Short strong hydrogen bonds: can they explain enzymic catalysis?

J Peter Guthrie

It has been proposed that some remarkable enzymic catalytic effects can be explained by the existence of unusually strong hydrogen bonds within the enzyme's active site. Although such hydrogen bonds may be short, and may have unusual properties, there is no evidence that unusually strong hydrogen bonds exist in solution or in enzyme active sites. Thus there is no basis for invoking strong hydrogen bonds to explain enzymic rate enhancements.

Address: Department of Chemistry, University of Western Ontario, London, Ontario N6A 5B7, Canada.

Chemistry & Biology March 1996, **3**:163–170

Introduction

In 1994 Cleland and Kreevoy [1] proposed that, "Formation of a short (less than 2.5 angstroms), very strong, low barrier hydrogen bond in the transition state or in an enzyme–intermediate complex can be an important contribution to enzymic catalysis. Formation of such a bond can supply 10 to 20 kilocalories per mole and thus facilitate difficult reactions such as enolization of carboxylate groups." This hypothesis has received considerable attention [2–18] and has been invoked to explain catalysis by a number of enzymes [4,5,8]. In this review I will examine the evidence concerning the existence and nature of short hydrogen bonds, the existence of strong hydrogen bonds in condensed phases, and the significance of short strong hydrogen bonds in enzyme mechanisms.

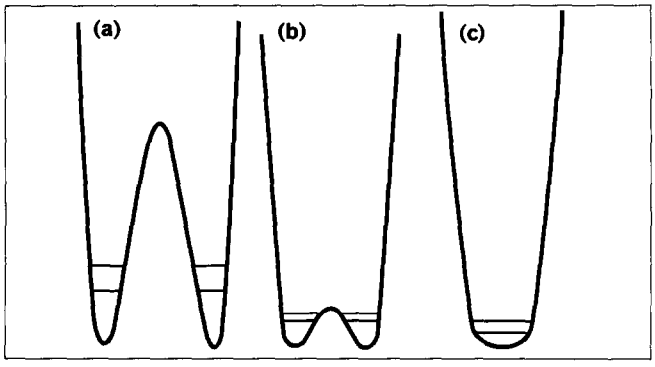
Hydrogen bonds are important in many ways in biochemistry; about this there is no argument. Hydrogen bonds have traditionally been considered to be weak interactions, with energies in the range of 3 to 7 kcal mol^{-1}. Such hydrogen bonds are important in peptide structures, DNA base pairing, and enzyme substrate binding. The point of contention is the claim that some hydrogen bonds in solution, and in particular in the active sites of enzymes, have energies of 10–20 kcal mol^{-1}. Commonly, hydrogen bonds have the proton in a double-well potential; as the atoms joined by the hydrogen bond come closer the barrier separating the two wells becomes lower, then disappears, leaving a single-well potential [19]. It has been proposed that when the barrier is low or non-existent the hydrogen bond becomes remarkably strong [1] (Fig. 1).

Existence and nature of short hydrogen bonds

Emsley, in a review article [20], made a case that certain hydrogen bonds were strong, and quantitatively different from normal hydrogen bonds. This argument was repeated in a review by Hibbert and Emsley [21]. The arguments depended very strongly on the strengths of hydrogen bonds in species such as FHF^-. In the gas phase the FHF^- ion is held together by a very strong hydrogen bond. Larson and McMahon [22] reported a value of –39 kcal mol^{-1} for $F^- + HF = FHF^-$, while more recently Wenthold and Squires [23] reported a value of –46 kcal mol^{-1}. The discrepancy was attributed to an accumulation of errors in the ladder of fluoride affinities used in the earlier work.

That FHF^- has a very strong hydrogen bond in the gas phase is widely known. What is less well known, although it has been determined by four groups over the years [24–27], is that FHF^- has a relatively weak hydrogen bond in aqueous solution, –0.82 kcal mol^{-1} for $F^- + HF = FHF^-$

Figure 1



Potential wells for hydrogen bonds. **(a)** Double well hydrogen bond; **(b)** low-barrier hydrogen bond; **(c)** single-well hydrogen bond. The horizontal lines show the energy levels for the lowest vibrational energy for hydrogen (upper) or deuterium (lower).

[27]. The gas-phase hydrogen bond of FHF^-, though one of the strongest known, is by no means unique: a number of symmetrical BHB^+ (where B is a neutral Lewis base) hydrogen bonds in the gas phase have energies of 30 ± 2 kcal mol^{-1} [28]. The strengths of asymmetrical hydrogen bonds of this type show a linear dependence on acid/base strength [28]. Hydrogen bonds between anionic Lewis bases (A, A') of the form AHA' also show a linear dependence of hydrogen bond strength on acid/base strength when one of the bases is F or Cl [29,30]. Thus, in the gas phase the existence of strong hydrogen bonds has been well established.

There are numerous examples of short hydrogen bonds, as determined by X-ray crystallography, including those systems which have been shown to have very strong hydrogen bonds in the gas phase [20,21,31].

I believe that there has been a misleading pseudosyllogism at work in this area. It takes the form:

FHF^- has a strong hydrogen bond (in the gas phase)

FHF^- has a short hydrogen bond (in the solid phase)

Therefore, all short hydrogen bonds are strong.

One thing leading to confusion is that ions in the gas phase are high energy species with a drastic need for

Table 1

Substituent effects on acidity in the gas phase.

Alcohol	ΔH_i (gas) kcal mol^{-1}
$(CH_3)_3COH$	373.3
HOH	390.8
$(CH_3)_3C$ vs H	17.5

Table 2

Substituent effects on basicity in the gas phase.

Amine	Proton affinity (gas) kcal mol^{-1} [33]
$(CH_3)_3CNH_2$	225.7
NH_3	207.0
$(CH_3)_3C$ vs H	18.7

stabilization, so that surprisingly large effects result from small structural changes; these effects are not predictive for the same structural changes in solution. For example, data from Bartmess *et al.* [32] on the heats of ionization of alcohols in the gas phase (Table 1) show that the *t*-butyl substituent stabilizes an oxide ion by 17.5 kcal mol^{-1} relative to hydrogen. Since 1.37 kcal mol^{-1} equates to a difference of ~1 pK_a unit, if the *t*-butyl substituent had the same stabilization effect in solution, one would expect that the pK_a of *t*-butyl alcohol would be $17.5 \div 1.37$, or 12.8 pK_a units more acidic than water; in fact, it is less acidic.

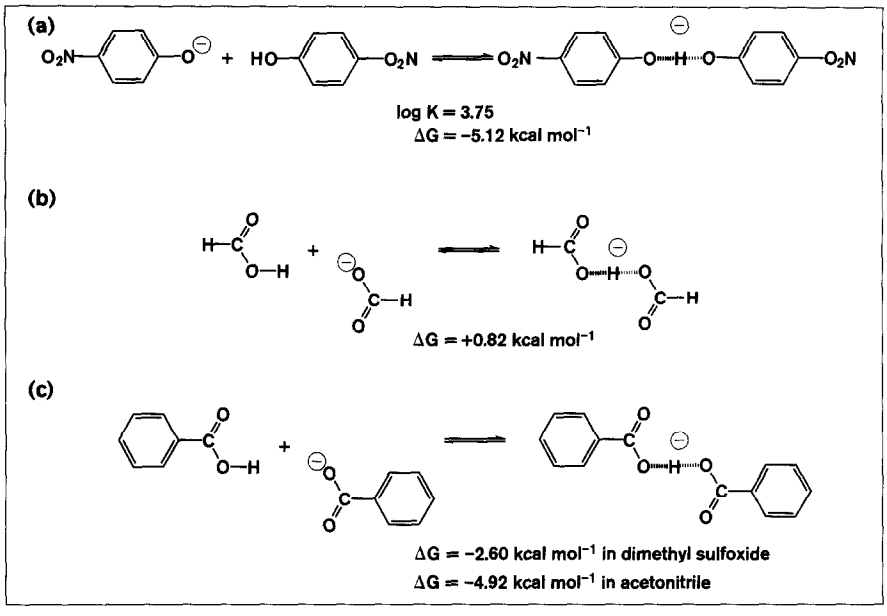
A similar substitutent effect is seen for cations, for example in the studies of Auc *et al.* [33] on the proton affinities of amines (proton affinity is the absolute value of the enthalpy released upon protonation in the gas phase) (Table 2). Again, the gas-phase data would lead us to predict that *t*-butyl amine would be more basic than ammonia by 13.7 pK_a units; in fact, the difference is only 1.3 pK_a units.

Thus, despite the unambiguous experimental evidence for large substituent effects in the gas phase, we should not expect similarly large substituent effects in solution, even in the active site of an enzyme. Similarly, the fact that ions with charges localized on small atoms in the gas phase show dramatic stabilization by hydrogen bonding, does not imply that similar hydrogen bonding will be so energetically significant in condensed phases, where there are many other ion-stabilizing mechanisms at work.

Kreevoy and Liang [19] studied the nature of the hydrogen bond between *p*-nitrophenol and *p*-nitrophenoxide. In acetonitrile they could demonstrate that the isotopic fractionation factor (the preference of a hydrogenic position for deuterium over hydrogen, relative to the preference of a hydrogenic position in a solvent molecule, in this case water) for the hydrogen bonded hydrogen was 0.31, an unusually low value, that the ^{1}H-NMR chemical shift for this hydrogen was 16.8, which is remarkably high, and that the IR spectrum showed a very broad continuum band that is missing in either *p*-nitrophenol or *p*-nitrophenoxide. Such a band is considered diagnostic for a short hydrogen bond with a centralized bridging hydrogen [19,20,34]. There is crystallographic evidence

Figure 2

Symmetrical hydrogen bonds and their properties. **(a)** The hydrogen bond formed between *p*-nitrophenol and *p*-nitrophenoxide in acetonitrile is strong compared to other hydrogen bonds [35], but falls far short of the energies claimed for short strong hydrogen bonds. **(b)** The hydrogen bond formed between formic acid and formate in aqueous solution is actually rather weak, but there is good evidence that it does in fact form in solution [36]. **(c)** The hydrogen bond formed between benzoic acid and benzoate in dipolar aprotic solvents varies in strength according to the degree of solvation of the anion and the acid [37,38].



that such very short hydrogen bonds are symmetrical, with a central hydrogen [31]. There is clearly something very special about this sort of symmetrical hydrogen bond. On the other hand, other researchers have determined the equilibrium constant for formation of this hydrogen-bonded species in the same solvent [35], and found that, although it is strong (for a hydrogen bond) at log K = 3.75, it is in no sense remarkable (see Fig. 2a).

It thus seems clear that there is a class of hydrogen bonds that, in solution, show unusually low deuterium fractionation factors and remarkably high ^{1}H-NMR chemical shifts, and, in the solid state, show IR spectra with a broad continuum absorption and short heavy atom–heavy atom distances. But what is the relevance of this observation to the strength of the hydrogen bond?

A second pseudosyllogism has been constructed, building on the first:

Short hydrogen bonds (in the solid state) are strong (in solution);

Systems with short hydrogen bonds have unusual isotopic fractionation factors, unusual ^{1}H-NMR chemical shifts and unusual IR vibration frequencies;

Therefore, any system with any of these unusual properties has a strong hydrogen bond.

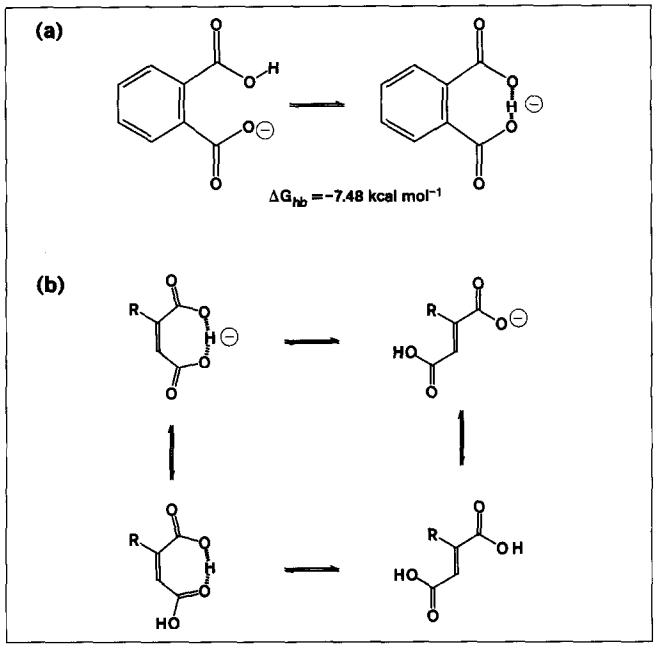
The problem here is that although the second premise appears to be soundly based on fact, the first is not, and thus the syllogism does not hold up.

Energetics of hydrogen bond formation in solution

How strong can a hydrogen bond be, in solution? It is well established that the equilibrium constants for formation of hydrogen-bonded complexes are more favorable in dipolar aprotic solvents than in water. In water the solvent competes with the acid to form hydrogen bonds to the hydrogen-bonding base; in both water and dipolar aprotic solvents, the solvent competes with the base to form hydrogen bonds with the hydrogen-bonding acid. The magnitude of this solvent effect is shown by comparison of measured equilibrium constants. Hydrogen-bonded complex formation is so weak in water that it is difficult to measure the equilibrium constants. Hand and Jencks [36] reviewed the evidence for carboxylic acid–carboxylate hydrogen-bonded complexes and concluded that there was good evidence for kinetically significant complex formation between formate and formic acid, though not for acetate and acetic acid (Fig. 2b). In dipolar aprotic solvents it is much easier to measure equilibrium constants for such complex formations; one value, for an acid of similar pK_a, was reported by Bordwell *et al.* [37] in dimethylsulfoxide, where the hydrogen-bonded complex is more favorable than the free acid/base by 3.4 kcal mol^{-1}, because there is no competing solvation of the anion. In acetonitrile, hydrogen bonding of this acid/base system is even stronger, by 5.7 kcal mol^{-1} [38], because solvation of the acid is weaker and there is no competing solvation of the anion (Fig. 2c).

There are no experimental data for the equilibrium constant for the formation of a phenol–phenoxide hydrogen

Figure 3



The strongest solution phase hydrogen bond known is an intramolecular hydrogen bond, but not all intramolecular hydrogen bonds are strong. **(a)** The intramolecular hydrogen bond of monohydrogen phthalate has a ΔG_{hb} of −7.48 kcal mol^{-1} in acetonitrile [40]. **(b)** Intramolecular hydrogen bonding in the *cis* isomer in the *cis/trans* isomerization systems of maleic/fumaric and citraconic/mesaconic acids makes little difference to the equilibrium constants when the solvent is water or methanol ($\Delta G_{acid} - \Delta G_{anion}$ = 0.7 kcal mol^{-1}), but has a larger effect ($\Delta G_{acid} - \Delta G_{anion}$ = 4.5 kcal mol^{-1}) in aprotic solvents such as dimethylsulfoxide.

bond in aqueous solution, but we can estimate such an equilibrium constant for complexation of *p*-nitrophenol with *p*-nitrophenoxide (Fig. 2a) after Stahl and Jencks [39] as ΔG_{hb} = +1.5 kcal mol^{-1}. In dimethyl sulfoxide solution the value has been measured as ΔG_{hb} = −2.3 kcal mol^{-1} [37], and in acetonitrile solution as ΔG_{hb} = −5.1 kcal mol^{-1} [35]. The value in dimethyl sulfoxide is more favorable than that in water because the phenoxide is not hydrogen bonded in a dipolar aprotic solvent. The value in acetonitrile is still more favorable because, in addition to the absence of hydrogen bonding to the phenoxide, competitive hydrogen bonding to the solvent is less important in a solvent that is a weaker base. The difference in ΔG_{hb} is only of the magnitude expected for the absence of competing hydrogen bonds to water.

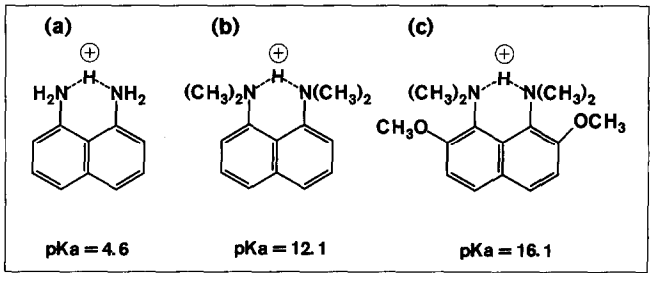
Weak interactions are often studied in intramolecular situations, where there can be a higher effective molarity, making the effect easier to demonstrate. Kolthoff and Chantooni [40, 41] have studied intramolecular hydrogen bonding in diacids, and found, for example, that for phthalic acid monoanion ΔG_{hb} = −7.48 kcal mol^{-1} in acetonitrile (Fig. 3a). There have been numerous other studies, which have found similar, but generally smaller, equilibrium constants [40–43]

Recently Schwartz and Drueckhammer [13] determined equilibrium constants for *cis/trans* isomerization of unsaturated diacids and their monoanions. The systems studied were maleic/fumaric and citraconic/mesaconic acids (Fig. 3b). In water or methanol there was little difference between the equilibrium constants for the acids and monoanions: $\Delta\Delta G \approx 0.7$ kcal mol^{-1}. In aprotic solvents, such as dimethylsulfoxide, the difference was larger: $\Delta\Delta G \approx 4.5$ kcal mol^{-1}. Although this was attributed to the increased hydrogen bond strength when pK_a values are matched, at least part of the effect must be due to the absence of competing hydrogen bonding by the anion to solvent in dimethylsulfoxide.

If short strong hydrogen bonds exist, then in a suitable solvent there must be large homoconjugation constants. A homoconjugation constant is the equilibrium constant for hydrogen bond formation between a conjugate acid : conjugate base pair. A hydrogen bond with a strength of 20 kcal mol^{-1} implies log K = 20 ÷ 1.37, giving a ΔpK_a of ~15. It is common to find that pK_a measurements in dipolar solvents are made more difficult by homoconjugate formation [44,45], but the homoconjugation constants are never as large as 15 pK_a units. The intramolecular shift observed by Kolthoff and Chantooni [40] is the largest such shift known.

A major difficulty for the hypothesis that short strong hydrogen bonds exist in solution is that no hydrogen bond energies of > 10 kcal mol^{-1} have been measured for complex formation in solution. (This statement is based on measurements of ΔG_{hb}; in the case of CCl_3–COOH + $(C_6H_5)_2SeO$ in CCl_4, the observed ΔH_{hb} is −15.4 kcal mol^{-1}, but ΔG_{hb} = −7.3 kcal mol^{-1} [46]. The equilibrium constant, or ΔG_{hb} is the better criterion for bond strength.) All claims for strong hydrogen bonds are in the gas phase or in solids, referenced to the gas phase [21]. One example in solution, which is quoted by

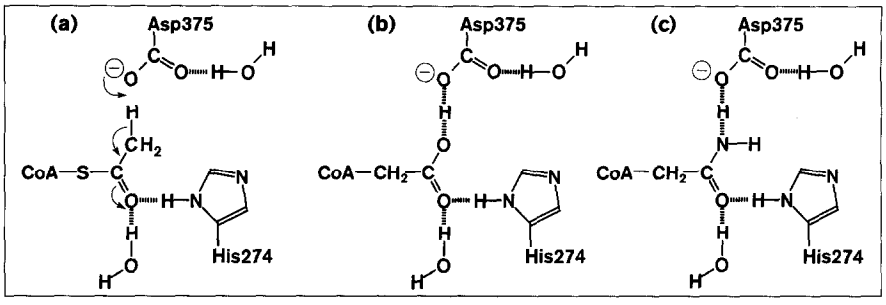
Figure 4



Variants of proton sponge and their pK_a values [47,48]. **(a)** Diaminonaphthalene; **(b)** 1,8-bis(dimethylamino)naphthalene; **(c)** 1,8-bis(dimethylamino)-2,7-dimethoxynaphthalene.

Figure 5

Hydrogen bonding at the citrate synthase active site. Complexes of citrate synthase with **(a)** acetyl CoA; **(b)** an isosteric inhibitor containing a carboxylic acid functional group; **(c)** an isosteric inhibitor containing a carboxamide functional group.



proponents of the short strong hydrogen bond hypothesis, is that of proton sponge, [2,47] for which the pK_a is remarkably high (Fig. 4b,c; [48]). This has been attributed by Cleland and Kreevoy [2], and by Frey [2] to the strength of the hydrogen bond in the cation. The problem with this explanation is that, if this is so, the hydrogen bond in 1,8-diaminonaphthalene should also be strong, yet the pK_a of this species is normal (Fig 4a). A more likely explanation is that the 1,8-bis(dimethylamino)naphthalenes are severely crowded [47] and have both a steric inhibition of resonance (which increases basicity) and a serious destabilizing interaction involving the lone pairs, which is relieved by protonation [21]. Buttressing by the 2,7-methoxy groups makes this destabilizing interaction worse. If the 1,8-diaminonaphthalene monocation could form a strong hydrogen bond simply by distorting itself to the same geometry as the dimethyl species (by rotating the amino group out of conjugation with the naphthalene, which is known to cost only 2.6 pK_a units [49]), this would happen, and 1,8-diaminonaphthalene would have a pK_a of at least 9.

Recent studies [50] have shown that in dimethylsulfoxide or tetrahydrofuran as solvents, hydrogen bond energies are linear in ΔpK_a through $\Delta pK_a = 0$, so that there is no special effect of $\Delta pK_a = 0$. This contradicts one of the assumptions of the short strong hydrogen bond hypothesis [1].

Enzyme mechanisms

I will now turn to some of the proposals for the involvement of short strong hydrogen bonds in the mechanism of rate enhancement by enzymes, focusing on those proposals that have been tested experimentally.

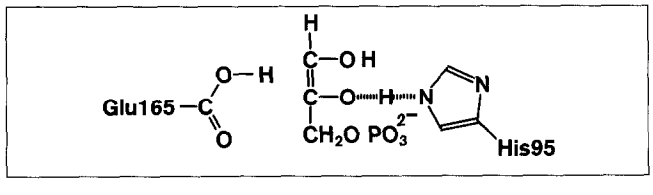
Gerlt and Gassman [51,52], in attempting to explain the enzymic rate enhancement observed for the inherently difficult process of removing a proton from carbon α to a carboxylate in mandelate, have made several proposals. Initially they suggested that "concerted general acid–general base catalysis provides the low-energy route consistent with the observed rates" of enzymic reactions, and that the crucial step might be "concerted general acid–general base catalyzed enolization of the carbon acid to generate an enol intermediate ..." [51,52].

Guthrie and Kluger [53], expanding on a criticism by Kresge [54] argued that the enol was in fact less stable than the enolate, and that general acid catalysis could not explain the observed rate enhancements. Because the enzyme active site constitutes a low polarity medium, they argued that electrostatic interactions would be stronger than in water and could be of sufficient magnitude to explain the observed rate effects.

Gerlt and Gassman [4,5] then argued that ΔG° is reduced by short strong hydrogen bonds (> 20 kcal mol^{-1}), and that $\Delta G^{\ddagger}_{int}$ (the Marcus intrinsic barrier) is reduced by prepositioning an electrophilic catalyst. They went on to use these proposals to explain catalysis in mandelate racemase, citrate synthase, serine proteases, ribonuclease, and other enzymes. They proposed [4] that for mandelate racemase "the general method for reducing ΔG° is differential hydrogen bonding.... When removed from aqueous solvent, short strong hydrogen bonds are formed when a proton is shared by two heteroatom bases whose conjugate acids have equal pK_a's." Similarly for citrate synthase they, and Cleland and Kreevoy [1], propose pK_a matching, between the enol of acetyl CoA and a neutral histidine imidazolyl group, of perturbed pK_a.

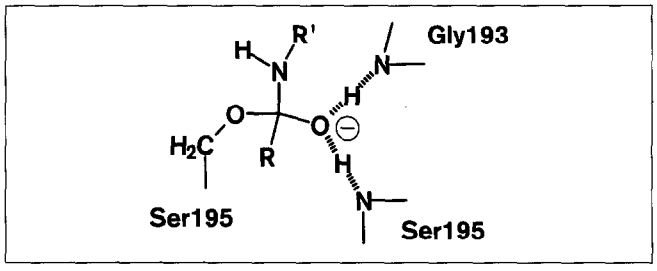
X-ray crystallography of complexes of isosteric inhibitors with citrate synthase found that these inhibitors have

Figure 6



Proposed short strong hydrogen bond in the active site of triose phosphate isomerase [1,4].

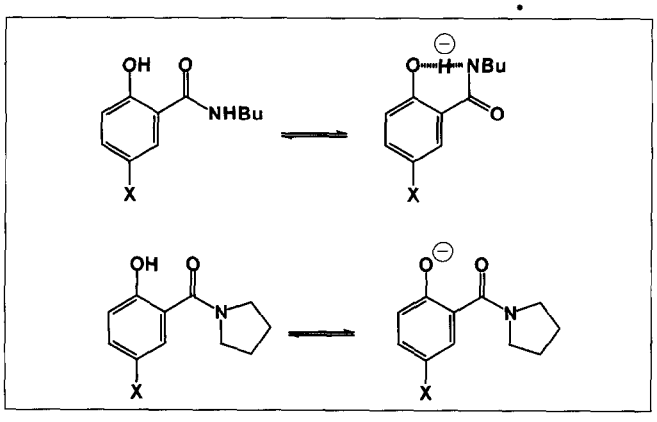
Figure 7



Proposal for stabilization of the anionic tetrahedral intermediate by short strong hydrogen bonds in reactions catalyzed by serine proteases [4].

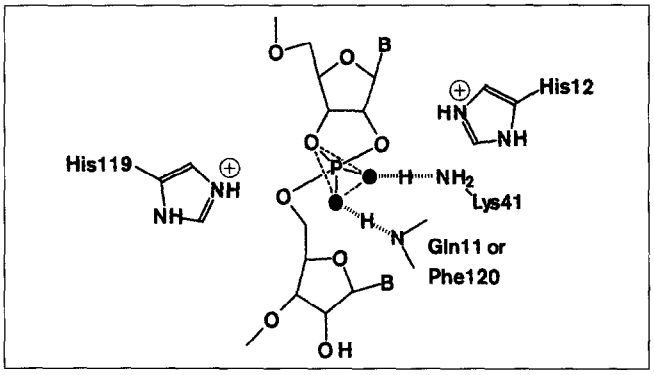
short hydrogen bonds to the carboxylate of Asp375, which is believed to act as a general base in the reaction with acetyl CoA [17]; see Figure 5. The oxygen–oxygen distance for the inhibitor with carboxylate is 2.38 Å. For the inhibitor with a carboxamide, the corresponding oxygen–nitrogen distance is 2.49 Å. Hibbert and Emsley [21] have suggested that O–H–O hydrogen bonds with an O–O distance less than 2.45 Å fall into the very strong category. But, in fact, the binding by these inhibitors is not unusually strong, nor is it very sensitive to the exact pK_a of the hydrogen-bonding group on the inhibitor. Binding of the inhibitor with a carboxylate is pH dependent, and from the pH dependence one can extract an inhibition constant, $K_i = 1.5 \times 10^{-9}$ M; for the inhibitor with a carboxamide, $K_i = 2.8 \times 10^{-8}$ M. These are both stronger than for acetyl-CoA, for which the K_M is 1.6×10^{-5} M, but the difference is reasonable given that substrate binding puts a CH_3 next to the carboxylate of Asp375, whereas inhibitor binding puts a hydrogen-bonding group there.

Figure 8



Stabilization of an oxyanion by hydrogen bonding has relatively small effects on pK_a. Different substituents were used to vary the phenolic pK_a of salicylamides with (top) and without (bottom) hydrogen bonding in the anion, and the difference in pK_a between the two systems due to hydrogen bonding, ΔpK_a, was measured. ΔpK_a was linear with respect to pK_a, with a slope of ~0.12.

Figure 9



Proposal for stabilization of the phosphorane intermediate in the reaction catalyzed by ribonuclease by short strong hydrogen bonds [4]. It was later shown, however, that replacing either of the non-bridging oxygen atoms (shaded) in 2',3'-cyclic UMP with sulfur gave only a five-fold rate reduction [56]; this change would be expected to prevent the formation of short strong hydrogen bonds.

Cleland and Kreevoy [1] and also Gerlt and Gassman [4] have proposed that catalysis by triose phosphate isomerase involves a short strong hydrogen bond between a neutral histidine and the enediolate intermediate (Fig. 6). Alagona *et al.* [18] argue, on the basis of quantum mechanics calculations, including solvation effects, that the proton transfer from histidine to the endiolate would be very unfavorable, and that the enzyme active site stabilizes the transition state by electrostatic interactions, not involving pK_a matching or short strong hydrogen bonds.

Gerlt and Gassman [4] proposed that short strong hydrogen bonds are formed in the oxyanion hole of chymotrypsin and other serine proteases (Fig. 7). Bryan *et al.* [55] studied the role of hydrogen bonding in the oxyanion hole in subtilisin by site-directed mutagenesis, and found that loss of one of the hydrogen-bonding groups (Asn155→Leu155) did not affect K_M, but lowered k_{cat} by ~300 fold. Mock and Chua [7] suggest that the actual effect of the loss of the hydrogen bond is only a 30-fold rate reduction. This is based on a study of the effect of phenolic pK_a on the intramolecular hydrogen bond in substituted salicylamides (Fig. 8), both in water and in dimethylacetamide. In both solvents the pK_a perturbation, ΔpK_a, is linear in pK_a, with a slope of ~0.12. Assuming that this applies to the hydrogen bonds in the oxyanion hole of serine proteases, when the carboxamide of a substrate ($pK_{BH+} \approx 0$) becomes a tetrahedral intermediate ($pK_a \approx 12$) the increased stabilization per hydrogen bond would be $10^{0.12*12} = 27$ fold. Mock and Chua further state that "... any hypothesis of active-site 'low-barrier' transient hydrogen bonds as a universal component of enzyme catalysis would seem to need more substantiation before being adopted as a legitimate major explanation for kinetic acceleration within active sites."

Gerlt and Gassman [4] also proposed that short strong hydrogen bonds stabilize the phosphorane intermediate in the reaction catalyzed by ribonuclease (Fig. 9); Herschlag [56] examined the mechanism of this enzyme by examining the effect of replacing either of the non-bridging oxygens in 2'3' cyclic UMP by sulfur. This caused a five-fold rate reduction. If the enzyme depends on short strong hydrogen bonds to stabilize the transition state, then replacement of oxygen by sulfur, which is a much poorer hydrogen bond former, and of quite different basicity [57], would lead to a large rate effect. He concluded that "the results suggest that short, strong hydrogen bonds do not contribute substantially to RNase A catalysis".

Conclusions

According to Cleland and Kreevoy [1], "the requirements for forming low barrier hydrogen bonds appear to be the absence of a hydrogen bonding solvent such as water, and similar pK_a values of the two heteroatoms involved in the bond. The strongest bonds form when the two heteroatoms are the same (oxygen or nitrogen) ...". This may be the requirement for a short or even single-well hydrogen bond, but it clearly has nothing to say about strong hydrogen bonds, taken as meaning bonds that have a ΔG_{hb} of > 10 kcal mol^{-1}. Hydrogen bonds with perfect pK_a matching are not remarkably strong in dipolar aprotic solvents such as acetonitrile. The strength of hydrogen bonds involving different species is linearly dependent on the acid or base strength of the species which varies, both in solution [39,50] and even in the gas phase where strong hydrogen bonding is a reality [29,30]. Theoretical calculations addressing this point led to the conclusions that "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." [3]

On the basis of the evidence available to date, one must thus conclude that there is no evidence for strong hydrogen bonds in solution, and therefore that there is no basis for invoking such hydrogen bonds to explain enzymic catalysis.

Acknowledgements

I thank the Natural Sciences and Engineering Research Council of Canada for financial support of this work.

References

1. Cleland, W. & Kreevoy, M. (1994). Low-barrier hydrogen bonds and enzymic catalysis. *Science* **264**, 1887–1890.
2. Warshel, A., Papazyan, A., Kollman, P., Cleland, W., Kreevoy, M. & Frey, P. (1995). On low barrier hydrogen bonds and enzyme catalysis. *Science* **269**, 102.
3. Scheiner, S. & Kar, T. (1995). The nonexistence of specially stabilized hydrogen bonds in enzymes. *J. Am. Chem. Soc.* **117**, 6970–6975.
4. Gerlt, J. & Gassman, P. (1993). An explanation for rapid enzyme-catalyzed proton abstraction from carbon acids – importance of late transition states in concerted mechanisms. *J. Am. Chem. Soc.* **115**, 11552–11568.
5. Gerlt, J. & Gassman, P. (1993). Understanding the rates of certain enzyme-catalyzed reactions: proton abstraction from carbon acids, acyl transfer reactions, and displacement reactions of phosphodiesters. *Biochemistry* **32**, 11943–11952.
6. Tobin, J., Whitt, S., Cassidy, C. & Frey, P. (1995). Low barrier hydrogen bonding in molecular complexes analogous to histidine and aspartate in the catalytic triad of serine proteases. *Biochemistry* **34**, 6919–6924.
7. Mock, W. & Chua, D. (1995). Exceptional active site H-bonding in enzymes? Significance of the 'oxyanion hole' in the serine proteases from a model study. *J. Chem. Soc. Perkin II* , 2069–2074.
8. Frey, P., Whitt, S. & Tobin, J. (1994). A low barrier hydrogen bond in the catalytic triad of serine proteases. *Science* **264,** 1927–1930.
9. Xiang, S., Short, S., Wolfenden, R. & Carter, C. (1995). Transition state selectivity for a single hydroxyl group during catalysis by cytidine deaminase. *Biochemistry* **34**, 4516–4523.
10. Tong, H. & Davis, L. (1995). 2-Amino-3-ketobutyrate-CoA ligase from beef liver mitochondria: an NMR spectroscopic study of low barrier bonds of a pyridoxal 5'-phosphate-dependent enzyme. *Biochemistry* **34**, 3362–3367.
11. Zhao, Q., Mildvan, A. & Talalay, P. (1995). Enzymatic and nonenzymatic polarizations of a,b-unsaturated ketosteroids and phenolic steroids. Implications for the roles of hydrogen bonding in the catalytic mechanism of Δ^5-3-ketosteroid isomerase. *Biochemistry* **34**, 426–434.
12. Messmore, J., Fuchs, D. & Raines, R. (1995). Ribonuclease A; revealing structure function relations with semisynthesis. *J. Am. Chem. Soc.* **117**, 8057–8060.
13. Schwartz, B. & Drueckhammer, D. (1995). A simple method for determining the relative strengths of normal and low barrier hydrogen bonds in solution: implications for enzyme catalysis. *J. Am. Chem. Soc.* **117**, 11902–11905.
14. Usher, K., Remington, S., Marton, D. & Drueckhammer, D. (1994). Affinities of acid and amide analogs of acetylCoA to citrate synthase. *Biochemistry* **33**, 7753.
15. O'Connell, T. & Malthouse, J. (1995). A study of the stabilization of the oxyanion of tetrahedral adducts by trypsin, chymotrypsin, and subtilisin. *Biochem. J.* **307**, 353–359.
16. Perrin, C. (1994). Symmetries of hydrogen bonds in solution. *Science* **266**, 1665–1668.
17. Usher, K., Remington, S., Martin, D. & Drueckhammer, D. (1994). A very short hydrogen bond provides only moderate stabilization of an enzyme–inhibitor complex of citrate synthase. *Biochemistry* **33**, 7753–7759.
18. Alagona, G., Ghio, C. & Kollman, P. (1995). Do enzymes stabilize transition states by electrostatic interactions or pK_a balance: the case of triose phosphate isomerase (TIM)? *J. Am. Chem. Soc.* **117**, 9855–9862.
19. Kreevoy, M.M. & Liang, T.M. (1980). Structures and isotopic fractionation factors of complexes, $A_1HA_2^-$. *J. Am. Chem. Soc.* **102**, 3315–3322.
20. Emsley, J. (1980). Very strong hydrogen bonds. *Chem. Soc. Rev.* **9**, 91–124.
21. Hibbert, F. & Emsley, J. (1990). Hydrogen bonding and chemical reactivity. *Adv. Phys. Org. Chem.* **26**, 255–379.
22. Larson, J.W. & McMahon, T.B. (1982). Gas phase bifluoride ion. An ion cyclotron resonance determination of the hydrogen bond energy in FHF^-. *J. Am. Chem. Soc.* **104**, 5848–5849.
23. Wenthold, P. & Squires, R. (1995). Bond dissociation energies of $F2^-$ and HF_2^-. A gas phase experimental and G2 theoretical study. *J. Phys. Chem.* **99**, 2002–2005.
24. Broene, H. & DeVries, T. (1947). The thermodynamics of aqueous hydrofluoric acid solutions. *J. Am. Chem. Soc.* **69**, 1644–1646.
25. Baumann, E.W. (1969). Determination of stability constants of hydrogen and aluminum fluorides with a fluoride-selective electrode. *J. Inorg. Nucl. Chem.* **31**, 3155–3162.
26. Ellis, A.J. (1966). The effect of temperature on the ionization of hydrofluoric acid. *J. Chem. Soc.* , 4300–4304.
27. Kresge, A.J. & Chiang, Y. (1973). Solvent isotope effects on the ionization of hydrofluoric acid. *J. Phys. Chem.* **77**, 822–825.
28. Larson, J.W. & McMahon, T.B. (1982). Formation, thermochemistry, and relative stabilities of proton-bound dimers of oxygen n-donor bases from ion cyclotron resonance solvent-exchange equilibria measurements. *J. Am. Chem. Soc.* **104**, 6255–6261.
29. Larson, J.W. & McMahon, T.B. (1983). Strong hydrogen bonding in gas-phase anions. An ion cyclotron resonance determination of fluorid binding energetics to Bronsted acids from gas phase fluoride exchange equilibria measurements. *J. Am. Chem. Soc.* **105**, 2944–2950.

30. Larson, J.W. & McMahon, T.B. (1984). Hydrogen bonding in gas-phase anions. An experimental investigation of the interaction between chloride ion and Bronsted acids from ion cyclotron resonance chloride exchange equilibria. *J. Am. Chem. Soc.* **106**, 517–521.
31. Gilli, P., Bertolasi, V., Ferretti, V. & Gilli, G. (1994). Covalent nature of the strong homonuclear hydrogen bond – study of the O–H---O system by crystal structure correlation methods. *J. Am. Chem. Soc.* **116**, 909–915.
32. Bartmess, J.E., Scott, J.A. & McIver, R.T. (1979). Scale of acidities in the gas phase from methanol to phenol. *J. Am. Chem. Soc.* **101**, 6046–6056.
33. Aue, D.H., Webb, H.M. & Bowers, M.T. (1976). Quantitative proton affinities, ionization potentials, and hydrogen affinities of alkylamines. *J. Am. Chem. Soc.* **98**, 311–317.
34. Albrecht, G. & Zundel, G. (1984) Carboxylic acid–N base hydrogen bonds with large proton polarizability in acetonitrile as a function of the basicity of hydrogen bond donors. *Z. Naturforsch.* **39A** 986–992.
35. Magonski, J., Pawlak, Z. & Jasinski, T. (1993). Dissociation constants of substituted phenols and homoconjugation constants of the corresponding phenol–phenolate systems in acetonitrile. *J. Chem. Soc., Faraday Trans.* **89**, 119–122.
36. Hand, E.S. & Jencks, W.P. (1975). Nonlinearity in buffer-rate relationships. The significance of carboxylate–acid complexes. *J. Am. Chem. Soc.* **97**, 6221–6230.
37. Bordwell, F.G., Branca, J.C. & Hughes, D L. (1980). Equilibria involving organic anions in dimethyl sulfoxide and N-methylpyrrolidin-2-one: acidities, ion pairing, and hydrogen bonding. *J. Org. Chem.* **45**, 3305–3313.
38. Coetzee, J. (1967). Ionic reactions in acetonitrile. *Prog. Phys. Org. Chem.* **4**, 45–92.
39. Stahl, N. & Jencks, W.P. (1986). Hydrogen bonding between solutes in aqueous solution. *J. Am. Chem. Soc.* **108**, 4196–4205.
40. Kolthoff, I.M., and Chantooni, M. (1975). Intramolecular hydrogen bonding in monoanions of *o*-phthalic acid and the homologous oxalic acid series in acetonitrile. *J. Am. Chem. Soc.* **97**, 1376–1381.
41. Kolthoff, I.M. & Chantooni, M. (1976). Intramolecular hydrogen bonding involving hydroxyl groups in mono- and dianions of diprotic acids in acetonitrile and dimethyl sulfoxide. *J. Am. Chem. Soc.* **98**, 5063–5068.
42. Chantooni, M. & Kolthoff, I.M. (1975). Acid–base equilibria in methanol, acetonitrile, and dimethyl sulfoxide in acids and salts of oxalic acid and homologs, furmaric and *o*-phthalic acids. Transfer activity coefficients of acids and ions. *J. Phys. Chem.* **79**, 1178–1182.
43. Kolthoff, I.M & Chantooni, M. (1969). Some hydrogen bond relations between homoconjugates and heteroconjugates of phenols and phenolates in acetonitrile. *J. Am. Chem. Soc.* **91**, 4621–4625.
44. Harlow, G. & Bruss, B. (1958). Titration of weak acids in nonaqueous solvents. Potentiometric studies in inert solvents. *Anal. Chem.* **30**, 1833–1836.
45. Bruss, B. & Harlow, G. (1958). Titration of weak acids in nonaqueous solvents. Conductimetric studies. *Anal. Chem.* **30**, 1836–1839.
46. Hadzi, D. & Rajnvajn, J. (1973). Hydrogen bonding in some adducts of oxygen bases with acids. 7. Thermodynamic study by infra-red spectroscopy of the association of chloroacetic acids with some oxygen bases. *J. Chem. Soc., Faraday Trans. 1* **69**, 151–155.
47. Alder, R.W., Bowman, P.S., Steele, W.R.S. & Winterman, D.R. (1968). The remarkable basicity of 1,8-bis(dimethylamino)naphthalene. *Chem. Commun.*, 723–724.
48. Hibbert, F. & Hunte, K.P.P. (1983). Exceptional base strength of 1,8-bis(dimethylamino)- and 1,8-bis(diethylamino)-2,7-diemethoxynaphthalenes: kinetic and equilibrium studies of the ionization of the protonated amines in Me_2SO-H_2O mixtures with hydroxide ion. *J. Chem. Soc. [II]*, 1895–1899.
49. Wepster, B. (1952). The basic strength of benzoquinuclidine and its bearing on the basic strength and the mesomeric energy of aromatic amines. *Recl. Trav. Chim. Pays-Bas* **71**, 1171–1178.
50. Shan, S.-O., Loh, S. & Herschlag, D. (1996). The energetics of hydrogen bonds in model systems. Implication for enzymatic catalysis. *Science*, in press.
51. Gerlt, J.A., Kozarich, J.W., Kenyon, G.L. & Gassman, P.G. (1991). Electrophilic catalysis can explain the unexpected acidity of carbon acids in enzyme catalyzed reactions. *J. Am. Chem. Soc.* **113**, 9667–9669.
52. Gerlt, J.A. & Gassman, P.G. (1992). Understanding enzyme catalyzed proton abstraction from carbon acids: details of stepwise mechanisms for β-elimination reactions. *J. Am. Chem. Soc.* **114**, 5928–5934.
53. Guthrie, J.P. & Kluger, R. (1993). Electrostatic stabilization can explain the unexpected acidity of carbon acids in enzyme catalyzed reactions. *J. Am. Chem. Soc.* **115**, 11569–11572.
54. Kresge, A. (1991). Generation and study of enols and other reactive species. *Pure Appl. Chem.* **63**, 213–221.
55. Bryan, P., Pantoliano, M., Quill, S., Hsiao, H. & Poulos, T. (1986). Site directed mutagenesis and the role of the oxyanion hole in subtilisin. *Proc. Natl. Acad. Sci. USA* **83**, 3743–3745.
56. Herschlag, D. (1994). Ribonuclease revisited: catalysis via the classical general acid–base mechanism or a triester-like mechanism? *J. Am. Chem. Soc.* **116**, 11631–11635.
57. Jencks, W.P. & Regenstein, J. (1976). Physical and chemical data. In *Handbook of Biochemistry and Molecular Biology* (G. Fassman, Ed.), vol. 1, pp. 305–451. Chemical Rubber Company, Cleveland.

A response to this review has been invited, and will appear in a future issue of **Chemistry & Biology**.