Modification of pK values caused by change in H-bond geometry

(proteins/*ab initio*/molecular orbital/proton transfer)

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ABSTRACT The competition between various groups for a proton is studied by *ab initio* molecular orbital methods. It is found that reorientations of the two groups involved in a H-bond can reverse the equilibrium position of the proton shared between them. Specifically, the carbonyl and hydroxyl groups were modeled by H_2CO and HOH . In the H-bond between these two groups, association of the proton with the carbonyl ($\text{H}_2\text{COH}\cdots\text{OH}_2$)⁺ is favored over the hydroxyl ($\text{H}_2\text{CO}\cdots\text{HOH}_2$)⁺ when the latter group is situated along a lone pair of the carbonyl oxygen. However, displacement of the water to the C=O axis between the two carbonyl lone pairs reverses the situation and ($\text{H}_2\text{CO}\cdots\text{HOH}_2$)⁺ is more stable. A similar reversal of stability is observed in the H-bond involving a Schiff base (modeled by CH_2NH) and amine (NH_3). In one arrangement where the lone pairs of the two groups point toward one another, the proton prefers the Schiff base to the amine—i.e., ($\text{H}_2\text{CHNH}\cdots\text{NH}_3$)⁺ is more stable than ($\text{H}_2\text{CHN}\cdots\text{HNNH}_3$)⁺. On the other hand, rotation of the lone pair of the amine away from the Schiff base nitrogen results in proton transfer across to the amine. These shifts in stability correspond to reversal of relative pK of the groups involved. A fundamental principle emerging from the calculations is that ion-dipole electrostatic interactions favor transfer of a proton to the group that is positioned as closely as possible to the negative end of the dipole moment vector of the other. The ideas developed here suggest a number of means by which conformational changes may be utilized to shift protons from residue to residue within a protein molecule such as an enzyme or bacteriorhodopsin.

The concept of pK has been an extremely useful one in analyses of protein structure and function (1, 2). Comparison of the pK of several residues leads to predictions as to the protonation state of each at a given pH. For example, since the pK of the histidine side chain (6.0) is higher than that of aspartic acid (3.9), it would normally be expected at intermediate pH such as 5 that the imidazole portion of histidine be positively charged and aspartic acid contain a negative COO^- moiety (1, 2). It is of course well recognized that structural and environmental features of the protein can influence the protonation state of each residue. These effects are frequently referred to as perturbations of the pK and are understood to arise from a number of factors. The first concerns the general character of the local environment in which the residue finds itself. Since the unperturbed pK is measured as an equilibrium in aqueous solution, it is not surprising that shielding the residue from solvent in the interior of a protein can modify its pK. As an example, the pK of aspartic acid is generally accepted to rise as the environment becomes more hydrophobic (1, 2) since lowering of the dielectric constant will preferentially destabilize the charged COO^- . Another factor here is the removal of the H-bonds that would normally form between aspartic acid and water in

solution. These H-bonds are an example of specific interactions involving the residue that can also affect its observed pK, changing it in such a direction as to reflect the added stability of one charge state or the other. Thus, H-bonding will preferentially stabilize COO^- over COOH (lower pK) and imidazole H^+ over imidazole (higher pK). Similar reasoning can be used to infer the effects of placing electrically charged species in the vicinity of the residue.

Let us now consider a pair of residues R_1 and R_2 that are linked together by a H-bond. In the competition for an added proton, it would be generally considered that the residue with the higher effective pK would win. Although this may very well be the case, we will attempt to demonstrate in this communication that the proton can be shifted from one residue to the other merely by changing the geometrical details of the H-bond connecting them. If the protonation states of the two residues are discussed in terms of pK, it is therefore possible to reverse the order of pKs of the two residues by changing the relative orientation of the H-bonding groups without any additional factors—e.g., motion of charged groups. This principle leads to the possibility of an attractive means of coupling pK shifts in a protein to conformational changes that affect the H-bond geometry.

It is quite difficult to examine the effect of intermolecular orientation upon protonation state equilibria experimentally for a number of reasons. When placed in solution, the pK of a molecule is obtained as a weighted average of many different orientations that the solvent molecules adopt relative to the solute over the time of the measurement. A similar problem of being unable to study a single intermolecular orientation is encountered in gas-phase work as well (3). It is possible in principle to synthesize molecules containing spatially restrained intramolecular H-bonds but this is difficult to do in a systematic fashion. An example of an experimental examination of this problem is provided by recent work of Menger *et al.* (4), who synthesized a series of three rigid carbon frameworks holding an oxygen base near a mobile C—H proton in well-defined angular and distance relationships. Although proton transfer was associated with a 24° shift in orientation, the interpretation of the results was ambiguous due to a concomitant 0.7-Å variation in distance between the C and O atoms in the three molecules.

In contrast to experimental limitations, specific geometries may be precisely specified and investigated systematically by using *ab initio* theoretical procedures (5, 6). It is a straightforward matter to study the intrinsic properties of the H-bond without complications involving solvent molecules. Of particular importance, it is possible to obtain calculated results comparable in accuracy to experimental data by appropriate choice of quantum chemical technique (5, 6).

In the present communication we consider two different proton transfer equilibria. The first involves the competition for a proton between a hydroxyl group, as might occur in water or serine, and the carbonyl C=O functionality of, for example, the peptide backbone or glutamic acid and aspartic acid side chains. The second case examines the Schiff base

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functionality due to its great importance in the proton pumping action of bacteriorhodopsin.

All calculations were carried out by using the GAUSSIAN-80 *ab initio* computer codes (7). The 4-31G* basis set (8, 9) was employed since its double- ζ description and the *d*-functions added to C, N, and O are expected to provide the flexibility necessary to adequately represent all electronic structures. In addition, as described below, this basis set closely reproduces the relative intrinsic proton affinities of the various molecules. All geometry optimizations made use of the gradient procedures contained within the GAUSSIAN-80 program.

CASE 1: CARBONYL AND HYDROXYL

In order to carry out the calculations it is necessary to choose small model systems that retain the essential features of the groups being studied. The water molecule is an obvious choice to model the hydroxyl group both because of its direct involvement in H-bonds and its small size, which allows very accurate calculations. Formaldehyde H_2CO is selected as our prototype carbonyl-containing molecule.

As the primary matter of interest here concerns the ability of each molecule to attract a proton, it is essential that the theoretical procedure being applied accurately reflects the difference in proton affinity between them. The proton affinities of both H_2O and H_2CO have been measured experimentally (3, 10, 11) and are equal to 170 and 177 kcal/mol (1 cal = 4.184 J), respectively. The theoretical proton affinities of both H_2O and H_2CO were calculated as the difference in energy between these fully optimized molecules and their respective protonated analogs H_3O^+ and H_2COH^+ . To this was added the contribution of zero-point vibrational energies (12, 13) and thermal correction terms. The proton affinities calculated for H_2O and H_2CO were 169 and 176 kcal/mol, respectively. These values are quite close to the experimental measurements; more important, the difference between them is 7 kcal/mol, identical to the experimental difference. We thus feel confident that our theoretical approach presents an accurate picture of the true situation.

The competition between the carbonyl and hydroxyl groups for a proton is modeled here by placing the proton in the H-bond connecting H_2CO and H_2O . The question to which we address ourselves is the relative stability of the situation where the proton is covalently bound to the carbonyl ($\text{H}_2\text{COH}\cdots\text{OH}_2^+$)⁺ as compared to the opposite case where the proton is primarily associated with the water ($\text{H}_2\text{CO}\cdots\text{HOH}_2^+$)⁺. The geometry of the H-bond is illustrated at the top of Fig. 1, from which the H atoms have been deleted for purposes of clarity. The angle θ is equal to the deviation of the O atom of water from the $\text{C}=\text{O}$ internuclear axis. In order to focus our attention on this angular feature of the H-bond, the intermolecular R(OO) distance is frozen at 2.75 Å in all calculations described herein. All other geometrical features of each configuration are fully optimized.

Surveys of a large body of crystallographic data from organic and biological molecules containing the carbonyl group (14, 15) have demonstrated a wide range of H-bond angles over which θ is found to occur. We have accordingly investigated the energetics of the proton position equilibrium for a series of angles varying over the range observed. Potential energy curves calculated for the motion of the central proton between the carbonyl and hydroxyl are presented in Fig. 1 for each of three different values of θ . Let us focus our attention first on the leftmost proton transfer potential pertaining to an angle of 72° , which corresponds to the hydroxyl being situated approximately along the direction of an sp^2 lone pair of the carbonyl oxygen. [This angle is also the optimized value for the ($\text{H}_2\text{COH}\cdots\text{OH}_2^+$)⁺ configuration.] This potential contains two wells; the left represents the situation where the proton is bound covalently to the carbonyl group ($\text{H}_2\text{COH}\cdots\text{OH}_2^+$)⁺. This minimum may be seen to be 8.8 kcal/mol more stable than the right well where the proton is associated with the water ($\text{H}_2\text{CO}\cdots\text{HOH}_2^+$)⁺. This fact is not surprising in light of the naturally greater proton affinity of H_2CO than of H_2O pointed out above. The middle potential represents the situation where the hydroxyl lies 40° from the $\text{C}=\text{O}$ axis and it may be seen that in this case, the two wells are separated by only 2.1 kcal/mol. Even more dramatic is the fully linear ($\theta = 0^\circ$) H-bond depicted on the right. Here the right well is lower in energy than the left,

FIG. 1. Calculated energy curves for transfer of a proton between a carbonyl and hydroxyl group. All energy differences are in kcal/mol. Hydrogens not directly involved in the transfer are not shown explicitly but rather by the lines denoting $\text{C}-\text{H}$ and $\text{O}-\text{H}$ bonds. Full geometry optimizations were carried out subject to the constraints described in the text. The COH bond angle in all ($\text{H}_2\text{COH}\cdots\text{OH}_2^+$)⁺ configurations is within 5° of 120° ; similarly, the central proton lies within 5° of the $\text{O}-\text{O}$ axis in all ($\text{H}_2\text{CO}\cdots\text{HOH}_2^+$)⁺ configurations. The $\text{O}-\text{H}$ bond lengths to the central proton are within 0.03 Å of 1.00 Å in both configurations. The angle between the plane of the righthand OH_2 subunit and the $\text{O}-\text{O}$ axis lies in the range 155° to 170° in the left wells of the potentials; this angle is between 130° and 136° in the right wells.

corresponding to a preference of the proton for the water by 7.5 kcal/mol. Thus, by changing the relative orientation of the two groups—i.e., the angular features of the H-bond—the relative stabilities of $(\text{H}_2\text{COH}\cdots\text{OH}_2)^+$ and $(\text{H}_2\text{CO}\cdots\text{HOH}_2)^+$ have been reversed by a total of 16.3 kcal/mol. This reversal may be described as a shift in relative pK of the carbonyl and hydroxyl groups induced merely by a change in H-bond geometry.

CASE 2: SCHIFF BASE AND AMINE

As our model of the Schiff base connecting the retinal chromophore of bacteriorhodopsin with the ϵ -amino group of lysine we take methylenimine $\text{H}_2\text{C}=\text{NH}$, which contains both the double and single bonds to the nitrogen atom. In analogy with the previous case, NH_3 is used for the other group that competes with the Schiff base for the proton. This amine group is expected to serve as a suitable prototype for various protein groups such as lysine, arginine, or histidine side chains, which form H-bonds via N atoms. The appropriateness of using the 4-31G* basis set was again tested by evaluation of the proton affinities of the two molecules. The calculations predict the methylenimine to have a greater proton affinity than NH_3 by 5 kcal/mol. The experimental correlate of this quantity remains rather uncertain due to difficulties in measurement of the proton affinity of CH_2NH ; however, a difference of 5 kcal/mol does appear to be within the range of uncertainty of the measurements (10).

As in the previous case, the distance between first-row atoms was held fixed at 2.75 Å. The angle θ was chosen to be that between the N—N axis and the local C_3 symmetry axis of NH_3 . We may speak of this latter axis also as the direction of the single N lone pair of NH_3 . Thus, θ represents the angle between the amine lone pair and the nitrogen of the Schiff base. Proton transfer potentials were calculated for θ angles of 0° and 55° and the results are illustrated in Fig. 2. All geometries were fully optimized subject to the above constraints and to a fixed $\theta(\text{CNN})$ angle of 129° , corresponding to the Schiff base lone pair pointing toward the amine N atom.

It may be noted from Fig. 2 that for $\theta = 0^\circ$, the $(\text{H}_2\text{CHNH}\cdots\text{NH}_3)^+$ configuration is more stable than $(\text{H}_2\text{CHN}\cdots\text{HNNH}_3)^+$ by 2.5 kcal/mol. Thus, the central proton

FIG. 2. Proton transfer potentials (in kcal/mol) involving methylenimine and ammonia. $R(\text{NN}) = 2.75$ Å and $\theta(\text{CNN}) = 129^\circ$. The NH_3 subunit was held in local C_3 symmetry; the angle between the C_3 axis and N—N is denoted as θ . The central proton lies 4° and 11° from the N—N axis in the left wells for $\theta = 0^\circ$ and 55° , respectively. The corresponding angles are 1° and 50° in the right wells. The $r(\text{NH})$ bond lengths for this proton are within 0.02 Å of 1.03 Å in all cases.

prefers association with the Schiff base to the amine group. However, the situation is reversed when the amine lone pair is rotated by 55° away from the Schiff base nitrogen; in this case the former configuration is *less* stable by an equal amount of 2.5 kcal/mol. At the same time, the latter rotation has substantially raised the barrier to proton transfer, due largely to the misalignment of the N lone pairs. Thus, an angular reorientation of the H-bond is again capable of reversing the equilibrium position of the proton and shifting it between the two groups involved in the bond. Rotation of the lone pair of the NH_3 subunit in the opposite direction—i.e., downward—produced an even greater destabilization of the $(\text{H}_2\text{CHNH}\cdots\text{NH}_3)^+$ configuration. In this case, not shown in Fig. 2, the left well was found to be higher in energy than the right by 7.1 kcal/mol.

DISCUSSION

The first question to which we address ourselves is the underlying reasons that account for these reversals in stability of the two wells in each potential. We begin our discussion with case 1 involving the carbonyl and hydroxyl groups. Let us consider the $\text{H}_2\text{COH}\cdots\text{OH}_2$ configuration with the proton residing on the carbonyl group with $\theta = 72^\circ$. Displacement of the OH_2 subunit to the $\text{C}=\text{O}$ axis ($\theta = 0^\circ$) raises the energy of the system by distorting the H-bond such that the $\text{O}\cdots\text{H}\cdots\text{O}$ angle is substantially smaller than 180° . A similar transition of θ from 72° to 0° produces the opposite result of a small *decrease* in energy of the $\text{H}_2\text{CO}\cdots\text{HOH}_2$ configuration for two primary reasons. First of all, there is little distortion of the H-bond since the central proton can remain along the O—O axis for both values of θ . Second, and equally important, is the fact that when $\theta = 0^\circ$ the positively charged HOH_2^+ subunit is situated directly along the dipole moment vector of H_2CO , stabilizing the righthand well via a charge-dipole interaction. In summary, decreasing θ from 72° to 0° raises the energy of the $\text{H}_2\text{COH}\cdots\text{OH}_2$ configuration but stabilizes $\text{H}_2\text{CO}\cdots\text{HOH}_2$, leading to the observed reversal in relative energy.

One clear distinction between H_2CO and H_2CNH is the number of lone electron pairs. H_2CO has two pairs on the oxygen; an additional proton will align itself along one of these pairs and the ensuing O—H bond will deviate by 62° from the dipole moment vector that lies along the $\text{C}=\text{O}$ axis of H_2CO . In contrast, the nitrogen of H_2CNH has only one lone pair that roughly coincides with the molecular dipole moment. When an excess proton is added to H_2CNH , the N—H bond makes an angle of 122° with the $\text{C}=\text{N}$ bond; hence, the CNN angle in $(\text{H}_2\text{CHNH}\cdots\text{NH}_3)^+$ will assume a comparable value in order to maintain a linear H-bond. This situation corresponds to the left potential in Fig. 2. Displacing the NH_3 subunit towards the $\text{C}=\text{N}$ axis will destabilize this configuration by destroying the linear character of the H-bond as noted above for $(\text{H}_2\text{COH}\cdots\text{OH}_2)^+$. The analogy between the two systems is removed when the proton is shifted across to the other subunit, however. Whereas the $(\text{H}_2\text{CO}\cdots\text{HOH}_2)^+$ configuration is stabilized by the ion-dipole attraction when the HOH_2^+ lies along the $\text{C}=\text{O}$ direction, this is not the case for $(\text{H}_2\text{CHN}\cdots\text{HNNH}_3)^+$ where the dipole of the left subunit does *not* point along the $\text{C}=\text{N}$ axis. Consequently, displacement of the NH_3 subunit to the $\text{C}=\text{N}$ axis was found incapable of reversing the relative stability of the $(\text{H}_2\text{CHNH}\cdots\text{NH}_3)^+$ and $(\text{H}_2\text{CHN}\cdots\text{HNNH}_3)^+$ configurations.

On the other hand, the energies of these two configurations *can* be affected in the desired manner by rotation of the ammonia, keeping its position fixed with respect to the Schiff base. As pointed out in Fig. 2, a rotation of the ammonia by 55° did in fact reverse the stability of the two configurations. The reason for this reversal is that the rotation turns the amine dipole moment away from the positively charged

H_2CHNH^+ subunit in the $\text{H}_2\text{CHNH}\cdots\text{NH}_3$ configuration, thereby destabilizing the left well by a large amount. The greater destabilization of the left well than of the right is particularly notable in light of the more severe distortion of the linear $\text{N}\cdots\text{H}\cdots\text{N}$ arrangement in the latter configuration, which one might normally expect to raise its energy by a greater amount.

It was pointed out above that a downward rotation of the dipole of the NH_3 subunit produces a greater preferential destabilization of the $(\text{H}_2\text{CHNH}\cdots\text{NH}_3)^+$ than does the upward rotation depicted in Fig. 2. This observation is due to the greater fraction of positive charge of the $(\text{H}_2\text{CHNH})^+$ subunit localized on the CH_2 group than on the imine H atom (as revealed by a Mulliken population analysis). Consequently, rotation of the negative end of the NH_3 dipole downward toward the less positively charged H raises the electrostatic energy more than when the dipole is turned toward the CH_2 .

The changes in equilibrium position of the central proton are clearly associated with energy increases imposed on each configuration by angular "distortions" of geometries that would otherwise be preferred in the absence of macromolecular steric constraints. An important question thus concerns the degree to which these angular deformations weaken the H-bond existing between the two subunits. In other words, is the interaction attractive even in the face of considerable rotations of one group or the other? The answer to this question is yes. Specifically, the interaction energy in the $(\text{H}_2\text{COH}\cdots\text{OH}_2)^+$ configuration illustrated on the left side of Fig. 1 with $\theta = 72^\circ$ is 28.1 kcal/mol, relative to the isolated $(\text{H}_2\text{COH})^+$ and H_2O subunits. Even after suffering a distortion energy of 14.6 kcal/mol as a result of reducing θ to 0° , the interaction remains attractive by 13.6. The data for the imine-amine system are rather similar. The strength of the H-bond in the $(\text{H}_2\text{CHNH}\cdots\text{NH}_3)^+$ configuration for $\theta = 0^\circ$ in Fig. 2 is 25.2 kcal/mol. Rotation of the NH_3 subunit by 55° weakens this interaction by only 13.4 kcal/mol, leaving a net attractive force of 11.8.

There is a clear pattern emerging from analysis of the calculated data that may be quite useful in predicting trends in pK shifts in terms of angular features of the relevant H-bonds. The main principle underlying these trends is that a proton is more likely to be located on group B when this group is located as close as possible to the dipole moment direction of group A—that is, the energy of $\text{A}\cdots\text{HB}$ is lowered relative to $\text{AH}\cdots\text{B}$ by the electrostatic ion-dipole interactions in such an arrangement. A corollary of this statement leads to a second means by which group B may enhance its probability of capturing the proton: by turning its dipole moment away from A, as was seen in case 2 above. In the case of oxygen atoms, an additional factor to be considered is their two lone pairs, neither of which points along the dipole moment of the group. Since the proton, when on the oxygen atom of group A, prefers a lone pair position, motion of the other group B toward the dipole vector of A further shifts the equilibrium position of the proton towards B by destroying the linear character of the $\text{O}-\text{H}\cdots\text{B}$ arrangement, thereby destabilizing the H-bond of $\text{AH}\cdots\text{B}$. Indeed, the general nature of these principles is underscored by their applicability to calculations involving other molecules (16).

The calculations described above pertain directly to an isolated H-bond in a gas-phase situation. It is hence important to discuss the changes to be expected from placing the H-bond in the interior of a protein. The "dielectric" effect of the surrounding atoms and bonds with their associated charges and polarizabilities has a tendency to level off the large energy differences typically observed in the gas phase. Therefore, the energy differences of 8.8 and 7.5 kcal/mol in Fig. 1 would probably be somewhat reduced within a protein. On the other hand, it is extremely unlikely that the environ-

ment will alter the conclusion that realignment of the H-bond can reverse the relative pK values of the groups involved. This statement is based in part on the fact that the two states of interest—namely, $(\text{H}_2\text{COH}\cdots\text{OH}_2)^+$ and $(\text{H}_2\text{CO}\cdots\text{HOH}_2)^+$ in case 1 or $(\text{H}_2\text{CHNH}\cdots\text{NH}_3)^+$ and $(\text{H}_2\text{CHN}\cdots\text{HNH}_3)^+$ in case 2—are electrically quite similar. All contain a H-bond between a positively charged species and a neutral molecule. There is hence no reason to expect preferential stabilization of either state by the surrounding protein lattice.

The data presented to this point have assumed a H-bond length of 2.75 Å as representative of bonds typically observed in proteins. It should be emphasized, however, that our finding of reversals in relative stabilities is *not* restricted to this particular bond length. For example, as mentioned above for $R = 2.75$ Å, the $(\text{H}_2\text{CHNH}\cdots\text{NH}_3)^+$ configuration is more stable than $(\text{H}_2\text{CHN}\cdots\text{HNH}_3)^+$ by 2.5 kcal/mol when $\theta = 0^\circ$ but the situation reverses and $(\text{H}_2\text{CHN}\cdots\text{HNH}_3)^+$ is lower in energy by 7.1 when the NH_3 subunit is rotated downward by 55° . A similar reversal in relative stabilities is observed even when the H-bond is stretched to 3.2 Å; rotation of the NH_3 changes ΔE from +3.3 kcal/mol to -3.1. The capability of changes in the H-bond geometry to influence the relative energies of the two wells is quite long-range in character. Calculations on model systems (16) have shown strong effects for H-bond lengths of >4 Å.

Another point concerns the magnitudes of the barriers to proton transfer. As may be seen in Fig. 1, the barrier for transfer from the carbonyl to the hydroxyl is progressively lowered as the hydroxyl is shifted towards the $\text{C}=\text{O}$ axis. At the same time, the barrier for transfer in the reverse direction is enlarged. Both of these trends would favor the occurrence of the proton on the hydroxyl group. The barrier changes in case 2 are somewhat different in that rotation of the lone pair of the amine group away from the Schiff base nitrogen raises the barrier for transfer in both directions simultaneously. Nevertheless, the height of this barrier is unlikely to prevent the proton shift since the light mass of this particle facilitates quantum mechanical tunneling through even rather high barriers within time scales much faster than the requirements of the biological process (17). Moreover, any vibrations that transiently shorten the H-bond would also act to speed up the transfer by diminishing the barrier.

Our finding that alteration of the H-bond geometry can result in transfer of a proton may have profound implications for protein function. This notion leads to a simple mechanism whereby a protein can control the protonation states of various residues. As an example, proton transfers are known to be an essential component in the catalytic pathway of a large number of enzymes (1, 2, 18–21). Relatively small conformational changes that affect the alignment of residues within the active site could enable the enzyme to transfer protons from one group to another (or to the substrate) at an appropriate time in the reaction. For example, the proton transfers occurring between Ser-195 and His-57 and between the former residue and substrate of chymotrypsin could be coordinated with conformational changes taking place in the enzyme-substrate complex (21) that alter the geometries of the appropriate H-bonds.

In another vein, generation and utilization of protonmotive force by proteins such as bacteriorhodopsin and H^+ -ATPase have been suggested to involve proton conduction along chains of H-bonds within the protein (22–26). The entire process entails a number of proton transfers from one residue to the next along the chain. Since various different groups are expected to participate in the chain, a situation would likely be encountered where the pK of residue i is higher than that of the next along the chain, $i + 1$, retarding the proton conduction at that point. In such a case, the transfer from i to $i + 1$ could easily be facilitated via a conformation-induced

change in the H-bond geometry between the two residues that reverses their relative pK values. This ordering of events may be thought of as "pumping" a proton to a group with nominally lower pK. From the opposite perspective, since proton transfer changes the orientational preference of the H-bond, a protonmotive force that causes such a transfer may be used to power a conformational change within the protein much as an "engine" converts external energy to mechanical force.

At the present, it is widely accepted that protonation/deprotonation of the Schiff base linkage is an essential component in the proton pumping activity of bacteriorhodopsin (27–34). Moreover, light absorption is known to lead to isomerizations about bonds within the retinal chromophore that alter the orientation of the Schiff base with respect to its protein environment (30–34). The principles described here suggest various mechanisms by which these two processes may be coupled together. For example, the Schiff base proton could easily be transferred to an acceptor group by the light-induced isomerization that changes its orientation with respect to this group. A conformational change occurring at a later stage in the pumping cycle could pull a proton from another group onto the Schiff base.

Protein residues that have been suggested as donating or accepting protons from the Schiff base in bacteriorhodopsin include lysine (27, 28, 31) as well as tyrosine and carboxylate residues (30, 32, 35–37). Our calculations apply directly to the amine group of lysine; although we have not explicitly examined a pairing of a Schiff base with an oxygen base, it is expected that the precepts delineated here would apply with only minor modifications.

The concept of reversal in relative pK values caused by reorientation is predicated on a pair of groups with fairly similar unperturbed pK (such as $\text{H}_2\text{C}=\text{O}$ and HOH). It is anticipated that although an actual reversal may not occur when the groups have very different initial pK values, the mechanism of H-bond geometry change can still produce substantial alterations in the pK difference.

It should be noted finally that the principle described here is not limited to proteins. Indeed, it may apply to any H-bond satisfying certain criteria. If the pK values are to be reversed, it would be necessary for some force external to the H-bond itself to maintain or change the relative orientations of the two groups. This function is served in proteins by the macromolecular structure that maintains the peptide backbone. In the case of a drug–receptor interaction, the structural constraints imposed by the many van der Waals and electrical contacts might serve a similar role.

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