

Ab Initio Calculations on a Critical Part of a Protein, with an H₅O₂ Partially Charged Group in a Central Role

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Received: December 7, 2000; In Final Form: March 28, 2001

The X-ray structure has been determined by Doyle et al. for the KcsA K⁺ channel (Doyle, D. A.; Cabral, J. M.; Pfuetzner, R. A.; Kuo, A.; Gulbis, J. M.; Cohen, S. L.; Chait, B. T.; MacKinnon, R. *Science* **1998**, 280, 69–77).¹ which is derived from the bacterium *Streptomyces Lividans*. As it is a channel protein, it offers an opportunity to examine in at least semiquantitative fashion the details of a possible gating mechanism. A model has been proposed for the transition to the open state on the basis of ESR data of Perozo et al. (Perozo, E.; Cortes, D. M.; Cuello, L. G. *Science* **1999**, 285, 73–78).² The X-ray structure includes four glutamates in the gating region, and the diameter of the channel seems wide enough that it takes some effort to explain why this structure is not in fact open. Dielectric or Born barriers have been proposed, although it is difficult to know the value to be inserted for a dielectric constant in so inhomogeneous a region. We propose that the water in the small volume defined by the glutamates holds the key. The calculations presented here show that if there is at least one available proton, an H₅O₂ forms with partial positive charge, bridging three of the four glutamates. The asymmetry is sufficient that the ion would in all likelihood rotate, averaging its position among the four glutamates (something that cannot be seen in ab initio calculations); consequently, it would be unlikely that the water could be seen in an X-ray structure. The bridging of the glutamates by the cation is, in this model, responsible for holding the structure together. When the number of protons is large enough that the net charge drops to 1, only two of the glutamates are ionized, and they are consequently no longer held in position, allowing the small twist observed by Perozo and co-workers (Perozo, E.; Cortes, D. M.; Cuello, L. G. *Science* **1999**, 285, 73–78) in the transition to the open position. The structures produced by ab initio calculations are shown, together with the consequent energies and potential distributions. The geometry is not considered exact, but there should be no qualitative errors, so the fundamental process is believed to be correct. However, the model is truncated to the approximately 1 nm closest to the H₅O₂, and the polarizability of the surroundings is not included, so some caution in interpretation is required. The calculation suggests that the geometry would be influenced by the H₅O₂ so that possible uncertainties in the X-ray structure would very likely be compensated for. The proton shift from carboxyl to water has a second consequence: simulations using point charge models of water are unlikely to prove correct in such a constricted region, with possibility of ionization; whether the gating mechanism is correct or not, channel simulations in this region would have to account for quantum effects.

Introduction

The mechanism of gating of K⁺ and Na⁺ voltage-dependent channels has been the subject of modeling and other studies over decades; Hille³ gives an introduction to the subject. More recently, many workers have made gating model proposals. Almost all have some form of conformational change of one or another part of the protein in response to the membrane depolarization, typically based on the accessibility of various residues. The accessibility is determined by mutating residues, generally to cysteine, and testing the mutant for ability to react with a specific reagent (a methanethiosulfonate, typically, for cysteine). Bezanilla⁴ has recently reviewed the subject in considerable detail while presenting a new conformational change model. We have earlier argued^{5–7} that there is an important role to be played by water in blocking the channel and that proton transfer is important in creating the movement of charge preceding the opening of the channel, that is, the gating

current. There is extensive literature on theoretical studies of water under a variety of conditions, of hydrogen bonding in water and between water and other small molecules, and of quantum effects in proton transfer. Acetic acid–water species have been studied to elucidate the role of hydrogen-bonded networks and the consequent self-assembly of such species.⁸ Tsuzuki et al. calculated water complexes with dimethyl ether, formic acid, and methanol to high accuracy.⁹ Xu considered an ice surface on which a proton transfer takes place.¹⁰ Cytosine has been shown to adopt a different conformation in a different ionization state when hydrated;¹¹ this may be important for our study, as we also find that there may be a different conformation, and certainly ionization state, for the section of channel we are studying, in the presence of water. Electric fields are also known to produce significant effects on hydrogen bonded structures.^{12–14} Many of these studies have shown good agreement with experimental results. There is also evidence of changes in the pK_a of acids in charged membranes. Although the system considered in this paper is larger than any discussed above

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except that of the hydrated cytosine, we perform the calculations at high level for the energies and charges by using the geometry partially optimized at the lower level and completed in the most sensitive region at the high level and by using the B3LYP DFT method.

Two recent experimental cases may be relevant here as examples of the behavior of pK_a in proteins or other charged membrane systems: one in bacteriorhodopsin¹⁵ and the other in a charged membrane in which the pK_a of acetic acid is altered by nearly 1 full unit in a polymeric membrane.¹⁶ In addition, Bezrukov and Kasianowicz¹⁷ found that protonating the α -hemolysin channel partially blocks the channel, a fact attributed by the authors to water tied up on the channel wall. This is consistent with the idea that the water in a channel could block the channel under the right conditions of charge.

The existence of an X-ray structure for the KcsA channel offers an opportunity of testing in at least a semiquantitative manner the possibility that water is tied up in the channel pore, thereby blocking it. This channel is itself proton-gated and lacks four of the transmembrane segments found in each domain in standard eukaryotic voltage-gated channels. We have argued that these may form a voltage-to-proton current transducer,⁵ so on the basis of this model, it should not be surprising that a channel lacking these segments would be gated by protons directly. What we have not previously considered is the details of the mechanism by which a proton would be able to remove a block of the small part of the channel by water. Neither have we considered the question of whether amino acid side chains might be held in place and prevented from relaxing by the water. The KcsA channel offers the opportunity to do this on a semiquantitative basis. If protons are to gate the channel, one should expect there to be acidic or basic residues to accept the proton in the gating region of the channel. There are. The four residues numbered 118 (E118, numbered 96 in the pdb file for the channel) are glutamates and point toward the center of the channel. The fact that residues 117 are arginines does not affect the glutamates, as the arginines do not point toward the center of the channel and their charges appear to be quite distant. Therefore, we are able to focus on the glu residues; to obtain truly quantitative results, it would be necessary to include more than these. In fact, to make a practical calculation, we must truncate the glu to the side chains, leaving the last two carbon atoms, in effect, only acetic acid. At that, the calculation is sufficiently extensive so that the structure of the water in the channel cannot be determined fully quantitatively even within the parameters of the model; we have 47–50 atoms (depending on the number of hydrogens), of which 22 are heavy (not hydrogen). Most of the high level calculations cited above are done on systems with fewer than 15 atoms, generally 6–8 of them heavy. The results of Mautner et al.⁸ strongly suggest that the work at the level we are using here is adequate for ionic complexes, where electrostatic energy is the primary term. It is possible to be quite clear here as to how the proton would interact with the water between the acid residues and how the charges would be distributed among the waters and the acid residues, and this is the essence of the problem. Knowing these facts in the different protonation states makes it possible to see how the acid groups are held together until the charges are neutralized by protons and, thus, how the water/acid combination stays together until neutralization destroys the electrostatic binding. While the water should then become mobile and the acid groups may be able to relax away from each other, that step cannot be seen in this calculation.

Methods

The four E118 residues for the KcsA channel, based on the Doyle et al. results,¹ were truncated to the last two carbon atoms. A hydrogen was substituted for the last truncated carbon at a bond distance of 1.08 Å, and two other hydrogens were added to the carbon (originally by using the parameters of CHARMM, the molecular dynamics program, setting the bond distances at 1.08–1.11 Å, together with standard sp^3 angles). The net effect was to produce four acetic acid molecules, with the carbon and oxygen relative positions determined by the X-ray data on the KcsA channel. One to four hydrogens were added at positions that seemed reasonable. These were to be optimized in position, so it was sufficient to choose plausible initial positions. As expected, they later moved appreciably, at least in some instances.

With four water molecules, “holes” were left in the structure that were incompatible with either a representation of a plausible system or further optimization. Therefore, two more water molecules were added so that the four acetic acids could be bridged by water, and two water molecules were left to fill the space between. The space between turned out to be an excellent fit for an $H_5O_2^+$ ion, not a result that had originally been anticipated. The four “acetic acid” residues were frozen (the two carbons, the two oxygens, and the methyl hydrogens, for a total of 28 atoms); the waters and the hydrogens originally located at the carboxyl groups were optimized using the semiempirical PM3 method¹⁸ to obtain a reasonable starting geometry for the six water molecules plus the hydrogens that partially or completely neutralized the acids. From this point on, refinements of structure were carried out for the two central water molecules plus the 1–4 hydrogens that were added to (partially) neutralize the acid moieties. The PM3 output was used as input to the low level ab initio “chemistry” HF/3-21G*, that is, using HF (self-consistent field) calculations with the limited basis set of starting wave functions 3-21G*. These lacked the diffuse wave function contributions that would be needed to properly refine a system with intermolecular nonbonded interactions but were better than a semiempirical method. The geometry is expected to be essentially correct, as the corrections would alter energy somewhat but would be very unlikely to effect major change in geometry. See also the discussion of ionic hydrogen bond effects in Mautner et al.,⁸ in which geometric optimization in such systems is considered to be satisfactory at the HF/4-31G level; this is not likely to be significantly more thorough than HF/3-21G*. The major point is that the electrostatics dominate the forces in ionic systems and, hence, are the principal determinant of the geometry. From this point on, all atoms other than those in the “center” of the four acetic acids were frozen (i.e., the 28 atoms of the 4 acetic acids, plus the 4 water molecules on the outside), while the two waters and the hydrogens that (partially) neutralized the acids were optimized. We defined the “center” as the average position of the four closer oxygens on the four acetic acid groups (or, very nearly equivalently, the other four oxygens, or all eight: results differ by less than 0.2 Å). It must be understood that the two waters nearest the center, by any of the three definitions, were those critical for binding the system, while those on the periphery made the overall system stable against the effects of the absence of any contacts for many atoms, leaving molecules near enough to have bad van der Waals contacts if they were frozen or to have incorrect geometry otherwise. With the waters in place, these alternatives were avoided. In addition, the higher-level calculation corrected the geometry to a small, but possibly critical extent, relative to the

TABLE 1: Net Charge and O–O Distance on H₅O₂ for Four Charge States

| total charge | 0 | −1 | −2 | −3 |
|--|-------|-------|-------|-------|
| O–O distance (Å) | 2.591 | 2.678 | 2.392 | 2.418 |
| H ₅ O ₂ charge (net) | 0.474 | 0.553 | 0.599 | 0.673 |

fixed acid residues. The density functional theory (DFT) calculation was done using the B3LYP^{19–21} method with the D95V basis set²² and the Gaussian98²³ program on a Compaq DS20 with two processors. All quantitative results shown are the output of this higher-level calculation. This method includes some of the correlation energy (that part of the energy beyond that produced by self-consistent field calculations). Electron correlation effects are important for precise energy and atomic charges. The lower-level calculation was necessary, however, as input; with less accurate geometry, the higher-level calculation generally failed entirely.

Results

The first question that must be answered concerns differences in geometry and binding with changes in total charge (equivalently, with changes in number of protons). If the waters are involved in gating, they must block the pore. In addition, they may act as a tight spring at high negative charge, holding the acid residues in place. The spring is suggested by the data of Perozo et al.,^{2,24} showing that the gating of the KcsA channel appears to be accompanied by an outward twist of the side chains and entire gating region to the extent of a few angstroms. The water may hold the channel from twisting apart. If this is to be the case, the central waters will have to be relatively positive, while the negative charge in the closed state must reside on the acid moieties. This sounds paradoxical, requiring more positive charge in the center when the overall charge is more negative. One way in which it might happen is to have the one free hydrogen reside near the center in the most negatively charged case, while the multiple hydrogens in the low-charge case move to the acid groups, leaving the center with loosely bound water. The most charged case considered here has three negative charges: −4 is so improbable that it seemed not worth computing, and comparison of −3 to −2 supports the original estimate; −4, in any case, cannot contribute to holding the acid groups together by any plausible mechanism, even if its energy were not too high for it to exist.

Probably the most interesting result of the entire calculation is the fact that, with only one neutralizing H in a system with four acid groups and hence total charge −3, the hydrogen moved away from its original position (as assigned in the input file) to a position near the center, forming a H₅O₂ that had a partial positive charge, just as would be required to hold the negatively charged system together. From here on, we will refer to H₅O₂ for the system computed here rather than H₅O₂⁺; it holds a partial charge, which varies with the total charge (see Table 1). It turns out that the space between the oxygens of the acids provides almost the exact space needed for the H₅O₂. Table 1 shows that the net positive charge on the central H₅O₂ does increase as the total negative charge increases.

Furthermore, the O–O distance in the −3 and −2 forms was approximately 2.4 Å, at least 0.2 Å shorter than that in a normal hydrogen-bonding system and almost exactly equal to that obtained for isolated H₅O₂⁺ by ab initio calculations.^{25,26} This distance relaxed to 2.6–2.7 Å in the less charged cases, exactly as though there were no binding force holding the two together, beyond that of an ordinary hydrogen bond. The individual water molecules are still strongly attached to the acid moieties (single charge case, bond lengths 2.400 and 2.511 Å; see Table 2c)

TABLE 2: Interatomic Distances

| (a) Charge = −3 | | | | | | | | |
|-----------------|--------------|----------------|--------------|--------------|----------------|--------------|-------|--------|
| | H | O _w | H | H | O _w | H | H | H |
| H | 0 | | | | | | | |
| O _w | 0.996 | 0 | | | | | | |
| H | 1.700 | 1.086 | 0 | | | | | |
| H | 1.761 | 1.040 | 1.925 | 0 | | | | |
| O _w | 2.760 | 2.418 | 1.363 | 3.280 | 0 | | | |
| H | 3.227 | 2.920 | 1.950 | 3.782 | 1.012 | 0 | | |
| H | 2.856 | 2.834 | 1.962 | 3.788 | 0.994 | 1.612 | 0 | |
| O _a | 1.781 | 2.716 | 2.994 | 3.512 | 3.440 | 3.849 | 2.992 | |
| O _a | 4.324 | 4.201 | 3.405 | 5.072 | 2.585 | 1.616 | 2.830 | |
| O _a | 4.862 | 3.941 | 3.664 | 3.756 | 4.115 | 3.981 | 5.103 | |
| O _a | 3.016 | 2.486 | 3.340 | 1.467 | 4.663 | 5.202 | 5.166 | |
| (b) Charge = −2 | | | | | | | | |
| | H | O _w | H | H | O _w | H | H | H |
| H | 0 | | | | | | | |
| O _w | 1.007 | 0 | | | | | | |
| H | 1.707 | 1.160 | 0 | | | | | |
| H | 1.730 | 1.035 | 2.001 | 0 | | | | |
| O _w | 2.718 | 2.392 | 1.248 | 3.237 | 0 | | | |
| H | 3.204 | 2.916 | 1.907 | 3.820 | 1.038 | 0 | | |
| H | 2.885 | 2.876 | 1.875 | 3.760 | 1.002 | 1.632 | 0 | |
| H | 3.917 | 2.970 | 2.838 | 2.982 | 3.347 | 3.362 | 4.332 | 0 |
| O _a | 1.767 | 2.698 | 2.870 | 3.431 | 3.306 | 3.731 | 2.910 | 5.495 |
| O _a | 4.307 | 4.133 | 3.294 | 5.057 | 2.521 | 1.497 | 2.859 | 4.159 |
| O _a | 4.886 | 3.921 | 3.754 | 3.824 | 4.142 | 4.119 | 5.136 | 0.986* |
| O _a | 3.063 | 2.533 | 3.432 | 1.501 | 4.625 | 5.255 | 5.139 | 3.702 |
| (c) Charge = −1 | | | | | | | | |
| | H | O _w | H | H | O _w | H | H | H |
| H | 0 | | | | | | | |
| O _w | 1.048 | 0 | | | | | | |
| H | 1.888 | 1.722 | 0 | | | | | |
| H | 1.612 | 0.987 | 2.281 | 0 | | | | |
| O _w | 2.609 | 2.678 | 0.995 | 3.222 | 0 | | | |
| H | 3.285 | 3.387 | 1.904 | 4.131 | 1.218 | 0 | | |
| H | 2.751 | 3.110 | 1.680 | 3.509 | 1.017 | 1.869 | 0 | |
| H | 4.070 | 3.407 | 3.453 | 4.140 | 3.941 | 3.668 | 4.903 | 0 |
| H | 2.330 | 1.602 | 3.136 | 1.918 | 4.086 | 4.545 | 4.651 | 3.079 |
| O _a | 1.597 | 2.589 | 2.759 | 2.835 | 3.030 | 3.718 | 2.605 | 5.496 |
| O _a | 4.310 | 4.473 | 3.101 | 5.282 | 2.400 | 1.209 | 2.852 | 4.116 |
| O _a | 5.020 | 4.294 | 4.329 | 4.942 | 4.780 | 4.461 | 5.770 | 0.984 |
| O _a | 3.189 | 2.511 | 4.108 | 2.516 | 5.078 | 5.571 | 5.613 | 3.746 |
| (d) Charge = 0 | | | | | | | | |
| | H | O _w | H | H | O _w | H | H | H |
| H | 0 | | | | | | | |
| O _w | 1.100 | 0 | | | | | | |
| H | 2.006 | 1.599 | 0 | | | | | |
| H | 1.640 | 0.985 | 2.119 | 0 | | | | |
| O _w | 2.780 | 2.591 | 1.016 | 3.057 | 0 | | | |
| H | 3.246 | 3.178 | 1.767 | 3.854 | 1.081 | 0 | | |
| H | 2.851 | 3.007 | 1.732 | 3.315 | 1.112 | 1.754 | 0 | |
| H | 4.101 | 5.906 | 3.199 | 3.871 | 3.730 | 3.691 | 4.784 | 0 |
| H | 2.498 | 5.656 | 3.073 | 1.931 | 4.075 | 4.523 | 4.673 | 2.928 |
| H | 5.055 | 5.480 | 3.884 | 5.443 | 4.833 | 3.134 | 4.818 | 2.459 |
| O _a | 1.480 | 2.542 | 3.007 | 2.799 | 3.437 | 3.829 | 3.015 | 5.498 |
| O _a | 4.218 | 4.299 | 3.082 | 5.089 | 2.459 | 1.389 | 2.880 | 4.297 |
| O _a | 5.080 | 4.214 | 4.025 | 4.767 | 4.430 | 4.321 | 5.524 | 0.991 |
| O _a | 3.334 | 2.602 | 3.983 | 2.507 | 4.990 | 5.500 | 5.559 | 3.565 |

Italics: bond between one hydrogen originally on a carboxyl group and one of the mobile oxygens. Boldface: Short oxygen–oxygen distances, <2.6 Å. Italics + boldface: Oxygen–hydrogen distances intermediate between normal-bond (~1.0 Å) and hydrogen-bond distances (~1.7 Å).

but do not bind to each other particularly strongly (bond length 2.678 Å); it is easy to see how the system could move apart with increasing protonation. The shift of nearly 0.3 Å suggests a strong force. In the standard water models used for simulations, there is a rise in energy of 20–40 kJ/mol as the intermolecular

distance drops from 2.6 to 2.4 Å, which is roughly the closest one can expect two oxygens to approach in water.²⁷ The short distance found in this calculation may well be a consequence of the energy for the spring that holds the acid groups together in the closed state of the channel; the binding energy is presumably comparable to that found in H_5O_2^+ , although it is not possible to separate it from the system energy in this case. There is another fairly short distance, in the 2.5 to 2.6 Å range, to a neighboring oxygen belonging to one of the acid moieties (see Table 2). This adds to the binding energy of each water, albeit to a more limited extent, as the bonds are not as short; the bond length would be more like that in H_7O_3^+ or H_9O_4^+ .^{25,26} With further protonation, the spring relaxes (the central O—O distance returns to normal), presumably permitting a small conformational change that also allows the water to move relatively freely, so that it is no longer blocking the channel.

Table 2 has additional bond distances, showing that the two waters are still tightly held by the acid groups. There are several anomalous distances in addition to the one we have just discussed between the oxygens of the two water molecules optimized in the center of the group of molecules. The oxygen—oxygen distances between the two central waters and two of the fixed oxygens remain fairly short at all charge states, in one case shrinking all the way to 2.400 Å. This is important in that it shows the strength of the binding of these waters to the acid groups that would form part of the wall of a channel. As they are held only at one end, they presumably would exchange rapidly, much more so than would waters held by both ends, as in the more highly charged case. Those waters, judging from the energy required to produce two such short O—O distances, are not quite covalently bound but approach that state.

There are anomalous O—H distances as well (1.15–1.55 Å), generally one for each short O—O distance; these distances are intermediate between normal hydrogen bonds (1.7 Å) and normal covalent bonds (1.0 Å). These bond lengths suggest that some of the O—H bonds have partially covalent character. While these were not investigated in detail, it is clear from the bond lengths that these bridging hydrogens are involved in bonds that differ from the types considered in standard molecular models, for example, the type that might be used in MD calculations.

Discussion

In considering the consequences of the calculation, it must be understood that some factors have been necessarily omitted, especially the effect of all parts of the channel more than ~1 nm from the center of the region for which the calculation was done. Therefore, the interpretation must be considered to be somewhat tentative, pending the inclusion of more of the channel. Solvation effects from water above and below the section that is calculated, which is only about 1 nm thick, may be of some significance. Changes in the nearby amino acids would also affect the central group in two ways: (1) the potential, electric field, field gradient, and higher terms would be altered at the position of this group, and (2) the transport of the proton needed to change the charge would be affected. However, the essential bonds are unlikely to be disrupted, especially as there is relatively little difference between the -3 and -2 charge cases, followed by a huge change to -1 , and then not much more change to 0 charge. This is consistent with the expectation for a gate and makes the calculation at least a very plausible basis for understanding the gating process. Presumably, modifying the glu residues involved would be a critical test of the model. Small alterations in the starting positions of the E118 residues would be unlikely to matter, as

the H_5O_2 is likely to pull them into position in the -2 or -3 charge cases; in the -1 charge case, the positions would change appreciably, and the calculation only shows that the configuration would be able to change.

Assuming that the calculation does describe the physical situation reasonably plausibly, two major conclusions would follow. First, the channel is likely to be impervious to the passage of almost anything when the charge is -2 , and probably -3 . Since the crucial O—O bond distance is a little shorter and the bonding apparently a little stronger with the charge of -2 than with -3 , it would appear that the -2 state is the better candidate for the closed state. However, -3 would also be closed, and it is possible that any pH that kept the chemical potential of the hydrogen ion below that required to have a charge of at least -2 would lead to a closed channel. The disappearance of the short O—O bond between the oxygens of the two waters in the H_5O_2 is likely to mean the loss of the block in the channel. The maintenance of the short bond length to the oxygens in the carboxyl groups suggests that there is additional binding energy of the separate water molecules to the carboxyl groups; if the groups swing apart, these waters may reasonably be expected to maintain their positions relative to those of the carboxyl groups, probably on a time scale that is large compared to that for ordinary place exchange in water, around 10^{-11} s. However, this cannot be taken as evidence for the stability of the binding of the water on a time scale of, say, milliseconds. Additional work on possible transition states for the exchange of water would be needed to obtain the correct time scale. One would expect that the time scale for this exchange would be much smaller than that for the exchange in which the water is bound as part of an H_5O_2 with both ends held by carboxyl groups and with additional binding energy coming from electrostatic effects absent in bulk water. For the H_5O_2 /carboxyl case, even with the exchange, the water positions would be essentially stable, as the new water would replace the leaving water in the same position (or possibly rotated to another carboxyl, but not otherwise changed).

As a result, the KcsA channel “gate” is likely to be the H_5O_2 . The proton responsible for gating the channel would do so by changing the total charge from -2 to -1 , with loss of the H_5O_2 as an entity holding the carboxyls of the glutamates in the center of the channel. The central waters would separate, and the glu residues would no longer be held in place, allowing the channel to open. The glu residues themselves do appear to hold the waters and may be solvated.

The second major consequence of this calculation is the necessity for caution in simulations. The water models used in standard simulations have been generally point charge models, polarizable or not, in which charge exchange is impossible. We see here that charge exchange can occur when there is a high electric field and acidic or basic groups, such as carboxyl. Furthermore, O—O distances may be appreciably different than those in bulk water. This suggests that in narrow crevices, additional moves should be allowed in simulations to account for possible charge exchange. “Narrow crevices” may include active sites of enzymes, for example. It is, so far, impossible to even make an informed guess as to whether such effects play a role in protein folding.

Suggesting in detail how to include these effects in simulations is beyond the scope of this calculation, but the fact that such moves are required is strongly suggested by the results. When in the vicinity of an acidic or basic group, it would be necessary to test for the electric field and to have an algorithm for the probability of charge exchange; obviously, it is not

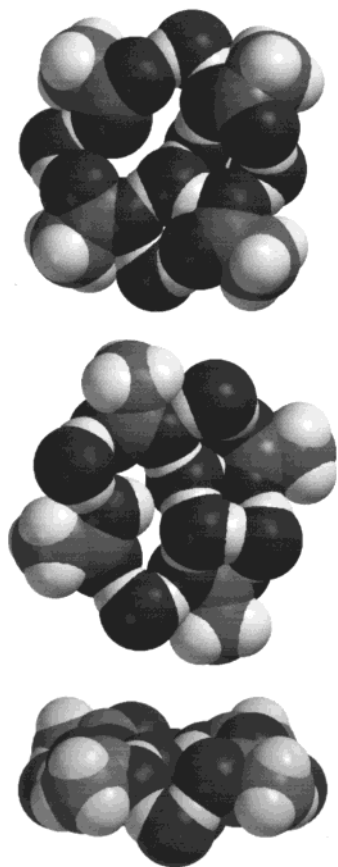


Figure 1. Views of the van der Waals surface of the atoms in the computation; black is oxygen, medium gray is oxygen, and light gray is hydrogen: (a) A view of the triply charged form. The carboxyl not bonded to the H_5O_2 has no bond at all. (b) A view of the doubly charged form; note the extra hydrogen attached to the carboxyl group that was empty in the triply charged form (on the left in the figure). Note also that there are still small gaps in which the normal van der Waals radii of the atoms do not quite touch. These are too small to allow passage of an ion. (c) The same as panel b, rotated to show the near planarity of the entire system.

possible to do an ab initio simulation step in the course of a simulation. With such groups, it may make sense to have the result of such a step for the relatively closed regions of a protein built in to the behavior and structure of water in these regions. The local potential energy surface for water and for protons could be included as a lookup table for moves in the relevant region. To continue this discussion would carry us too far afield, but it does seem clear that such a procedure is necessary. The bonding, or at least the bond length, between hydrogen and oxygen is, in some cases, apparently intermediate between normal covalent bonds and normal hydrogen bonds. These presumably partially covalent bonds exist where the high fields and partially charged species prevent the existence of ordinary forms of bonds.

One other point is almost certainly important: the distribution of the H_5O_2 in the center of the grouping is not symmetric. There is one acid group that is left with no bonds to the H_5O_2 . This may have some relation to the apparently stronger bonding with -2 charges than with -3 ; -3 leaves the lone carboxyl with no bond, while with -2 , there is a leftover H that can make the last carboxyl neutral, likely to be a preferred state with two other negative charges present (see Figure 1). The potential as calculated does show the odd carboxyl oxygens going from slightly more negative than all the others to more positive as total charge changes from -2 to 0 , although the

effect is not large. In no case is the position of the waters centered. One should expect that the configuration might average out over times as long as those involved in gating, as the waters, fairly tightly bound as they are, will nevertheless be able to rotate within the grouping; that is, they may be able to exchange partners, varying which of the four carboxyl groups does not bond to the H_5O_2 . This may be responsible for the failure of the waters to appear in the X-ray structure of the KcsA channel, as the one water in the selectivity filter does. Instead, the electron density in the central region would be fairly evenly distributed on the relevant time scale. In systems with total charge -1 or 0 , putatively open structures, the water should be fairly free to move.

Conclusions

There are two principal conclusions:

- (1) There is a partially charged H_5O_2 bonded to three (two relatively tightly, one hydrogen bonded) of the carboxyl groups of the four acetic acids held in the relative positions of the corresponding atoms of the four KcsA glutamates (E118). The H_5O_2 itself has a short, and presumably strong, H bond when the total charge is -2 or -3 ; when the charge is -1 , the central bond lengthens and, presumably, weakens. The most probable explanation of the gating action of the proton in the KcsA channel is the destruction of this H_5O_2 (at least the severe weakening of the central bond), with the two waters remaining partially bonded to separate carboxyl groups. The carboxyl groups can then swing apart, as the "spring" holding them together is broken. The bonding in this complex is significantly distorted from that normally found in water.
- (2) The charge transfer involved suggests that simulations, whether MD or MC, cannot use normal water models in the vicinity of (<7 Å) acidic or basic moieties that are tightly grouped. This may apply in channels and possibly in the active sites of some enzymes.

Acknowledgment. This work has been supported in part by the PSC/CUNY internal faculty research grant program at City University of New York and by a SCORE subgrant from NIH. I wish to thank Dr. J. Ochterski of Gaussian, Inc., for assistance in the application of the Gaussian software to this problem and Professor T. Lazarides for assistance with CHARMm in the placement of hydrogens.

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