collisional activation will result in ligand detachment rathter than oxidative addition of a CH or CC bond to the π-complexed Fe^+. If this hypothesis holds true, the observation of the m/z 83 ion from the RNC/Fe^+ system would then imply that the binding energy of a *side-on* complexed isonitrile to Fe^+ is larger than that of a nitrile function. Obviously, this tempting suggestion represents a challenge for theoretical chemistry.

A referee has raised the question whether the reactivity of RNC with Fe^+ may be due to an isomerization of the type RNC → RCN, a reaction which has been known for more than a century[17] and which indeed occurs under a variety of conditions.[18] In the present case this possibility can be ruled out on the following ground. The reactivity of $C_nH_{2n+1}CN/Fe^+$ complexes for alkyl nitriles with $3 \leq n \leq 6$ is such that *both* H_2 and C_2H_4 loss occurs upon collisional activation, presumably in competition from a common intermediate.[2,3] This is also borne out by the data shown in Figure 2. In contrast, the RNC/Fe^+ system behaves distinctly different in that with increasing chain length the loss of C_2H_4 does not any longer correspond to the elimination of H_2. It is the latter reaction which dominates the chemistry of the RNC/Fe^+ system while for the isomeric RCN/Fe^+ complexes elimination of both H_2 and C_2H_4 occurs.

Acknowledgment. Financial support of our work by the Deutsche Forschungsgemeinschaft, Fonds der Chemischen Industrie, Stiftung Volkswagenwerk, and Technische Universität Berlin, is gratefully acknowledged.

Registry No. C_2H_5CN, 624-79-3; n-C_3H_7CN, 627-36-1; n-C_4H_9CN, 2769-64-4; n-$C_5H_{11}CN$, 18971-59-0; n-$C_6H_{13}CN$, 15586-23-9; Fe^+, 14067-02-8.

(17) Weith, W. *Ber. Dtsch. Chem. Ges.* **1873**, *6*, 210.
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Models for Strong Interactions in Proteins and Enzymes. 1. Enhanced Acidities of Principal Biological Hydrogen Donors

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Abstract: The acid dissociation energies of several key biological hydrogen donors are found to fall into a narrow range, $\Delta H°_{acid}$ = 352–355 kcal/mol. The strong acidities of these donor groups enhance the hydrogen bond strengths involved in the protein α-helix, imidazole enzyme centers and DNA. Specifically, the peptide link is modeled by the dipeptide analogue CH_3CO-Ala-OCH_3. Its acidity is strengthened, i.e., $\Delta H°_{acid}$ is decreased by 8 kcal/mol compared with other amides, due to electrostatic stabilization by the second carbonyl in the peptide $-CON^-CH(CH_3)CO-$ grouping. The acidity of imidazole is also strengthened by 8 kcal/mol compared with that of the parent molecule, pyrrole, primarily due to resonance stabilization of the ion. Hydrogen donor NH_2 groups of adenine and cytosine are modeled by 4-aminopyrimidine, and the acidity of this amine group is strengthened by ring aza substitution. An intrinsic acidity optimized for hydrogen bonding strength therefore emerges as a common property of the diverse hydrogen donors in the protein α-helix, enzymes and DNA. This property may therefore be in part responsible for the natural selection of these molecules as principal biological hydrogen donors.

Hydrogen bonding plays a central role in the structures and energetics of biopolymers. For example, the α-helix structure of proteins involves hydrogen bonds where the amide NH function serves as a hydrogen donor. Hydrogen bonds in DNA and the interactions of imidazole in enzymes also involve NH·O and NH·N interactions. Zeeger-Huyskens demonstrated that a correlation exists between the strength of hydrogen bonds and the proton affinities of the components (in this case, the proton affinity of N^-, i.e., the acidity of NH).[1] The intrinsic NH acidities of biomolecules are therefore relevant to NH·O and NH·N hydrogen bonds that determine the α-helix structure of proteins and base pairing in DNA.

While the above interactions involve neutral molecules, hydrogen bonds involving ions are also relevant to biological systems. Intermediates in enzyme reactions go through ionic transition states. These transition states are stabilized by hydrogen bonding to polar groups in the environment.[2] The strengths of these ionic hydrogen bonds are in turn related to the acidity difference $\Delta\Delta H°_{acid}$ of the components.[3-9] These interactions take place in protein interiors from which the solvent is partially or fully excluded and therefore can be modeled by gas-phase ionic complexes.

This and the following paper[3] will present gas-phase studies relevant to hydrogen bonding of biomolecules. The first paper will deal with intrinsic acidities, which are related to the hydrogen donor strengths in both neutral and ionic hydrogen bonds. The second paper will deal with the strength of ionic interactions of

(1) Zeeger-Huyskens, T. *J. Mol. Struct.* **1986**, *135*, 93 (*THEOCHEM* **1986**, *28*, 93). Hydrogen bond strengths are shown to be inversely correlated to the difference in the proton affinities of the components. For cationic complexes AH^+·B, the relevant parameters are the relative PAs of the neutral, deprotonated species A and B. For neutral complexes AH·B, the relevant parameters are similarly the proton affinities of the deprotonated components, i.e., PA(B) and PA(A^-), where the latter is equal to $\Delta H°_{acid}$(AH). For neutral hydrogen bonds, the correlation between PA(A^-) - PA(B) and $\Delta H°_D$ shows a slope of about 0.1.
(2) Warshel, A. *Acc. Chem. Res.* **1981**, *14*, 284.
(3) Meot-Ner (Mautner), M. *J. Am. Chem. Soc.*, following paper in this issue.
(4) Meot-Ner (Mautner), M. *J. Am. Chem. Soc.* **1984**, *106*, 1257.
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model molecules and relate these to aspects of bioenergetics of enzymes.

Proteins and peptides are too involatile for gas-phase measurements. Fortunately, the amino acid derivative CH_3CO-Ala-OCH_3, i.e., $CH_3CONHCH(CH_3)COOCH_3$ (also denoted below as AAlaNH), is sufficiently volatile for these purposes. The important feature of this compound is that it contains the peptide-like –CONHCHRCO– grouping. This model compound was used successfully previously in studies on cationic hydrogen bonds of peptide-like molecules.[10] Imidazole and 4-methylimidazole will be models for the imidazole moiety of histidine, and 4-aminopyrimidine will be the model for hydrogen donor NH_2 groups of nucleic bases. This work will present a ladder of gas-phase acidities involving these model biomolecules.

Experimental Section

The experiments were done on the NBS pulsed high-pressure mass spectrometer.[11] The components of interest are introduced in a gas mixture to the ion source. An ionizing electron pulse is followed by a sequence of ion–molecule reactions, which lead to equilibrium in the proton transfer or association reactions. Temperature studies in the range 100 to 670 K are used to determine reaction enthalpies and entropies. The method was described in detail elsewhere.[11]

The carrier gas in the present experiments was CH_4. Anions were generated by adding up to 5% N_2O. Electron capture yields O^- which reacts with CH_4 to give OH^-, which in turn reacts with the sample AH or with the reference compound BH to give A^- or B^-. Apparently, one step in this sequence has a positive activation energy, since we found that the yield of ions by this method decreases substantially below about 180 °C. The signal also decreases at high temperatures, where an ion of *m/e* 42, presumably CNO^-, becomes dominant.

An alternative method to generate ions was to add trace amounts of CH_3ONO to the gas mixture. In this method electron capture yields CH_3O^- which reacts to give A^- and B^-. CH_3ONO was synthesized by mixing about 1 mL of CH_3OH and *tert*-amyl nitrite in a vial.[12] About 1 mL of the head-space vapor was introduced to a 3-L bulb which was heated to 180 °C and which contained about 100–500 Torr of the gas mixture. The amides and reference compounds were added to the bulb with a syringe and constituted 0.01% to 1% of the reaction mixture.

The mixture was allowed to flow to the ion source through stainless steel tubing heated to 200 °C. Low-volatility compounds such as CH_3CO-Ala-OCH_3 and azoles may become absorbed on the walls of the stainless steel or glass flow lines, similar to absorption in a gas chromatograph column. With some of these compounds, it was necessary to allow the mixture to flow up to 30–60 min until the apparent equilibrium ion ratio did not change with further flow time, which suggested that the sample concentration in the ion source reached the nominal value. As a check, rate constants for exothermic reactions involving low-volatility components were measured. The apparent value, calculated by using the nominal concentration, always reached the expected collision rate, which showed that the nominal and real concentrations were equal. Further, the usual checks were also performed to verify that the equilibrium constant did not vary with mixture composition and with total gas pressure in the range 0.8–2.4 Torr.

The reagents used were obtained from commercial sources, were of nominal purities >98%, and were used as purchased.

Results

The gas-phase acidities of the compounds AH relative to reference compounds BH were measured by proton transfer equilibria (reaction 1).

$$B^- + AH \rightleftarrows A^- + BH \qquad (1)$$

Van't Hoff plots for the temperature studies are shown in Figures 1 and 2, and the results are summarized in Figure 3.

The gas-phase acidity of a compound is defined as $\Delta G°_{acid}(AH) = \Delta G°_D(A^-–H^+)$. The related enthalpy change is $\Delta H°_{acid}(AH) = \Delta H°_D(A^-–H^+)$, which is equal to the proton affinity of A^-. Stabilization of A^- lowers the acid dissociation energy, and therefore lower $\Delta H°_{acid}$ values represent strengthened acidity.

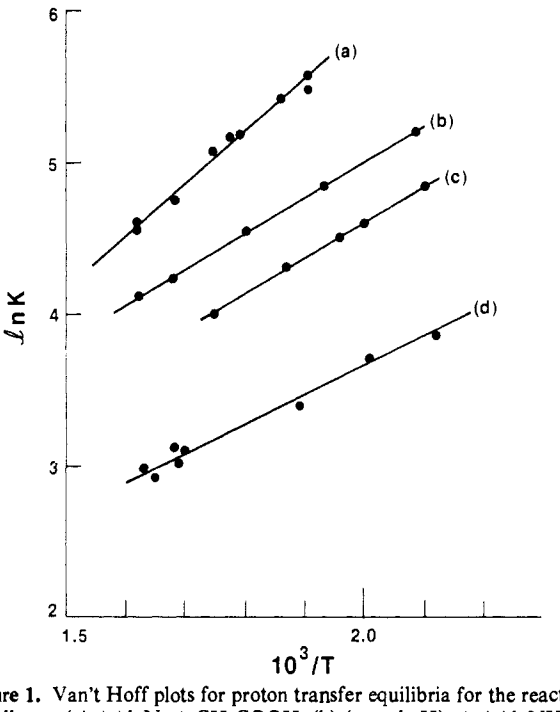

Figure 1. Van't Hoff plots for proton transfer equilibria for the reactants as follows: (a) $AAlaN^- + CH_3COOH$; (b) $(pyrrole\text{-}H)^- + AAlaNH$; (c) $CH_2CHO^- + CH_3CONHCH_3$; (d) $CH_3CONCH_3^- +$ pyrrole.

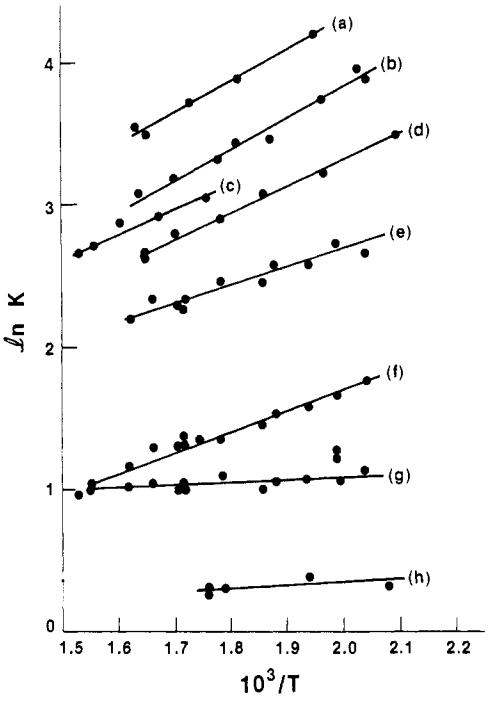

Figure 2. Van't Hoff plots for proton transfer equilbria for the reactants as follows: (a) $imidazoleH^+ + (C_2H_5)_2NH$; (b) $(pyrrole\text{-}H)^-$ + pyrazole; (c) $(imidazole\text{-}H)^- + CH_3COOH$; (d) $(pyrazole\text{-}H)^- + HCN$; (e) $CF_3COCH_2^- + CH_3COOH$; (f) $(indole\text{-}H)^- + CH_3COOH$; (g) $CF_3COCH_2^-$ + indole; (h) CN^- + imidazole.

Temperature studies are desirable because $\Delta H°_{acid}$ values can be obtained from single-temperature measurements only by the use of estimated or calculated entropies, and structural information is not always available for such calculations. In the present study temperature studies were performed to create an interlocking ladder of $\Delta H°_{acid}$ values (Figure 3). From the consistency of the ladder, the accuracy of the relative $\Delta H°_{acid}$ values is estimated as ±1 kcal/mol.

Because of decomposition or low vapor pressures, temperature studies were not performed on $HCONH_2$, $HCONHCH_3$, 4-

(10) Meot-Ner (Mautner), M. *J. Am. Chem. Soc.* **1984**, *106*, 278.
(11) Sieck, L. W. *J. Phys. Chem.* **1985**, *89*, 5552.
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(13) (a) Bartmess, J. E.; Scott, J. A.; McIver, R. T. *J. Am. Chem. Soc.* **1979**, *101*, 6047. (b) Bartmess, J. E. *J. Phys. Chem. Ref. Data*, 1987, in press (compilation of anion thermochemistry).

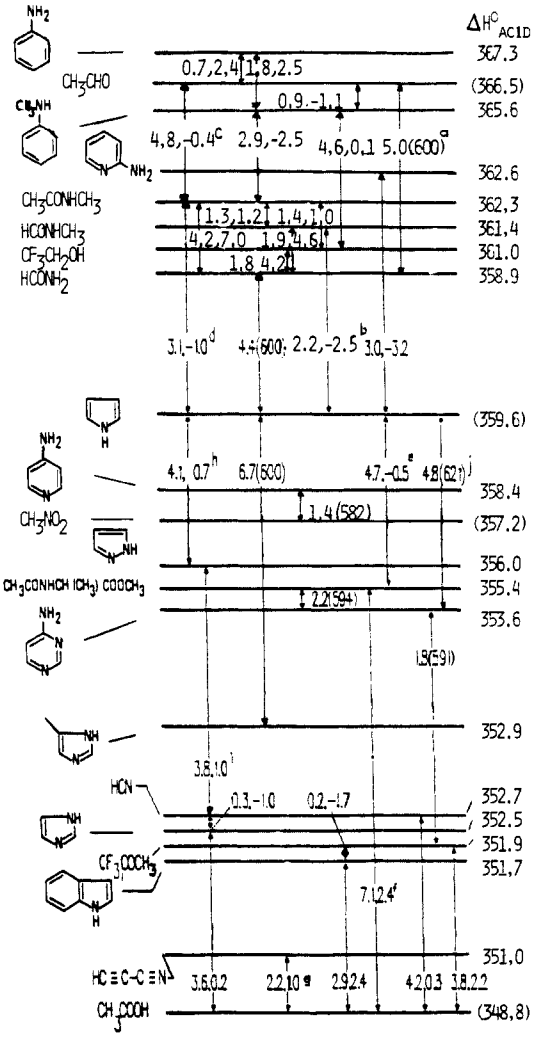


Figure 3. Ladder of gas-phase acidities. Numbers in arrows are $\Delta H°$ (kcal/mol) and $\Delta S°$ (cal/(mol·K)) for $A^- + BH \rightarrow B^- + AH$, where AH is the bottom compound and BH is the top compound connected by the arrow. Numbers followed by temperature in parentheses are $\Delta G°$ measured at a single temperature. Absolute $\Delta H°_{acid}$ values are in reference to values shown in parentheses from ref 13b. Rate constants for some of the exothermic reactions, and the temperature at which they are measured, are as follows ($\times 10^{-9}$ cm^3 s^{-1}, K): (a) 1.2 (600); (b) 2.2 (600); (c) 1.2 (600) and 1.2 (480); (d) 1.1 (600) and 1.1 (480); (e) 4.0 (480); (f) 1.8 (620) and 1.8 (540); (g) 0.7 (650) and 0.7 (600); (h) 1.0 (626) and 1.0 (487); (i) 2.0 (600) and 2.0 (490); (j) 2.4 (621).

methylimidazole, and 4-aminopyrimidine. For single-temperature equilibrium measurements $\Delta S°$ is often calculated from rotational symmetry changes, but the results in Figure 3 show that other factors are also significant and the entropy of rotational symmetry is not a good approximation. Where measured, the observed entropy changes were small, and for single-temperature measurements we assume $\Delta H° \cong \Delta G°$.

The accuracies of the absolute $\Delta H°_{acid}$ values depend on those of the reference compounds. Here we use as reference $\Delta H°_{acid}(CH_3COOH) = 348.5 \pm 2$ kcal/mol from the tabulations of Bartmess.[3] Standard deviations of the slopes of van't Hoff plots and reproducibility in replicate measurements suggest an error of ±0.5 kcal/mol for relative $\Delta H°_{acid}$ values. The error estimates for relative $\Delta G°_{acid}$ values are ±0.5 kcal/mol and for $\Delta S°_{acid}$ ±1 cal/(mol·K). The $\Delta H°_{acid}$ values for imidazole and pyrazole are in good agreement with recent ion cyclotron resonance values obtained by Taft et al.[14]

The ladder in Figure 3 contains six reactions which include the transition $CH_3COOH \rightarrow CH_3COO^-$. This entails the loss of an OH rotor, and Cumming and Kebarle[15] calculated $\Delta S° = -4.4$ cal/(mol·K) for this half-reaction. However, after accounting for $\Delta S°_{rotational\ symmetry}$ of the other reactants in these equilibria, the average $\Delta S°$ measured for the $CH_3COOH \rightarrow CH_3COO^-$ half-reaction is only -1.2 ± 1 cal/(mol·K). Of particular relevance are the reactions with HCN and HCCCN which show small $\Delta S°$ values, and which involve linear reactants whose entropy of deprotonation also should be small.

Rate constants for some of the proton transfer reactions were obtained from the approach to equilibrium using standard reversible kinetics. The results are shown in Figure 3. The conditions were not optimized for kinetic measurements. For example, the approach to equilibrium was sometimes too fast for an accurate rate constant determination. The rate constants are accurate therefore only within a factor of 2. Within these limits all the reactions proceed near the collision rate of about 10^{-9} cm^3 s^{-1}.

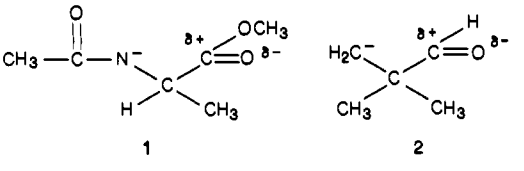
Reaction 9 shows an exceptionally large rate constant. This may be due to the large size of the reactant, $CH_3CONHCH(CH_3)COOCH_3$. It may be expected that unusually fast reactions could occur when the combined radii of the reactants exceeds the capture collision radius. The further study of reactions of large ions and molecules is of interest.

Discussion

1. Acidities of Amides and the Peptide Link. Methyl substitution affects gas-phase acidities in two opposing ways. In some saturated compounds, such as CH_3OH vs H_2O, the increased polarizability increases the stability of the ion, resulting in strengthened acidity.[13] On the other hand, in many unsaturated compounds, such as CH_3CCH vs HCCH and CH_3COOH vs HCOOH, the electron donating ability of the methyl group decreases the stability of the ion, resulting in weakened acidity.[15] In the amides, methyl substitution in going from $HCONH_2$ to $HCONHCH_3$ and $CH_3CONHCH_3$ has a small effect, decreasing the acidity by about 2 kcal/mol on each step.

The observation of main interest is that in contrast to methylation, the addition of a carbonyl has a major effect on the acidity. This is expected when acylation is directly on the nitrogen, as in proceeding from NH_3 ($\Delta H°_{acid} = 404.2$) to CH_3CONH_2 ($\Delta H°_{acid}$ estimated as 364 kcal/mol from the other amides) to $CH_3CONHCOCH_3$ (346.7 kcal/mol).[15] In the latter, the deprotonated form is stabilized by delocalization of the negative charge over the two carbonyls and the bridging N^- center. In contrast, in the $CH_3CON^-CH(CH_3)COOCH_3$ the intervening methylene group allows delocalization involving only one carbonyl.

It is surprising therefore that a significant strengthening in acidity is observed nevertheless from $CH_3CONHCH_3$ to $CH_3CONHCH(CH_3)COOCH_3$. The substantial stabilizing effect of 6.9 kcal/mol could be due to the through-bond electrostatic interaction of the anionic moiety with bond dipoles of the $C(O)OCH_3$ substituent, which are oriented favorably in **1**. The conformation of **1** is similar to the ion $CH_2(CH_3)_2CCHO^-$ (**2**). In the latter Noest and Nibbering found that carbonyl substitution strengthened the acidity, compared with CH_4, over 20 kcal/mol, and electrostatic calculations showed a charge–dipole interaction of 21 kcal/mol.[16] In the deprotonated peptide the substituent dipole interacts with a more delocalized charge and the substituent effect is smaller.



1 **2**

A more stable conformation may be in the five-membered ring involving the enolic resonance form as in ion **3**. This is similar to the intermediate in nucleophilic displacement reactions on

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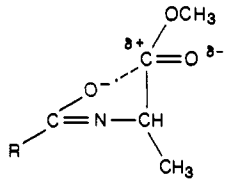
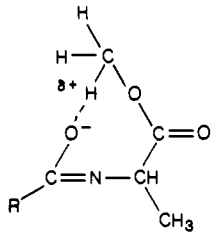
carbonyls. Another possibility might be through-space electrostatic interactions such as in ion **4**.



3



4

Ion **4** involves a $CH^{\delta+}\cdots O^-$ interaction. This can be viewed as a specific hydrogen bond or, more likely, an electrostatic interaction with the partial positive charge of one or several hydrogens on the methyl group. Such $CH^{\delta+}\cdots X^-$ bonds can be as strong as 12–15 kcal/mol.[17,18] However, constrained structures such as **3** and **4** usually also involve a significant negative entropy of 5–15 cal/(mol·K),[10,19] but proton transfer reactions of AAlaNH in Figure 3 show no such changes. Therefore, the stabilizing effect is most likely due to charge–bond dipole interactions as in ion **1**. In any event, the acidity of the amide NH group of the peptide link is significantly strengthened compared with that of a regular amide function.

2. The Deprotonation Site of the Peptide Link. The peptide link in general and in the model compound AAlaNH contains a possible strong carbon acid center at the methylene unit which is adjacent to electron-withdrawing groups. Some carbon acids such as $CH_3COCH_2COCH_3$ are more acidic than amides,[13] and therefore it is conceivable that the molecule and peptide links in general are carbon rather than nitrogen acids. It is desirable therefore that the acidic site of the peptide link is established.

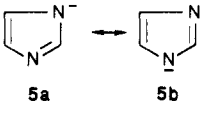
Protonation or deprotonation sites at carbon vs oxygen or nitrogen centers can be established through the clustering properties of the ions. This is a useful test since carbon is a weaker hydrogen bonding center than oxygen or nitrogen. For example, clustering was used as a test for the ring protonation of aniline[20] and pyrrole.[21]

The bonding energies in $AAlaN^-\cdot H_2O$ and $AAlaN^-\cdot CH_3OH$ are 15.2 and 18 kcal/mol, respectively.[3] If deprotonation occured on carbon in AAlaNH, these complexes would involve $C^-\cdot HO$ bonds, and if on nitrogen, then $N^-\cdot HO$ bonds. The experimental values can be compared with expected bonding energies for either type of hydrogen bond.

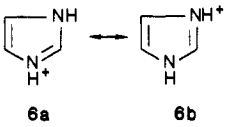
We investigated recently complexes of carbanions such as HC_2^- and $C_5H_5^-$, as well of nitrogen based anions such as pyrrolide, with hydrogen donors.[22] $\Delta H°_D$ vs $\Delta\Delta H°_{acid}$ relations from these studies predict 15.4 and 17.6 kcal/mol for $AAlaN^-$ bonded to H_2O and CH_3OH, respectively, through $N^-\cdot HO$ bonds. These are in good agreement with the experimental values. In contrast, the correlation for $C^-\cdot HO$ bonds would predict 9.8 and 13.6 kcal/mol, respectively, for these complexes, similar to, for example, the bonding energies of carbanion complexes such as $c\text{-}C_5H_5^-\cdot H_2O$, where $\Delta H°_D$ = 11.0 kcal/mol.[22] However, the present values are significantly higher than these values. Moreover, bonding strengths in $AAlaN^-$·pyrrole and $AAlaN^-$·AAlaNH are also consistent with $N^-\cdot HN$ rather than $C^-\cdot HN$ bonds.[3]

The clustering energies cannot differentiate between $N^-\cdot HO$ bonds and $O^-\cdot HO$ bonds that would be formed with the enolic resonance form of the ion, since these lie on the same $\Delta H°_{acid}$ vs $\Delta\Delta H°_D$ correlation line.[3] It is possible that the two forms are close in energy and may be in equilibrium in the reaction mixture. In any event, they both involve the ion generated by deprotonation on nitrogen. Therefore, the hydrogen bonding energies identify AAlaNH and probably peptide links in general as nitrogen bases.

3. The Acidities of Azoles. The acidities of the azoles may be compared to that of the parent compound, pyrrole, $\Delta H°_{acid}$ = 359.6 kcal/mol. In comparison, $\Delta H°_{acid}$ of imidazole is lowered, i.e., the acidity is strengthened by 7.1 kcal/mol. The strengthened acidity may be attributed in part to the electronegative aza substitution and to the resonance stabilization of the anion by the equivalent structures **5a** ↔ **5b**. The latter is similar to the case of the cation that can also be stabilized by two equivalent resonance structures **6a** ↔ **6b**.



5a **5b**



6a **6b**

In relation to this, we remeasured the proton affinity of imidazole by two proton transfer equilibria (reactions 2 and 3), one involving a temperature study.

$$(C_2H_5)_2NH_2^+ + \text{imidazole} \rightleftarrows \text{imidazoleH}^+ + (C_2H_5)_2NH \quad (2)$$

$$\Delta H° = 4.3 \text{ kcal/mol}, \ \Delta S° = 0.1 \text{ cal/(mol·K)}$$

$$c\text{-}C_6H_{11}NH_3^+ + \text{imidazole} \rightleftarrows \text{imidazoleH}^+ + c\text{-}C_6H_{11}NH_2 \quad (3)$$

$$\Delta G°(465) = -2.2 \text{ kcal/mol}$$

$$(\Delta S°_{\text{rot. sym.}} = 0.8 \text{ cal/(mol·K)}), \qquad \Delta H° = -1.8 \text{ kcal/mol}$$

The new results define PA(imidazole) as 222.3 ± 0.7 kcal/mol, in excellent agreement with 222.1 kcal/mol from the previous measurements.[23] The proton affinity of imidazole is higher than that of pyrrole by 8 kcal/mol. Therefore, the overall stabilizing effect on the two resonance forms seems to be similar in the anion and in the cation.

In relation to histidine, the effect of alkyl substitution on carbon 4 is of interest. The electron-releasing effect of the 4-methyl group weakens the acidity by 1.4 kcal/mol. However, polarization of larger alkyl groups may strengthen the acidity by stabilizing the charge in the anion, as in carboxylic acids.[15] Therefore, the gas-phase acidity of the imidazole group in a histidine residue is probably similar to that of imidazole itself.

Resonance stabilization of the charge is possible also in deprotonated pyrazole as in **7a** ↔ **7b**.

7a **7b**

However, here repulsion between the adjacent lone pairs is destabilizing. Consequently, the acidity of pyrazole is weakened by 3.5 kcal/mol compared with imidazole. The various factors that affect the acidities of imidazole and pyrazole were analyzed in greater detail by Taft et al.[14]

The effect of a fused ring strengthens the acidity of indole vs pyrrole by 7.9 kcal/mol. The nitrogen lone pair in $(\text{indole-H})^-$ cannot couple to the fused aromatic ring because of symmetry, and the stronger acidity may be assigned to charge stabilization by increased polarization. In comparison, we found that the effects of fused rings in other systems are the following:[17] benzene ($\Delta H°_{acid}$ = 400.7 kcal/mol) vs naphthalene (394.2 kcal/mol);[6] pyridine (391.0 kcal/mol) vs quinoline (385.6 kcal/mol). The strengthening of acidities by the fused rings is roughly similar, 4–8 kcal/mol, although the electronic structures are different. This suggests that the effect of the fused rings is mostly nonspecific, electrostatic in nature.

In summary, the acidities of azoles vary over a range of about 8 kcal/mol compared with the parent compound pyrrole, due to

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(21) Meot-Ner (Mautner), M.; Ross, M. M.; Campana, J. E. *J. Am. Chem. Soc.* **1985**, *107*, 4835.
(22) Meot-Ner (Mautner), M. *J. Am. Chem. Soc.*, submitted for publication.
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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–