ARTICLE

Symmetries of Hydrogen Bonds in Solution

Charles L. Perrin

The nuclear magnetic resonance method of isotopic perturbation can distinguish between single- and double-well potentials in intramolecularly hydrogen-bonded monoanions of dicarboxylic acids. These are classic cases of a "strong," symmetric hydrogen bond in the crystal. The observed carbon-13 isotope shifts induced by the substitution of oxygen-18 demonstrate that these monoanions exist as a single symmetric structure in a nonpolar solvent but as two equilibrating tautomers in aqueous solution. The change is attributed to the disorder of the aqueous environment. These are simple counterexamples to the hope that the crystal structure reveals the actual molecular structure in aqueous solution.

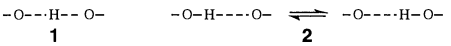
Hydrogen bonding is the attraction to an acceptor atom A of a hydrogen already bonded to a donor atom D (*1*). It is largely electrostatic in origin and arises from the stabilizing interaction between the D-H dipole (or monopole) and the dipole (or monopole) on A. This is unusually favorable for hydrogen because it is so small that the dipoles (or monopoles) can approach closely. There may also be a quantum mechanical contribution, corresponding to a resonance hybrid, D-H····A ↔ D^-··H—A^+, of two resonance forms (which must have identical internuclear distances). Alternatively, in terms of molecular orbital theory, the hydrogen bond corresponds to a delocalization of electron density into molecular orbitals constructed from the atomic orbitals on D, H, and A. For the hydrogen bond to provide significant attraction, both D and A must be small electronegative atoms, so that the dipole moments (or monopole charge densities) are large and so that they can approach closely. Also, the two resonance forms must not be of very different energies, or else the form of higher energy will not contribute to the hybrid and provide stabilization. In molecular orbital terms, this means that the atomic orbitals on D and A must be of similar energies, or that D^- and A must have similar basicity.

The hydrogen bond is a key feature of molecular structure, including that of biomolecules (*2*). It is responsible for the well-known base pairing in nucleic acids and for the secondary structure of proteins. Also, hydrogen bonding is important for the catalytic action of many enzymes (*3*) and for the binding specificity of enzyme inhibitors (*4*). Even in the synthesis of small molecules, hydrogen bonding has been recognized as a key design and control element. It can be important in enantioselective synthesis (*5*), for facial selectivity (*6*), and for molecular recognition by synthetic receptors of adenines (*7*) and barbiturates (*8*). Several groups have taken advantage of hydrogen bonding to design large self-assembling supramolecular complexes (*9*).

The author is with the Department of Chemistry, University of California at San Diego, La Jolla, CA 92093–0358, USA.

Symmetry of Hydrogen Bonds

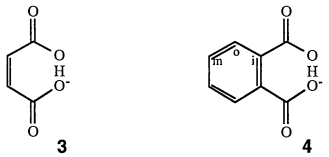
A fundamental question regarding hydrogen bonds is whether the potential energy for motion of the hydrogen has a single minimum ("well") or two minima (Fig. 1). If single, the hydrogen is centered between the two acceptor atoms (**1**), and the hydrogen bond is unusually strong. If there are two minima, the hydrogen is closer to one acceptor than to the other (to which acceptor it is closer depends on the relative energies of the two minima). There are then two different tautomeric forms (**2**), which equilibrate rapidly with each other, and the centered structure is merely the transition state for proton transfer, which interconverts the two tautomers (actually, this also requires that the zero-point energy be lower than the barrier between the two wells, as drawn in Fig. 1).



1 **2**

If the two acceptor atoms are identical, it might naively be thought that the hydrogen bond must be symmetric, so that the hydrogen would not need to choose which acceptor to be closer to. Nevertheless, both situations have been observed, even with identical acceptor atoms (*10*). An asymmetric hydrogen bond seems to be more common. One of the best studied examples is $CH_3C(OH)=CHCOCH_3$, the enol of 2,4-pentanedione, which is a mixture of two tautomers, each with the hydrogen covalently bonded to one oxygen and hydrogen-bonded to the other.

In contrast, a single symmetric structure is seen for hydrogen maleate (**3**) and hydrogen phthalate (**4**) monoanions in crystals. These anions have unusually strong hydrogen bonds, even in solution, as evidenced by large differences between their first and second acidity constants. One empirical generalization is that the symmetric form becomes favored only when the oxygen-oxygen distance is ≲2.5 Å. This is reasonable because as two energy wells approach each other, the barrier between them eventually disappears, and the two wells are transformed into a single one.



3 **4**

The best prospects for a symmetric hydrogen bond are when the two (deprotonated) acceptor atoms are of identical basicity, as in **3** and **4**. Then, if the hydrogen bond is symmetric, the two resonance forms are of identical energy, so that they contribute equally, leading to great resonance stabilization. There may also be additional covalent character to both of the O–H bonds (*11*).

Knowledge of the symmetry of hydrogen bonds is important for understanding the structure and action of biomolecules. It is essential to know all interatomic distances accurately because they are critical for biological function. Even minute changes, as small as 0.01 Å, may be significant, as judged from the sensitivity of enzymes to substrate structure. Because a symmetric hydrogen bond favors a shorter distance between the acceptor atoms and holds them closer, it is important to know when a hydrogen bond may be symmetric.

Potentially symmetric hydrogen bonds have become of great interest recently because of their role in enzyme action (*12*). It has been proposed that their high strength provides 10 to 20 kcal/mol of stabilization to otherwise unstable intermediates in enzyme-catalyzed reactions. A key requirement for such stabilization is the matching of the acidity constants (pK_a) of the two donors, so that the hydrogen will not bond more strongly to one than to the other.

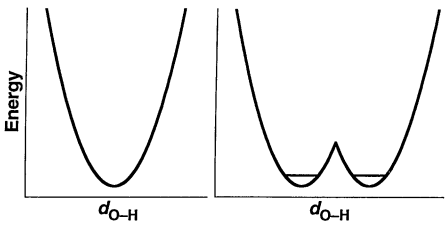


Fig. 1. Single- and double-well potentials for motion in a hydrogen bond. Energy is plotted versus the O–H distance. The horizontal line is the zero-point energy.

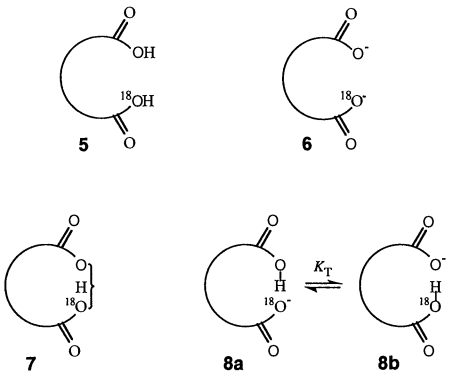
Knowledge of the symmetry of hydrogen bonds may also be important for the design of small molecules that are to exhibit intermolecular recognition (*13*). Ordinarily, a severe limitation to binding is that hydrogen bonding can provide little binding attraction in aqueous solution. This is because the formation of a hydrogen bond in water is at the expense of hydrogen bonds to water (*14*), so that the net binding is rarely sufficient to provide strong binding. To achieve in water the nanomolar dissociation constants characteristic of some biological macromolecules, it may be necessary to go beyond the ordinary hydrogen bonds and to use strong, symmetric ones.

Determination of Hydrogen-Bond Symmetry

Previous experimental methods for determining the symmetry of hydrogen bonds include microwave spectroscopy, which is applicable only in the gas phase, and x-ray or neutron diffraction, which is applicable only in crystalline materials. Most studies in solution have used infrared spectroscopy, but that does not provide an absolute answer because it relies on empirical correlations with other hydrogen bonds that are thought to be symmetric. Also, infrared spectroscopy is difficult to apply to water, a medium that is of special interest for hydrogen bonding. Two other methods applicable to solutions make use of the difference between chemical shifts of protium and deuterium (*15*) or the quadrupole coupling constant of deuterium (*16*), but these methods require independent knowledge of how these parameters might vary with the position of the hydrogen along the O-O axis. They too are rarely applicable to aqueous solutions because the hydrogen-bonded proton or deuteron ordinarily exchanges with solvent and cannot be seen. Even quantum mechanical calculations (*17*) are not reliable because the shape of the potential energy surface and the number and positions of its minima vary with the level of approximation. However, a new direct method that uses nuclear magnetic resonance (NMR) can give definitive answers to the question of the symmetry of hydrogen bonds, even in aqueous solution.

The familiar NMR methods are incapable of answering this question because even if there are two forms, they will interconvert so rapidly that only an averaged NMR spectrum will be seen, and this will be indistinguishable from the spectrum of a symmetric structure. Our ability to probe molecular symmetry depends on the method of isotopic perturbation of equilibrium (*18*). Saunders and co-workers (*19*) have applied this method extensively to carbocations, including the controversial norbornyl cation.

The following example illustrates this method. The ^{13}C NMR spectrum of a mono-^{18}O–substituted dicarboxylic acid (**5**) shows a signal near chemical shift δ 170, due to the carboxyl carbon. The signal of the dianion (**6**) is near δ 176. The change of 6 parts per million reflects the sensitivity of chemical shift to the state of protonation, which is what makes the method possible. The question we ask is whether the monoanion exists as a single structure (**7**) or as a mixture of two tautomeric forms (**8a** and **8b**) that differ in the position of the proton. Because of the rapid proton exchange from carboxyl to carboxylate, individual ^{13}C NMR signals of separate CO_2H and CO_2^- groups cannot be observed. Instead, only an averaged signal is seen, with an observed chemical shift that is the average of the chemical shifts of the protonated and unprotonated forms, weighted by their relative amounts. Ordinarily, that would be a 50:50 average, near δ 173. However, the isotope changes the vibrational frequencies of a carboxylic acid and of a carboxylate and thereby changes their zero-point energies. As a result, tautomer **8b** is very slightly more stable than **8a**. Indeed, it is known that ^{18}O decreases acidity (*20*). Thus, the carbon attached to ^{18}O is more likely to be a carboxylic acid, and the carbon attached to ^{16}O is more likely to be a carboxylate. Consequently, in the averaging, the chemical shift of the former is closer to that of carboxylic acid (δ 170), and the shift of the latter is closer to that of carboxylate (δ 176). This represents a small upfield shift for the carbon attached to ^{18}O and a small downfield shift for the carbon attached to ^{16}O, and thus a separation between these two carbon signals.



Actually, the situation is complicated by intrinsic isotope shifts, whereby a heavier isotope shifts its attached ^{13}C upfield (*18*, *21*). For a carboxyl group, the two signals are separated by ~0.026 ppm even in the diacid and the dianion. Therefore, the observed separation in the monoanion must be corrected for this instrinsic shift. This analysis implies that if the monoanion is a mixture of two tautomers (**8a** and **8b**), there will be an additional isotope shift in the monoanion, beyond the intrinsic shift, as a result of isotopic perturbation of the equilibrium. In contrast, if the monoanion is a single structure (**7**), there are no such complications, and only a constant intrinsic shift will be seen, independent of the state of protonation.

It is possible to be more quantitative for the case of a double-well potential, leading to two species **8a** and **8b**. The isotope shift Δ in the monoanion is related to the tautomeric equilibrium constant K_T by

$$\Delta = \Delta_0 + \frac{K_T - 1}{K_T + 1 + (4rK_T)^{1/2}} D \quad (1)$$

where Δ_0 is the intrinsic isotope shift as seen in the diacid or dianion, r is the ratio of second and first acidity constants of the diacid, and D is the difference between the chemical shifts of the carboxylic acid and carboxylate groups in the monoanion. The value of D is unknown but can be approximated by the difference between the chemical shifts of the diacid and the dianion.

To apply this method, several acids were titrated with small aliquots of KOH, and ^{13}C NMR spectra were obtained after each addition, with careful attention to resolving the small isotope shifts. According to the above analysis, the isotope shift is expected to increase upon addition of base, to reach a maximum at half neutralization, and to return to the initial intrinsic isotope shift at two equivalents of base. Indeed, in a test of the method, we observed (*22*) an increased isotope shift in the monoanion of succinic acid (**5**, linker = CH_2CH_2), and similar results were obtained for a statistical mixture of all succinic acid–^{16}O,^{18}O isotopologs (*23*). The tautomeric equilibrium constant K_T estimated from Eq. 1 agrees well with values previously measured for such acids (*20*) and also with values estimated (*22*) from the effect of isotopic mass on vibrational frequencies. Therefore, this method can detect a proton-transfer equilibrium between a carboxylic acid and a carboxylate.

An additional isotope shift of the same magnitude was unexpectedly also obtained for the monoanions of maleic (**3**) and phthalic (**4**) acids. Isotope shifts for phthalic acid are seen not only at the carboxyl carbon but also at the more distant ipso, ortho, and meta carbons, where the intrinsic shifts are too small to be resolved. These monoanions had been considered canonical examples of a symmetric hydrogen bond. They ought to have shown only an intrinsic isotope shift, independent of the extent of neutralization.

It might be thought that the method of isotopic perturbation is forced into yielding the conclusion that every structure appears asymmetric simply because the isotopic substitution creates an asymmetry. Not only is an ^{18}O needed for the perturbation, but in

addition, a ^{13}C is needed for the NMR detection, and only without these would the molecule be truly symmetric. However, it follows from the Born-Oppenheimer approximation (*24*) that the potential energy surface governing nuclear motion, whether containing a single or double well, is independent of nuclear mass. Therefore, isotopic substitution can probe whether the hydrogen bond is symmetric or asymmetric.

Because these results were so unexpected and the isotope shifts observed are hardly larger than the intrinsic shifts, several control experiments were obligatory (*22*). These experiments involve an inverse dependence of isotope shifts on temperature, a doubled effect with two substituting ^{18}O atoms, an increased isotope shift in D_2O compared with H_2O, and substantial isotope shifts at the ipso, ortho, and meta carbons of the hydrogen phthalate monoanion. Moreover, the isotope shifts of the latter two carbons are downfield, in sharp contrast to the upfield intrinsic shifts that were seen nearly universally. We therefore conclude that hydrogen maleate (**3**) and hydrogen phthalate (**4**) monoanions indeed have their hydrogens in double-well potentials.

Comparison of Crystalline and Aqueous Media

The conclusion that these hydrogen bonds are asymmetric contradicts previous x-ray and neutron diffraction results that are definitive; yet ours also seem incontrovertible. Perhaps crystallographers have been wrong for 70 years, and there is never a truly symmetric hydrogen bond. However, HF_2^- is unquestionably symmetric. We therefore rationalized this contradiction by recognizing that our study was carried out in aqueous solution and that water is different from crystals. Despite the importance of aqueous solutions, no studies of the symmetry had been carried out in water. However, a moment's reflection discloses the difficulty of doing so.

What makes water different is that it is a liquid that hydrogen bonds to the carboxylate group. On the average, both carboxyls must be solvated equally. However, water is a disorganized medium. It would cost considerable negative entropy to produce the long-range organization of the hydrogen-bonded network required to solvate both carboxyls identically. If instead one carboxyl group is more strongly solvated than the other, then it will prefer to be the carboxylate and the proton will then be attached to the other carboxyl. This differential solvation creates an asymmetry of the energy wells. Even though a single-well potential may be favored in the symmetric environment of a crystal, we proposed (*22*) that the disorder of the aqueous environment makes the hydrogen bond asymmetric. Indeed, an asymmetric hydrogen bond is seen in hydrogen maleate crystals where the cation is placed asymmetrically (*25*), and it has long been recognized (*10*) that asymmetric crystal forces can be strong enough to favor an asymmetric hydrogen bond. Nevertheless, it was unexpected that the seemingly minor change from crystal to water would be sufficient to do so.

Molecular orbital calculations support this proposal (*26*). The effect of solvation is to raise the energy of the symmetric structure relative to the asymmetric one. This is because a polar environment stabilizes a concentrated negative charge more than it does a delocalized one, as in a symmetric structure. However, these calculations modeled the solvent as a continuous dielectric, which does not take into account the dynamic and discrete aspects of water. Therefore, it is even more likely that aqueous solution might make asymmetric a hydrogen bond that is otherwise symmetric.

To test this possibility, we measured the isotope shifts of hydrogen maleate (**3**) and hydrogen phthalate (**4**) anions, with appropriate cations, in organic solutions, both in non–hydrogen-bonding solvents dimethyl sulfoxide (DMSO) and acetonitrile and in the nonpolar solvent tetrahydrofuran (THF). The intrinsic isotope shift remains unchanged in these solvents. In both DMSO and acetonitrile, the isotope shift is less than in aqueous solution. This may be because the environment is more symmetric, but it may also be the result of a reduction in the unknown D of Eq. 1 (*27*). In the still less polar THF, the hydrogen maleate anion, with tetrabutylammonium counterion, shows no additional isotope shift beyond the intrinsic shift. In this medium, this anion does have a symmetric hydrogen bond. This result is reassuring because it does not contradict previous ones in crystals (*10*) and in CH_2Cl_2 (*15*, *16*). Moreover, this result verifies that the isotopic substitution itself cannot be responsible for creating the asymmetry that is seen in aqueous solution.

Even tetrabutylammonium hydrogen succinate shows a symmetric hydrogen bond in THF. This is a double result because a symmetric hydrogen bond requires an intramolecular one, even though aqueous hydrogen succinate monoanion (**8**, linker = CH_2CH_2) has no such hydrogen bond (*28*). In so nonpolar a solvent, the best solvation for a carboxylate anion is a hydrogen bond from a carboxylic acid. Thus, the conformation must have changed to permit a hydrogen bond that is not only intramolecular but also symmetric.

These results do not distinguish whether hydrogen bonds become asymmetric in water because of its polarity or because of its disorder. It seems that polarity itself is not sufficient because a crystal, with its counterions, is also quite polar, having strong electric fields, and yet the hydrogen bond is symmetric. Therefore, we conclude that the hydrogen bonds become asymmetric in water because of its disorder, which makes it improbable that both of the carboxyls would be simultaneously solvated in an identical manner. Certainly, the importance of the local environment in determining the symmetry of the hydrogen bond is demonstrated by the fact that the symmetry seen in crystals or nonpolar solvents is broken in aqueous solution. This is a remarkably simple counterexample to the prevailing hope that a crystal structure describes the solution structure.

How likely is a single-well hydrogen bond in water? According to the crystal structures, monoanions **3** and **4** seemed like the most favorable cases, but they are asymmetric. Other candidates are derived from 1,8-naphthalenedicarboxylic acid, which permits a shorter oxygen-oxygen distance; quinolinic acid, which is a zwitterion; and 1,8-diaminonaphthalenes ("proton sponges") (*10*).

These results are of relevance to a recent proposal of a symmetric double-minimum hydrogen bond in crystalline amides such as *N*-methylacetamide and polyglycine (*29*). The customary view of a –C=O··H–N– fragment is that the proton is covalently bonded to the nitrogen and only hydrogen-bonded to the oxygen. The evidence for a new interpretation was the inelastic neutron scattering spectrum, which shows a 1575-cm^{-1} vibrational mode attributed to an N-H stretch, rather than the 3250-cm^{-1} mode that appears in the infrared spectrum and is now attributed to an overtone. This lower frequency was interpreted as meaning that the N–H bond is weakened by proton transfer to the neighboring oxygen, and

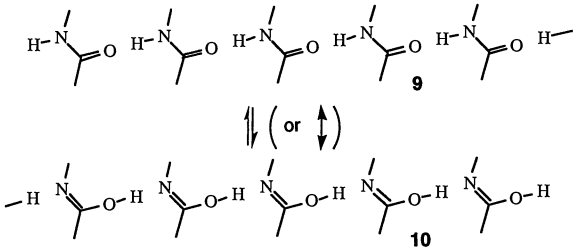


Fig. 2. Previously proposed mechanism for proton transfer down a chain of hydrogen-bonded peptide groups by means of high-energy imidic acid tautomers.

that there is dynamic proton exchange between amidic C(=O)N–H and imidic C(OH)=N forms. However, the rarity of symmetric hydrogen bonds makes this interpretation suspect. Certainly, the basicities of the two acceptor atoms are badly mismatched, inasmuch as the acidity constants of imidic acids and amides are <11 (inferred from isoelectronic enols) and 18 (extrapolated to aqueous solution), respectively (*30*). Therefore, the two resonance forms are of very different energies, as can also be recognized from the estimated tautomeric equilibrium constant of 10^{-8} between amide and imidic acid (*31*) and from the calculated energy difference of 12 kcal/mol (*32*). A symmetric NHO hydrogen bond, with the hydrogen equally likely on the N or the O, is therefore quite doubtful.

This proposal also arises frequently in connection with protein-mediated proton transport and proton exchange (*33*). It is often suggested that proton transfer proceeds along a chain of peptide groups (Fig. 2). Donation of a proton at one end of **9** causes the protons in all of the hydrogen bonds to shift from nitrogen to oxygen, forming **10** and permitting a different proton to be released at the other end. This is consistent with the above proposal of a symmetric double-minimum hydrogen bond, which thereby facilitates the proton transfer. In terms of that proposal, **9** and **10** are contributing resonance forms. However, **10** is composed of the unstable imidic acid tautomers of an amide. It is higher in energy by 12 kcal/mol per peptide fragment, and it is of too high an energy to contribute. As further evidence, such proton transfer is quite slow. For example, the rate constant for acid-catalyzed proton exchange of *N*-formylglycine, which proceeds through the imidic acid $HC(OH)=NCH_2COOH$ as intermediate, is only 2.2 M^{-1} s^{-1} (*34*). This means that it takes more than a month at pH 7 to produce such an intermediate.

Conclusions

The question of the symmetry of a hydrogen bond is more complicated than previously thought. The symmetry is not simply inherent in the molecular structure, depending only on the oxygen-oxygen distance. In addition, it can depend on the environment. The method of isotopic perturbation has demonstrated itself to be useful and effective for the study of hydrogen bonding, and it provides definitive answers to the question of the symmetry of the hydrogen bond.

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