 0.5$ to 9 kcal/mol.

In the resting state, the fourth interaction of Asp 102 is with the neutral imidazole group of His 57. By itself, this would be 27.7 kcal/mol, but the other three strong ligands will decrease it by a factor of $0.7^3 = 0.35$ to about 10 kcal/mol.

In relation to the imidazole, Roberts et al. showed that "the tautomer with the hydrogen on N3 predominates, and this is unusual for simple 4-substituted imidazole derivatives and for histidyl residues in proteins. Its predominance in α-lytic proteases was attributed to a hydrogen-bonded interaction between NH in the 3 position and the adjacent buried carboxylate group of aspartic acid."[15] The energetic feasibility of this ion-induced tautomer shift is explained by the present data. We estimated the stabilization gained by the Asp 102–imidazole hydrogen bond, after accounting for the other interactions of the Asp 102 carboxylate, was about 10 kcal/mol. The tautomer shift requires only a few kcal/mol,[16] and this can be still supplied by the hydrogen bond to Asp 102.

Altogether, the estimated solvation energy of the carboxylate group of Asp 102 by its four interactions with the protein environment is therefore about 65 kcal/mol in the rest state of the enzyme. Residual bounded water molecules can increase the solvation energy. In the activated state containing the tetrahedral intermediate, Asp 102 interacts with the cationic, protonated imidazole as in **3**. This ion–ion interaction should be substantial. However, it is moderated by the fact that the imidazole also interacts with the anionic tetrahedral intermediate.

The other anionic species in **3** is the tetrahedral oxyanion formed by the acylation of the substrate. This interacts with two peptide amide hydrogens according to the model of Warshell and Russell.[14] To estimate the hydrogen-bonding energies, the acidity of the tetrahedral oxygen is needed. Judging from the carboxylate-like O–C–O$^-$ arrangement, it may be similar to carboxylic acids. Therefore $\Delta H°_{acid}$ is about 348–355 kcal/mol. According to correlation 2, $\Delta H°_D$ for the O$^-\cdot$HN bond with a peptide link is 26 ± 2 kcal/mol for the first bond and $26 \times 0.7 = 20$ kcal/mol for the second bond. Considering the protonated imidazole as a strong ligand weakens both interactions by a factor of 0.7. Therefore, the total stabilization of the tetrahedral oxyanion by the peptide backbone is about 32 kcal/mol. This can be a major factor in the catalytic effect, as Warshel and Russell suggested.

8. Intrinsic Acidities and a Possible Direct Proteolytic Mechanism. The current mechanism for serine proteases assigns the location of protons without considering the intrinsic acidities and ionic hydrogen-bonding strengths of the participating groups. However, the present values suggest that the lowest energy arrangement of protons may be different. The central point is that the acidity and hydrogen donor strength of a peptide amide hydrogen is significantly greater than that of an alkoxy hydrogen such as that of the serine OH.

In the conventional reaction intermediate (Figure 4b), a proton shift occurred from the serine OH function to the imidazole nitrogen.[15,17] This was followed by nucleophilic attack of the serine O$^-$ group on the amide carbonyl of the substrate peptide link to create the anionic tetrahedral intermediate, and the serine residue was acylated by the substrate. A second proton shift to the amide nitrogen will then cause the dissociation of the peptide bond.

While there is strong evidence for this scheme, the gas-phase results suggest that the peptide NH group of the substrate hydrogen is a better hydrogen donor than the serine. Therefore, it should hydrogen bond to the imidazole nitrogen or transfer a proton to it, in preference to the serine OH group.

Quantitatively, a peptide amide group in the substrate is more acidic by over 20 kcal/mol than an alkoxy group. Considering the aspartate–imidazole complex as an anionic group, correlation 2 suggests that the amide link of the substrate should hydrogen bond to the imidazole nitrogen more strongly by about 6 kcal/mol than the serine OH function does. Therefore, the peptide NH function should displace the serine hydrogen bond (Scheme I, step **b**). This may lead to a charge alternation pattern in the substrate that will activate the peptide link for nucleophylic attack by the serine alkoxy oxygen (step **c**). Alternatively, the strong acidity of the amide may result in proton transfer to the imidazole, leading to an ionic four-center intermediate (step **c′**).

Intermediate **c′** can lead directly to the release of a free amine and the acylated serine, while **c** leads to the same products through an ionic tetrahedral intermediate **d**. All of the intermediates **c**, **c′**, and **d**, as conventional oxyanion in Figure 4b are strongly stabilized by hydrogen bonding to two peptide NH donor links. As we discussed, the interaction energy may be as strong as 32 kcal/mol.

The conventional mechanism and Scheme I **d** → **e** both involve proton transfer from protonated imidazole to the amide nitrogen of the substrate. However, the carbonyl oxygen of amides is a stronger intrinsic base than the nitrogen, so proton transfer should occur favorably to the carbonyl oxygen of the substrate peptide.

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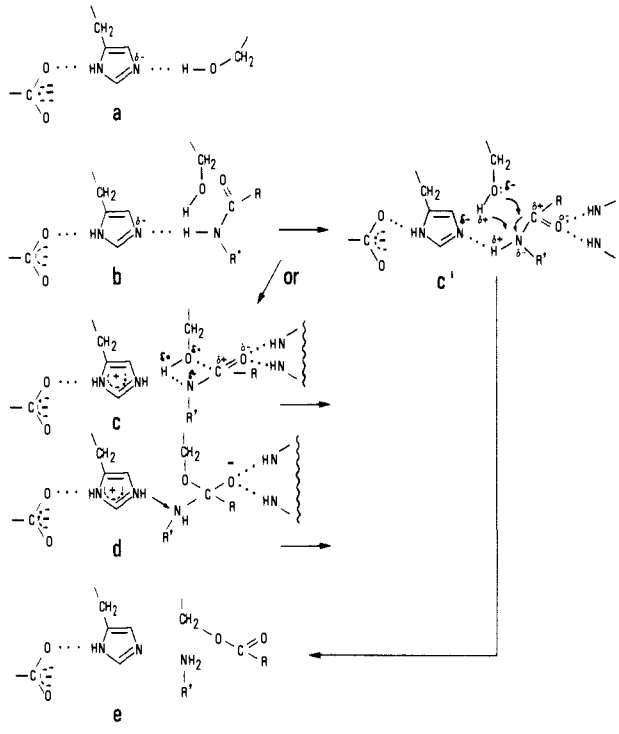
Scheme I



The actual mechanism may involve therefore a complex sequence of proton transfers at this stage.

In summary, the essential features of the suggested mechanism are the negative charge in the aspartate–imidazole complex and the strong intrinsic acidity of the substrate peptide NH. Together, these features lead to a mechanism equivalent to the base-catalyzed hydrolysis of the peptide. This is reasonable since, in essence, COO^- is a strong base and the substrate peptide link is a strong intrinsic acid.

In discussing these details, it is recognized that the lowest energy path may not be available in the enzyme because of steric constraints, and multiple interaction effects may also alter relative energies. In this case, the present lowest energy mechanism may not apply in natural enzymes but may be of interest in synthetic enzymes where lowest energy paths may be realized.

Conclusion

Data from ion clusters directly involving model biomolecules are applied to obtain quantitative estimates of ionic interactions in protein environments. The clustering studies show that protein environments can solvate ionic species substantially. Two amide links of a peptide backbone can stabilize an anion by 45 kcal/mol, and a cation by a similar amount, so that four such peptide units can stabilize a cation–anion pair at infinite separation by 90–110 kcal/mol. Together with further stabilization by nearby ionic and polar groups and water molecules, the protein can therefore provide a strongly stabilizing environment for ionic intermediates.

In the special case of serine proteases, the ionic intermediate involves a carboxylate stabilized by four hydrogen bonds, which may contribute as much as 60 kcal/mol, and a tetrahedral oxyanion intermediate bonded to two peptide backbone NH groups, which can contribute over 30 kcal/mol. These latter interactions are therefore substantial and may indeed be the main catalytic factor as suggested by Warshel and Russell. Further, the present results and considerations of intrinsic acidities suggest an alternative proteolytic mechanism where a substrate amide proton is transferred to the imidazole center of the enzyme.

The present data display the usual correlation between hydrogen bond strength and acidities. The hydrogen donor strength of peptide link in ionic hydrogen bonds is therefore enhanced by its high intrinsic acidity. The ion–neutral bond between aspartate and imidazole in enzyme centers is also enhanced by the high intrinsic acidity of imidazole. Further, we found similarly high intrinsic acidity in another essential hydrogen donor, i.e., aminopyrimidine that models nucleic base components. In addition to its effect in ionic systems, increased intrinsic acidities also strengthen key neutral hydrogen bonds. The intrinsic acidity therefore emerges as a unifying property that may have affected the natural selection of donor molecules for essential biological hydrogen bonds in both ionic and neutral systems.

Acknowledgment. I thank Dr. M. Krauss and Dr. A. Warshel for helpful comments and a critical review of this work.

Time-Resolved Slow Dissociation of Benzonitrile Ions by Trapped-Ion Ion Cyclotron Resonance Photodissociation

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Abstract: Benzonitrile ions were photodissociated, showing loss of HCN, by single pulses of 308-nm light in the ion cyclotron resonance ion trap. The extent of dissociation following the laser pulse was time resolved, giving a typical time constant of 200 μs for dissociation. The time-resolved photodissociation curve was found to follow very well the behavior expected from extrapolation (via RRKM calculations) of previous dissociation rate measurements in the 1–10-μs regime. It was concluded that a statistical, RRKM theoretical description applies to this dissociation process down at least to rates as slow as 10^3 s^{-1}.

Ion-trap methods have made possible the study of chemical processes in isolated molecules on unprecedentedly long time scales. The study of infrared–fluorescent relaxation on a time scale of tens or hundreds of milliseconds is an outstanding achievement of this approach.[1] Recently the time-resolved observation of the dissociation of chlorobenzene ions on a time scale of tens of microseconds in the ion cyclotron resonance (ICR) ion trap was described.[2] We describe here the a time-resolved study of dissociation of benzonitrile ions by the same technique but on a longer time scale. We believe this to be the slowest unimolecular dissociation process whose time profile has ever been displayed for

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