The Nonexistence of Specially Stabilized Hydrogen Bonds in Enzymes

Steve Scheiner* and Tapas Kar

Contribution from the Department of Chemistry, Southern Illinois University, Carbondale, Illinois 62901

Received February 16, 1995[⊗]

Abstract: Ab initio calculations are used to test a recent suggestion that enzymic catalysis can be aided by strengthening of a hydrogen bond in a key intermediate, occurring when this bond is shortened and the pK_a's of the two groups are equalized. The requisite amount of energy is not available in electrically neutral H-bonds; no additional strengthening can be accomplished by shortening such a bond. Interaction energies where one subunit is charged, on the other hand, can be very high. These bonds are intrinsically very short, and the proton transfer profile contains a very low energy barrier. There is no special stabilization associated with the disappearance of the transfer barrier or equalization of the pK_a's.

Introduction

The notion that the transition state or reaction intermediate of a substrate is more tightly bound by an enzyme than the reactants occupies a prominent place in our understanding of catalytic activity. There have been recent suggestions[1−4] that enhanced binding may occur when hydrogen bonds are strengthened in the reaction intermediate as a result of a shortening of the distance between the proton donor and acceptor, coupled to an equalization of their proton affinities. The proton transfer potentials in such short H-bonds are presumed to contain a very low barrier, or none at all, permitting essentially free motion of the proton between the donor and acceptor atoms. It is proposed in this "low barrier hydrogen bond (LBHB)" hypothesis[3,4] that a H-bond between enzyme and substrate may be initially weak due to a mismatch in the pK_a of the donor and acceptor. Factors that equalize the pK_a's in the transition state permit the strengthening of the H-bond by some 10−20 kcal/mol; this energy then becomes available to speed up the reaction.

This suggestion has encountered opposing viewpoints in the literature. Guthrie and Kluger,[5] for example, argue in the general and specific case of mandelate racemase that electrostatic stabilization of the enolate could supply the requisite energy to allow rapid reactions of carboxylic acids as carbon acids, without recourse to any particularly strong H-bonds. They invoke the low polarity of the environment within enzyme interiors[6,7] which would enhance the electrostatic interactions, as compared to aqueous solution.

As the LBHB idea is capable in principle of offering valuable insights into the detailed mechanism of various enzymes, it is important to examine its validity. X-ray crystal structures can be used as evidence that certain H-bonds are very short, as discussed by Cleland and Kreevoy.[3] With regard to the presence or absence of an energy barrier, proton peaks in the NMR spectra of certain short H-bonds in proteins are characteristic of a low or nonexistent barrier.[4] But neither of these types of measurements can directly assess the energetic aspects of the transition from a long to a very short H-bond, which is at the heart of any catalytic activity. Indeed, the literature of experimentally determined H-bond energies is rather sparse.[8]

One means of directly addressing the energetics is via ab initio molecular orbital methods which can be competitive in accuracy and reliability with experimental measurements. Precise control can be exerted over the length of the H-bond examined and the height of the barrier to proton transfer can be calculated, allowing all facets of the LBHB hypothesis to be critically examined. So as to be as complete as possible in our coverage, a number of different systems are considered here, with a variety of different attributes. Two of the complexes are uncharged, containing a pair of neutral molecules; transfer of a proton in a neutral AH··B system leads to an ion pair, $A^-\cdots{}^+HB$. The other principal type of H-bond pairs an ion with a neutral molecule. The latter interaction is considerably stronger than neutral H-bonds. The transfer of a proton does not cause any fundamental difference between reactants and products since $AH^+\cdots B$ and $A\cdots{}^+HB$ are similar in nature. With regard to the atoms involved in the H-bonds, we have examined a broad spectrum. OH···N and OH···O are representative of the typical types of bonds encountered in proteins; also examined for purposes of diversity is the less orthodox IH···N.

In each case below, the two chief precepts of the suggestion are examined in detail. The length of the H-bond is progressively shortened and the proton affinities of the two partners are varied with respect to one another, so as to determine how each of these two factors influences the nature of the proton transfer potential and, more importantly, the strength of the H-bond. These pK variations are accomplished by various means which include the strength of interaction with the environment or angular aspects of the H-bond connecting the two groups, or by simply allowing the chemical nature of the partners to control the process.

[⊗] Abstract published in *Advance ACS Abstracts,* June 1, 1995.

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0002-7863/95/1517-6970$09.00/0

Methods

Calculations were carried out with the ab initio GAUSSIAN-92 code.[9] The 6-31G* basis set was used for the carboxyl−imine system and 6-31G** for carbonyl−hydroxyl. The latter basis was used also for the N and H atoms of ($H_3N\cdot\cdot HI$), with I represented by ECP+DZ+d.[10-12] All geometries were fully optimized, subject only to the restrictions indicated. Correlation effects were included via second-order Møller−Plesset (MP2).[13,14] The interaction with the dielectric continuum in the first system is modeled by the self-consistent reaction field (SCRF) method,[15] which depends upon the dielectric constant ϵ chosen to represent the surroundings.

Results

Carboxyl−Imine. The first H-bond examined connects a carboxyl oxygen atom to the nitrogen of an imine. The carboxyl group is a particularly important one in protein function as Asp and Glu residues are common components of enzymatic reactions; the imine is less common but plays an essential part as the Schiff base in the functioning of visual proteins such as rhodopsin.[16] The carboxyl group is placed within the context of formic acid and the C=N double bond of the Schiff base is modeled by methyleneimine. The proton transfer in $HCOOH\cdots NHCH_2$ transforms a pair of neutral molecules to the ion pair, $HCOO^-\cdots {}^+HNHCH_2$.

The uppermost curve in Figure 1a illustrates the proton transfer potential computed for this system in the absence of any external influences or solvent and for a H-bond length $R(O\cdot\cdot N)$ = 3.25 Å. At this relatively long separation, the potential contains two wells, corresponding to the neutral and ion pairs, respectively, with a relatively low barrier between them. It is important to note that the neutral pair is much more stable than the ion pair, by some 50 kcal/mol, due to the difficulty in attaining the charge separation intrinsic to the latter. From the depth of the left well in the profile, the neutral pair may be seen to be more stable by 9 kcal/mol than a pair of isolated HCOOH and $NHCH_2$ molecules, so the latter quantity corresponds to the H-bond energy.

Of course, the situation wherein a H-bond occurs within a protein is quite different than the fully isolated setting to which the uppermost curve of Figure 1a corresponds. The ability of the surrounding atoms to interact with the charge distribution within the H-bonded system may be modeled to a first approximation by immersing the system in a dielectric medium via the self-consistent reaction field (SCRF) formalism.[15] This approach permits the surrounding medium to interact with and stabilize the electric field generated by the H-bond. Since the stabilization can be expected to rise as the charge separation within the H-bond increases, it is not surprising to note from Figure 1 that the ion pair on the right side is stabilized much more by the progressively increasing values of dielectric constant ϵ than is the neutral pair on the left.[17]

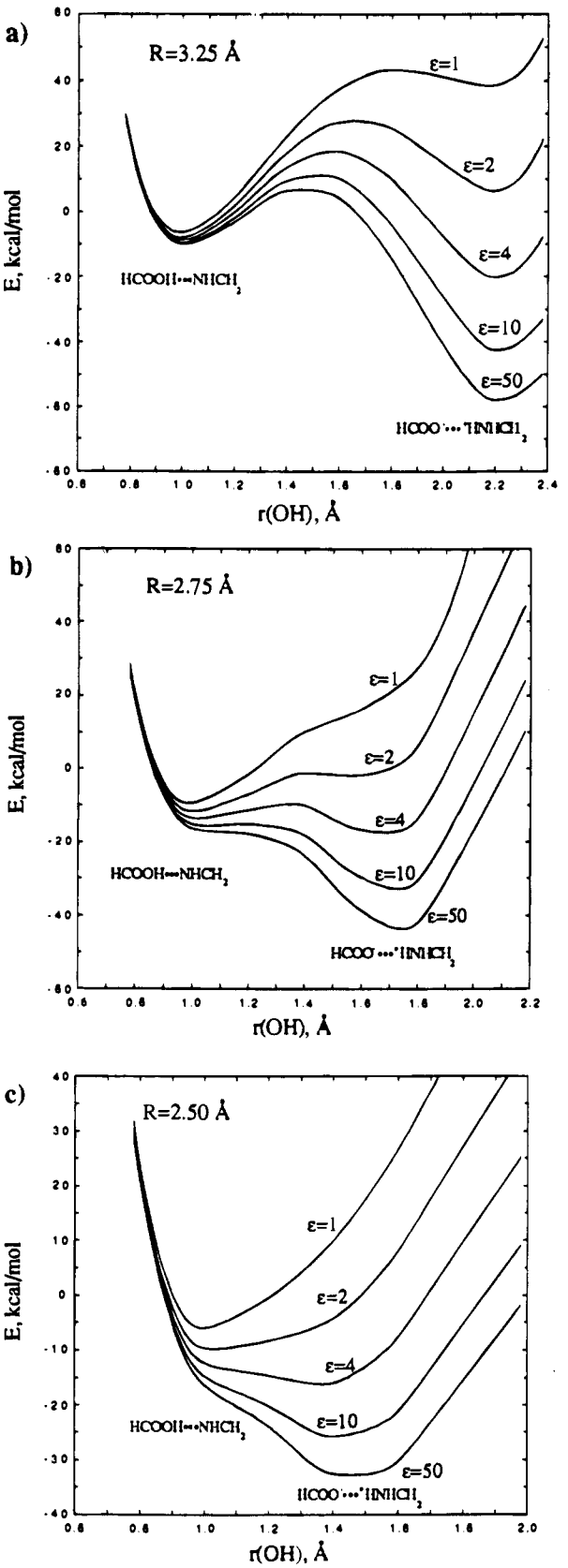

Figure 1. Energies computed for proton transfer between formic acid and methyleneimine for various H-bond lengths. The geometries of all configurations were fully optimized at the SCF level, subject only to the restriction of the chosen R(ON) length, after which correlation was added via MP2. ϵ refers to the dielectric constant of the continuum surrounding the system. The zero of energy for each curve corresponds to the total energy of the optimized isolated HCOOH and $NHCH_2$ molecules, within a dielectric medium of the same ϵ.

For large values of the dielectric constant, the interaction with the medium makes the ion pair more stable than the neutral

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pair. Perhaps most relevant to the discussion here, a point is reached (at approximately $\epsilon = 4$) where the two minima are equal in energy. This point corresponds most closely to the equal pK_a's that are proposed to lead to dramatic strengthening of the H-bond. It is also worthy of note that other studies have placed the average dielectric constant in the protein interior in the 2−4 range;[18] that of water is much higher. One can hence expect the neutral pair to be preferred in the context of a typical protein interior and the ion pair to predominate in aqueous solution. Other than the expected stabilization of the ion pair by the dielectric medium, which continues unabated for larger values of ϵ, beyond the point where the energies of the two minima are equalized, there is no particular strengthening observed.[19]

In addition to the equalization of proton affinity, the second tenet of the original proposal mandates a shortening of the H-bond, to the point where the proton transfer barrier vanishes. Clearly, the transfer barrier remains for all values of ϵ in Figure 1a for which $R = 3.25$ Å. Similar transfer profiles, obtained for a shorter R(N··O) of 2.75 Å, are illustrated in Figure 1b. Again, the neutral pair on the left is preferred for small ϵ, and the preference switches to the ion pair as ϵ surpasses 4. At this particular point, where the two minima are about equal in energy, the barrier is quite small, only some 5 kcal/mol. But again, there is no particular extra stabilization added to the system when the energies of the two minima equalize. The barrier vanishes for larger values of ϵ, where the ion pair is preferred, but the stabilization is due entirely to the greater interaction of the ion pair with the surrounding medium, not to any dramatic change in the transfer profile.

The H-bond is further contracted to the very short distance of only 2.5 Å in Figure 1c. In this case, the barrier is absent in the profile for each value of ϵ, leaving a single-well potential. The position of the minimum reveals a smooth shift from neutral to ion pair as the dielectric constant increases. But again the strength of the H-bond undergoes no drastic increase at any point in Figure 1c, and the value computed for $\epsilon = 4$ is simply intermediate between those obtained for smaller and larger dielectric constants.

It appears then that attaining a point where the two wells in the potential are equally stable, i.e. equalizing the pK_a's of the donor and acceptor group, offers no particular stabilization of the system. This is true regardless of whether the potential contains a barrier to proton transfer or not, at long or very short H-bond lengths. Nor is the H-bond energy necessarily enhanced when the H-bond becomes very short; indeed the opposite is observed, in contrast to the suppositions of the LBHB hypothesis. For example, the H-bond energy of the ion pair, with $\epsilon = 4$, is 22 kcal/mol when $R = 3.25$ Å, is 18 kcal/mol when R is reduced to 2.75 Å, and is reduced further to 16 kcal/mol when the H-bond is shortened to 2.5 Å, where the transfer barrier vanishes. Considering the neutral pair with unit dielectric constant, the H-bond energy is in the range of 8−11 kcal/mol for $2.5 < R < 3.25$ Å, and it is in fact smallest for the shortest H-bond length.

Carbonyl−Hydroxyl. We turn our attention now to a very different sort of system, ($H_2CO\cdot\cdot H^+\cdot\cdot OH_2$), where the carbonyl and hydroxyl oxygen atoms compete for the bridging proton. This H-bonded complex differs from the earlier example first in that two O atoms participate. A second difference is that the system is charged overall as compared to $HCOOH\cdots NHCH_2$ which does not contain a net charge. The ($H_2COH^+\cdot\cdot OH_2$) → ($H_2CO\cdot\cdot{}^+HOH_2$) transfer simply moves the net positive charge from one subunit to its partner so immersion in a dielectric continuum would have little influence upon the relative energies of the two configurations of ($H_2CO\cdot\cdot H^+\cdot\cdot OH_2$).

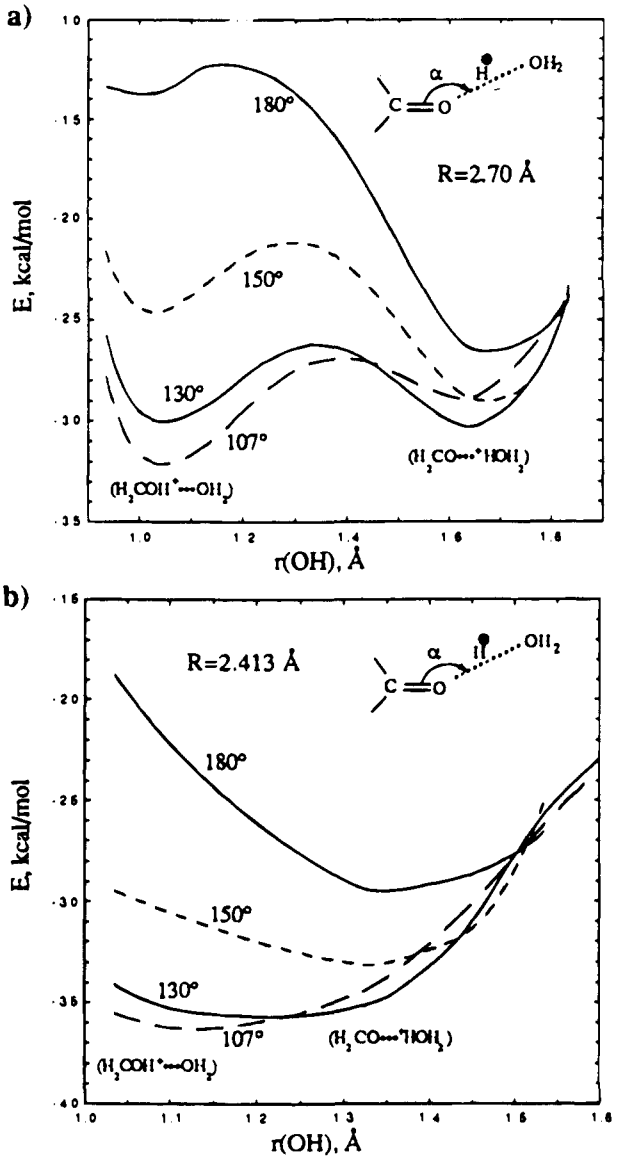

Figure 2. MP2/6-31G** proton transfer profiles computed for proton transfer in ($H_2CO\cdot\cdot H^+\cdot\cdot OH_2$). Geometries of all configurations were optimized at the MP2 level, subject only to the restriction of the chosen R(O··O) and angle α. The zero of energy for each curve corresponds to the total energy of the optimized isolated H_2CO and OH_3^+. (a) R(O··O) = 2.70 Å; (b) R(O··O) = 2.413 Å, the H-bond length in the global minimum.

Of particular importance here are the angular aspects of the H-bond. Crystal structure surveys of H-bonds in proteins demonstrate that proton donors can approach the carbonyl oxygen from a wide range of directions between the two lone pairs of this atom.[20,21] Consistent with prior findings,[22,23] Figure 2 demonstrates that the direction of approach can profoundly

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(19) It should be stressed that there is no special significance attached to the particular value of dielectric constant where the two pK_a's equilibrate. This value would change were a different model used to represent the interaction with surroundings, or a different level of ab initio theory. What is important here is the behavior of the system when the two minima in the potential are approximately equal in energy, regardless of the cause of this equalization. For example, previous calculations with a different basis set, and with no inclusion of correlation,[17] had noted the reversal occurs at a slightly different value of ϵ.

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influence the energetics of the proton transfer. As the water moves from the C=O axis toward a lone pair of the carbonyl oxygen, the bridging proton shifts its preferred position from the hydroxyl to the carbonyl. The right well of the uppermost curve in Figure 2a is more stable than the left by 13 kcal/mol, indicating the energetic preference for ($H_2CO\cdot\cdot{}^+HOH_2$) when α is equal to 180°, i.e. when the O atom of the water lies along the C=O axis. As this angle diminishes to 150°, the left well begins to stabilize and when α reaches 130° the carbonyl and hydroxyl O atoms have essentially the same pK_a.

In terms of the H-bond interaction, the more stable of the two minima (the right) has lowered from −27 to −30 kcal/mol as a result of this reorientation, a strengthening of only 3 kcal/mol. Equally important are the consequences of a further reduction in α below 130°. The lowest curve in Figure 2a illustrates that as the equilibrium position of the proton shifts toward the carbonyl and the pK_a's of the two groups begin to differ again, the H-bond energy continues to increase, reaching 32 kcal/mol when the energies of the two wells differ by 3 kcal/mol. This finding would certainly argue against any particular stabilization associated with equalization of pK_a.

Also examined once again was the effect of a shortening of the H-bond, so as to induce the double-well potential to change its character to single well. An example of such a short R(O··O) distance is 2.413 Å (the fully optimized global minimum for this complex). The proton transfer profiles in Figure 2b pertain to this shorter distance and exhibit the expected single-well character. As in the case of the longer H-bond, the minimum in the potential for $\alpha = 180°$ corresponds to the ($H_2CO\cdot\cdot{}^+HOH_2$) configuration, indicated by the long r(OH) at its nadir. As the hydroxyl oxygen deviates from the C=O direction, and α is lowered, the position of this minimum shifts toward the carbonyl oxygen on the left. At the same time, the minimum becomes deeper, representing a stronger H-bond. When the proton is located about equidistant between the two oxygens, when $\alpha = 130°$, and the pK_a's of the two atoms may be said to be equalized, the H-bond energy is 36 kcal/mol. Again as for the longer H-bond, further reduction in α and the ensuing disequalization of the the proton affinities results in a stronger H-bond. The contraction of the H-bond from 2.7 to 2.41 Å has only a small effect on the H-bond energy, increasing this quantity from 32 to 36 when $\alpha = 107°$, despite the transition from double- to single-well character of the transfer profile.

To summarize these results, the shortening of the H-bond from 2.7 to 2.4 Å does indeed strengthen the H-bond in this system. For $\alpha = 130°$, this shortening increases the H-bond energy from 31 to 36 kcal/mol. This stronger bond should not be attributed to the transition from double- to single-well character of the proton transfer profile. It is instead a natural feature of numerous ionic H-bonds between O atoms to be both short and strong, due chiefly to the strong ion−dipole electrostatic interaction. The single-well proton transfer profile is a direct result of the short R(O··O). Forcing further contraction of the bond would weaken it as the two subunits are pushed into van der Waals contact. There is no special stabilization of the system which results from equalization of the proton affinities of the two O atoms. Indeed, the opposite is observed: the H-bond energy increases as the pK_a disparity grows in favor of the carbonyl oxygen.

Ammonia−Hydrogen Iodide. The previous examples adjusted the relative proton affinities of the donor and acceptor groups by immersion in a dielectric medium or by modulation of the angular aspects of the H-bond. It is possible also to choose a system where the two wells in the proton transfer potential are nearly equally stable naturally, a simple result of the chemical nature of the groups involved. Such is the case

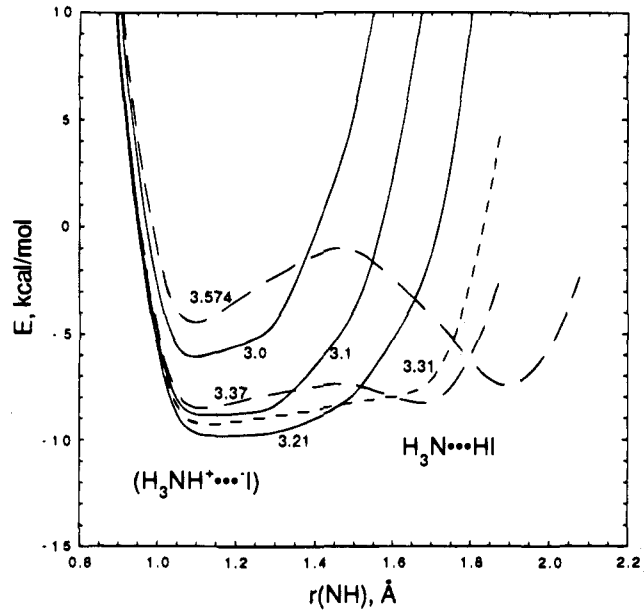

Figure 3. MP2 proton transfer profiles calculated for ($H_3N\cdot\cdot HI$). The zero of energy corresponds to the total energy of the optimized isolated NH_3 and HI molecules.

when NH_3 competes with I^- for the proton, where the neutral pair ($H_3N\cdot\cdot HI$) is approximately equal in energy to the ion pair ($H_3NH^+\cdot\cdot I^-$). The proton transfer profiles of this system are illustrated in Figure 3 for various H-bond lengths, ranging between 3.0 and 3.57 Å. At the longest H-bond length investigated, 3.574 Å, the profile contains a pair of minima, separated by an energy barrier of about 5 kcal/mol. The neutral pair is slightly more stable than the ion pair, by 2 kcal/mol. The H-bond energy, relative to the isolated NH_3 + HI pair, is 8 kcal/mol. Shortening of the N··I separation to 3.37 Å makes the two configurations nearly identical in energy, with only a small barrier separating them. The H-bond energy here is nonetheless virtually unchanged from what it is for 3.57 Å. The next contraction step to 3.31 Å eliminates the transfer barrier entirely, transforming the profile into a single-well potential; the geometry of this well corresponds to the ion pair. But the enhancement in binding energy accompanying this barrier elimination is negligible. Further reduction in the H-bond length retains the single-well character of the potential but has virtually no effect on the H-bond energy. Indeed, if the bond is contracted below 3.21 Å (the equilibrium length), the H-bond energy begins to diminish, as the bottom of the well rises in energy. For example, the H-bond energy for $R = 3.0$ Å is 4 kcal/mol less than that for 3.21 Å.

In summary, in the case where the intrinsic pK_a's of the two groups involved in the H-bond are quite similar, as evident by the nearly equal energy of the two wells in the transfer profile, the H-bond energy is affected to only a very minor degree by the shortening of the H-bond. Reduction of the H-bond length from 3.57 to 3.0 Å yields small changes in the maximal H-bond energy of 10 kcal/mol, despite transition from double- to single-well character of the transfer profile.

Discussion

It is important to draw a distinction between neutral H-bonds, in which neither partner bears an electric charge, and ionic complexes, wherein one or both may be charged. The H-bond energies of the former are typically quite a bit smaller than those of the latter. For example, two water molecules are bound together by only 5 kcal/mol, whereas the interaction enegy is magnified by a factor of 5 to 7, should one molecule be charged,

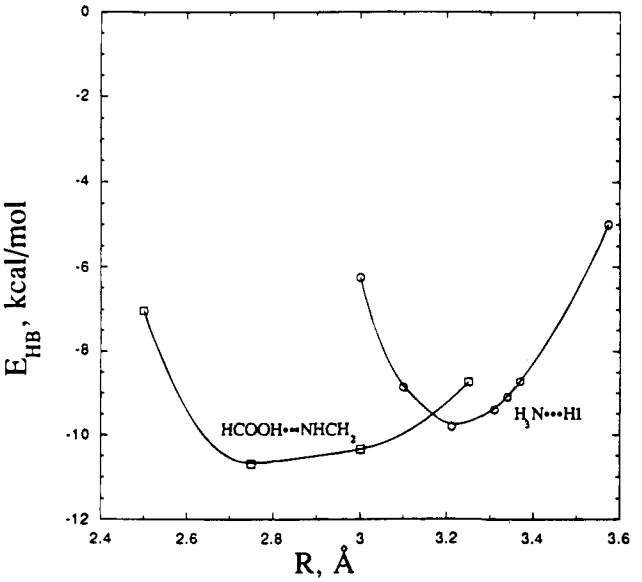

Figure 4. H-bond energies computed for two neutral pairs $HCOOH\cdots NHCH_2$ and $H_3N\cdots HI$ as a function of the distance separating them. Values are negative to reflect the greater stability of the complex than the isolated subunits. $H_3N\cdots HI$ undergoes a transition from neutral to ion pair when $R < 3.37$ Å, but this has little effect upon the interaction energy.

as in $(OH_3)^+\cdot\cdot OH_2$ or $(OH)^-\cdot\cdot OH_2$.[24] There is no way to enhance the binding of a neutral complex by 10−20 kcal/mol. Pushing the two molecules closer together than their optimal separation will detract from, rather than enhance, the binding, even if the H-bond is shortened to the point where the barrier to proton transfer is eliminated. The strongest neutral H-bonds are those pairing a strong acid with a strong base, as in the case of HI + NH_3 described above. H-bond energies of up to 10 kcal/mol or so are possible here.[25] Should the acid and base become sufficiently strong, the transfer of the bridging proton from the former to the latter occurs, and the complex takes on ion-pair character, as in $H_3NH^+\cdot\cdot{}^-I$. But the energy of the latter will generally be similar to that of the neutral pair, i.e. no dramatic stabilization occurs as a result of the proton transfer, whether the potential contains one or two minima.[26]

These trends are explicitly illustrated in Figure 4 for the $HCOOH\cdots NHCH_2$ and $H_3N\cdots HI$ systems. The maximum in the H-bond energy occurs at around 2.75 Å in the former case and at 3.2 Å for the latter, due to the larger I atom. The latter system passes from a neutral pair to ion pair as the intermolecular separation is reduced below 3.37 Å. Regardless of whether or not a proton transfer occurs, in either case, the H-bond energy becomes smaller as the two molecules are pushed closer together than their equilibrium distance.

H-bonds pairing an ion with a neutral molecule are significantly stronger, due chiefly to ion−dipole and higher order terms in the electrostatic interaction, coupled with the ability of the ion to favorably polarize its partner molecule. Interaction energies of as much as 37 kcal/mol, as noted for $(H_2COH^+\cdot\cdot OH_2)$ here, are not uncommon. In addition to their greater strength, ionic complexes such as these have considerably shorter H-bonds. For example, the two oxygen atoms are separated by 3.0 Å in the neutral water dimer, whereas R(OO) = 2.4 Å in $(OH_3)^+\cdot\cdot OH_2$ or $(OH)^-\cdot\cdot OH_2$. The shorter and stronger H-bond leads to a proton transfer potential that contains a single well (or two wells with a very shallow barrier separating them).[27]

If the two partners are artificially held further apart than their equilibrium separation, the binding energy will be reduced accordingly, and the transfer barrier will rise. It is only in this context that the LBHB hypothesis may be considered as valid. That is, a pair of partners, one of them charged, must first be held apart by the enzyme, thereby weakening the H-bond. The proton transfer potential in this artificially long H-bond will contain two distinct minima with a substantial energy barrier separating them. By later releasing this restraint, the two groups are freed to approach one another, thereby magnifying the H-bond energy to its "normally" greater value, and permitting the proton transfer potential to collapse to its intrinsic single-well character. It must be stressed that this sort of mechanism has a price to pay: energy is required to hold the two partners apart in the initial configuration. And it should be added that the much greater strength of the ionic H-bond in the gas phase is weakened in the context of a protein interior. Fersht et al.[28] estimate a differential of only 3 kcal/mol in free energy between ionic and neutral H-bonds within tyrosyl-tRNA synthetase. On the other hand, equalization of the pK_a's seems to be a minor factor; any extra stabilization resulting from equalization of the proton affinities of the two partners is small.

The latter conclusion, based largely upon theoretical and gas-phase measurements, has recently been confirmed within the context of an enzyme when the binding to two inhibitors with citrate synthase was compared.[29] In the first case, an ionic H-bond, 2.49 Å in length, is formed between a carboxylate of the enzyme and the amide group of the inhibitor, with markedly different pK_a's. When the amide is changed to carboxylate, the H-bond contracts to 2.38 Å, and the pK_a's of the the two carboxylate groups become quite similar. Despite this bond shortening and pK_a equalization, which is associated with the disappearance of the proton transfer barrier, only 1.8 kcal/mol extra stabilization is realized, far less than supposed by the LBHB hypothesis.

There are other ionic complexes which are by nature weaker and longer than $(H_2COH)^+\cdot\cdot OH_2$ or $(OH_3)^+\cdot\cdot OH_2$. A water molecule binds to protonated formamide or to $HCOO^-$ by less than 20 kcal/mol,[30,31] with R(OO) in the 2.6−2.8 Å range. As for the more strongly bound complexes, both of the latter contain intrinsic single-well proton transfer potentials which may be converted to double wells by stretching the interoxygen distance. Incorporating atoms less electronegative than O into the H-bond further reduces the strength of the complex. $(NH_4)^+\cdot\cdot NH_3$, for example, is bound by 10 kcal/mol less than $(OH_3)^+\cdot\cdot OH_2$, with a H-bond that is longer by 0.2 Å.[32] A triple bond to the N makes it somewhat more electronegative; the H-bond in $C{\equiv}NH\cdots{}^-N{\equiv}C$ is consequently about 25 kcal/mol and the internitrogen distance of 2.75 Å is just short enough so that the proton transfer potential contains a symmetric single well.[33]

While there is of course a definite relationship between the strength of the H-bond and the pK_a's of the two groups, it is a central conclusion of this study that there is no *special* stabilization associated with equalization of these two quantities.

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Consider the ionic $AH^+ \cdot\cdot B$ wherein B is initially less basic than A. This bond gains in strength as B becomes more basic, with no discontinuity observed when the pK_a's of A and B are equal. The only notable change occurring when the basicity of B surpasses that of A is a geometric one: the bridging proton transfers across to the more basic B, forming $A \cdot\cdot {}^+HB$. This behavior is supported by ab initio calculations and experimental observations.[34,35] A set of ionic $OH^+ \cdot\cdot O$ and $NH^+ \cdot\cdot N$ H-bonded systems was studied in which the proton affinity of one subunit was changed relative to the other by alkylation. It was demonstrated that the H-bond energy diminished smoothly, obeying a Marcus relationship that included as parameters only the proton affinity difference (ΔPA) and the H-bond energy of the symmetric system ($\Delta PA = 0$).[36] Indeed, this well-behaved relationship between H-bond energy and ΔPA had been noted earlier for a wide array of complexes, containing systems with both single- and double-well potentials.[34] It is worth stressing that the latter found no evidence of precipitous change in H-bond energy accompanying the transition from single- to double-well potential.

In summary, there is little possibility that interactions between neutral partners can be strengthened to the extent recently proposed.[3] Interaction energies seldom exceed 10 kcal/mol, even under optimal gas-phase conditions. Forcing closer contact, so as to eliminate the proton transfer barrier, only weakens the interaction by strong steric repulsions. In other words, a H-bond cannot be made stronger by compressing it to be shorter than its equilibrium length. A proton transfer which forms the ion pair can be stimulated under certain circumstances but no particular stabilization results therefrom.

Interactions between an ion and a neutral are much stronger, in excess of 30 kcal/mol in some cases. The H-bonds of the strongest such complexes would tend naturally to be very short, some less than 2.5 Å, when external restraints are absent. Experimental finding of a very short H-bond, or evidence that the proton oscillates around the center of this bond, is a strong indicator of such a strong energetic interaction. Energy would be required to stretch this H-bond and introduce a barrier into the proton transfer potential. As the proton affinity of the less basic partner is enhanced, the binding energy steadily increases; no particular stabilization is associated with the point of equal pK_a or with the disappearance of the proton transfer barrier. There is no dramatic or precipitous change in H-bond strength that occurs when the acidities are equalized. The three systems examined here by ab initio calculations are stronger than most typical H-bonds within proteins. If these intrinsically strong interactions cannot be made very strong by compression or by pK equalization, it is unlikely that weaker H-bonds can be made to do so.

Acknowledgment. This work was supported by the National Institute of General Medical Sciences (GM29391).

JA950553N

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