Ab Initio Molecular Dynamics Study of Proton Transfer in a Polyglycine Analog of the Ion Channel Gramicidin A

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ABSTRACT Proton transfer in biological systems is thought to often proceed through hydrogen-bonded chains of water molecules. The ion channel, gramicidin A (gA), houses within its helical structure just such a chain. Using the density functional theory based ab initio molecular dynamics Car-Parrinello method, the structure and dynamics of proton diffusion through a polyglycine analog of the gA ion channel has been investigated. In the channel, a proton, which is initially present as hydronium (H_3O^+), rapidly forms a strong hydrogen bond with a nearest neighbor water, yielding a transient $H_5O_2^+$ complex. As in bulk water, strong hydrogen bonding of this complex to a second neighbor solvation shell is required for proton transfer to occur. Within gA, this second neighbor shell included not only a channel water molecule but also a carbonyl of the channel backbone. The present calculations suggest a transport mechanism in which a priori carbonyl solvation is a requirement for proton transfer.

INTRODUCTION

Proton transfer is arguably the most fundamental and common of chemical reactions. Particularly important is proton diffusion in hydrogen-bonded systems. The anomalously high rate of proton diffusion in water relative to cations of similar ionic radius (i.e., K^+), has led to speculation concerning the existence of different mechanisms for the diffusion of protons versus other cations. The presence of a hydrogen bond network in water led Grötthus to suggest a proton hopping mechanism in which the proton jumps from one water to the next. The proton transfer process is then one of chemical rather than hydrodynamic diffusion. Other ions cannot take advantage of this network and must diffuse via Stoke's Law. The Grötthus mechanism is currently the most commonly accepted theory describing proton transfer in bulk water.

The mechanism of proton transfer in bulk water is a topic of current interest. One of the recent studies used a two-state empirical valence bond model (Lobaugh and Voth, 1996) to determine the ground state adiabatic surface for $H_5O_2^+$ solvated in water. Tuckerman et al. (1995) carried out a thorough study of the solvation of hydronium (H_3O^+) in bulk water using the Car-Parrinello (CP) molecular dynamics (MD) technique and identified a possible mechanism for proton transfer. The degree of coordination of the first solvation shell of hydronium was found to be the key factor determining the transfer of the proton. Although there needed to be strong hydrogen bonding with the first solvation shell nearest-neighbor acceptors, the hydrogen bond with the nearest-neighbor donor had to be broken for proton diffusion to occur. This finding is in agreement with the experimental evidence that rates of proton transfer increase as hydrogen bonding is disrupted (Agmon, 1995; Franck et al., 1965).

Proton transfer is also a ubiquitous process in biological systems. For example, the transfer of protons is coupled to the flow of electrons during the process of energy transduction. In biological systems, protons must often traverse hydrophobic regions, such as cell membranes or the interior of proteins. There is considerable evidence for the presence of water wires in such environments, along which a proton may travel. Examples of these systems include the photosynthetic reaction center (Deisenhofer and Michel, 1989), transmembrane ion channels, and the CF_0 channel in bacteriorhodopsin (Henderson et al., 1990; Cao et al., 1991; Papadopoulos et al., 1990), in which a proton must traverse on the order of 10–12 Å to go from aspartate-96 to the Schiff base.

Gramicidin A (gA) is a transmembrane ion channel that provides an ideal model for a proton-conducting pathway. Gramicidin A is a β-helical channel (Urry, 1971), within which there exists a linear chain of water molecules. Although the primary function of gA is to transport monovalent cations (i.e., K^+ and Na^+), the rate of transport of protons is considerably greater than for these ions. Furthermore, experiments have shown that the streaming potential, which accompanies the translocation of cations, is decidedly absent in the case of proton diffusion (Levitt et al., 1978). The streaming potential provides a measure of the number of water molecules coupled to ion diffusion; its absence suggests that proton diffusion through gA occurs via a different mechanism than that of cation diffusion. Furthermore, if one accepts the validity of the Grötthus mechanism, the process of proton diffusion through gA may be similar to that found in liquid water and ice.

Gramicidin A is composed of two 15-residue β-helices. The structure is such that carbonyl oxygens line the lumen of the channel, effectively solvating the chain of water molecules within. Molecular dynamics simulations using

Received for publication 18 December 1995 and in final form 3 June 1996.
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0006-3495/96/09/1172/07 $2.00

empirical interaction potentials have revealed the role of the carbonyl oxygens in the solvation of monovalent cations (Roux and Karplus, 1991) and more recently of hydronium itself (Sagnella and Voth, 1996).

As indicated above, the ab initio CPMD method has been a useful tool in the study of the proton transfer process in bulk water. It has also been applied to aqueous acid solutions (Laasonen and Klein, 1994). Among the strengths of CPMD are its ability to describe the breaking and formation of bonds, a process fundamental to proton transfer. It is our goal here to apply the ab initio approach to the study of proton transfer within a model protein similar to that of gA. Although gA is, in itself, a small protein (552 atoms), it is still too large to use the CP method with present day computational resources; therefore, a polyglycine analog (p-Gly) of a single gA monomer was employed. Polyglycine has been used in several previous classical MD studies (Pullman and Etchebest, 1983; Åqvist and Warshel, 1989; Lee and Jordan, 1984) as a model for gA.

Pullman and Etchebest investigated the effect of side-chains on the translocation of Na^+ in gA. They showed that the overall shape of the potential energy profile was the same with and without the inclusion of the side-chains, which indicates the central role of the protein backbone in the diffusion mechanism.

Although a p-Gly analog ignores the side-chains, it nonetheless has several important characteristics of the true ion channel, gA. Among them are the positioning of the carbonyl oxygens and the β-helical structure of the peptide. As with gA, the model p-Gly houses a single-stranded chain of water molecules, which are solvated by the carbonyl oxygens.

MATERIALS AND METHODS

The ab initio CP method (Car and Parrinello, 1985) we utilize treats explicitly only the valence electrons, whereas the core electrons are handled using Vanderbilt ultra-soft pseudo-potentials (Vanderbilt, 1990). The ultra-soft pseudo-potential we employ enables the simulation of a larger number of atoms compared with conventional pseudo-potentials because they require a much smaller basis set. The cutoff used in the present work was 23.2 Ry. The core radii used were 0.794 Å for oxygen, 0.714 Å for carbon, 0.767 Å for nitrogen, and 0.370 Å for hydrogen. The CP method uses density functional theory and the local density approximation (LDA) to describe the exchange and correlation energies. This approach is described in detail by Laasonen et al. (1993a). We added the gradient correction to the LDA proposed by Becke (1988) for the exchange. This scheme for gradient correction has been shown to reproduce the structural and dynamical properties of liquid water in semiquantitative detail (Laasonen et al., 1993b). In the latter study, there was clear evidence for the existence of the $H_5O_2^+$ species. The energetics of the $H_5O_2^+$ cluster has been analyzed by Mijoule et al. (1993) using various gradient correction schemes. The Becke-Perdew scheme yielded a binding energy of $\Delta E = -31.6$ kcal/mol (Mijoule et al., 1993), which compares favorably with the experimentally determined value of $\Delta E = -31.8$ kcal/mol (Kebarle, 1977; Meot-Ner, 1986).

Much work has been done to test the validity of density functional theory and the corrections used in the treatment of hydrogen-bonded systems, including peptide-water interactions (Sim et al., 1992; Johnson et al., 1993; Novoa and Sosa, 1995). Although the LDA treatment of electron exchange and correlation alone may be inadequate to accurately describe hydrogen-bonded systems, gradient corrections to the LDA have provided considerable improvement to the energies and geometries of such systems. For example, in the work of Sim et al. (1992), it was reported that the structure and binding energy of the water-formamide dimer are in much closer agreement with experiment and Hartree-Fock calculations when using the Becke-Perdew functionals than without such corrections. The binding energies of the covalently bonded systems were within 1.0 kcal/mol of the values obtained from experiment (Johnson et al., 1993), whereas the hydrogen-bonding energies were within 2.0 kcal/mol of experimental results or Hartree-Fock calculations with higher order Møller-Plesset treatments (Novoa and Sosa, 1995). The conventional wisdom is that gradient-corrected LDA is satisfactory for the energetics of hydrogen-bonded systems but that the barriers for proton transfer are likely too low (D. R. Salahub, private communication).

Simulation details

The p-Gly channel we employed was composed of 13 glycine residues twisted into a right-handed β-helix. The length of the helix is ~10 Å and its diameter is ~4 Å. All atoms were included in the calculation, including the nonpolar hydrogens of the α-carbons. The excess proton was introduced as H_3O^+ and placed at the center of the channel, along with two additional water molecules on either side. The system was placed in a box of dimension $11 \times 11 \times 15$ Å^3, and periodic boundary conditions were employed in all directions. Another five water molecules were added to solvate the ends of the channel and to act as a bridge between the periodic images of the channel. The size of the simulation box was as large as our local memory requirements would allow. Increasing the size of the system would necessarily increase the computational cost as $\sim N^2M$, where N and M are the number of electrons and plane waves, respectively. The memory requirement scaling is NM.

Another possibility that we considered was to model the channel as an infinite helix with the p-Gly channel ends connected over the boundary conditions so that there are no mouths. After having attempted to use such a model, this method was rejected because the oxygens of the channel waters necessarily point toward the hydronium and, therefore, were misaligned at the boundaries, unable to participate in a satisfactory hydrogen-bonding arrangement. The small size of the simulation system, together with these strong hydrogen bond correlations, mitigate against the use of an infinite helix in this study. Although the system size is admittedly small, it is nonetheless manageable for the CP method as currently implemented on parallel architecture computers.

The current set-up has also proven to be useful as it has features of the interior of the gA channel and a junction. Although this is somewhat reminiscent of the situation in the gA dimer, it should be kept in mind that our periodic system is not a head-to-head dimer, as in gA. As a result, the hydrogen bonding between our periodic images is different from that found at the junction in the true ion channel.

With the starting configuration in place, a classical MD simulation was run to relax the system at 300 K. The potentials used for the Gly-Gly and water-Gly interactions were obtained from CHAR-MM (MacKerell and Karplus, 1996; MacKerell et al., 1992). The potential energy function for the hydronium is defined elsewhere (Sagnella and Voth, 1996), and the water model we employed was a flexible version of Jorgensen's TIP3P water model (Jorgensen et al., 1983). A time step of 0.5 fs was used to integrate the classical equations of motion. The temperature was regulated by means of a complete reassignment of the atomic velocities according to the Boltzmann distribution.

After a 50-ps equilibration, examination of the structure indicated that the simulation system remained intact (i.e., the helix did not unravel). Two of the five water molecules originally placed to solvate the mouths of the p-Gly channel were no longer involved in the hydrogen-bonded chain of channel waters and were therefore removed. The hydronium was located at the center of the channel forming hydrogen bonds with the neighboring waters at 2.52 and 2.95 Å and a carbonyl oxygen at 2.94 Å. The final configuration (see Fig. 1 *a*) provided the starting point for our CPMD study.

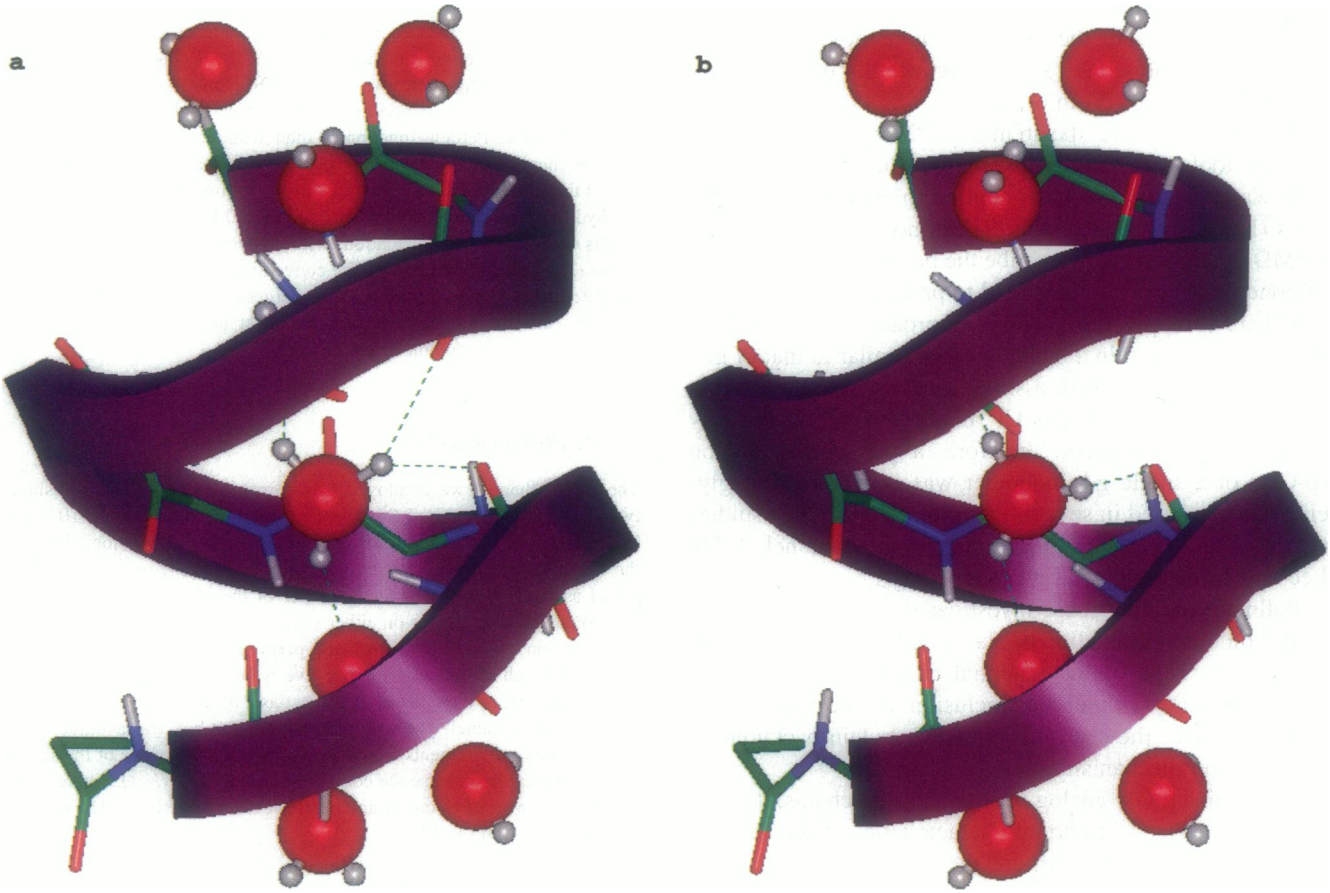

FIGURE 1 The methyl-terminated polyglycine channel. (*a*) After a 50-ps classical MD equilibration at 300 K. (*b*) After conjugate gradient minimization and 10 ab initio CPMD time steps at 150 K. The channel backbone is drawn with a combined stick-ribbon representation. The α-hydrogens have been omitted for clarity. The channel waters and hydronium are highlighted.

The β-helix used in this study contains more than 300 electrons. At the start of the CPMD run these electrons are allowed to relax to their ground state with the nuclei held in the configuration taken from the classical MD simulation. Afterwards, the positions of the nuclei were minimized using a conjugate gradient scheme because the potential felt by the nuclei in the CP simulation is different from that of the empirical potential. This reminimization allowed the backbone to relax, but the local configuration around the hydronium, namely, the $H_3O^+(H_2O)_2CO$ cluster, remained essentially unchanged with hydrogen bond distances of 2.52, 2.95, and 2.90 Å, respectively. In the subsequent CPMD simulations, the nuclei were treated classically, the fictitious electron mass was taken as 900 a.u., and to help maintain adiabaticity, the proton mass was actually that of a deuteron. These factors allowed a time step of 0.181 fs.

Three CPMD trajectories were run: one at 300 K and two others at 150 K. In two of the simulations, one end of the channel had an aldehyde group (CHO) and the other had an amine (NH_2) group. To rule out the possibility of the amine group ($NH_2 \rightarrow NH_3^+$) creating the driving force for proton transfer, a simulation was run with the terminal amine replaced by a methyl (CH_3). Each CP trajectory was run for a little more than 1.0 ps. Although the ab initio trajectories are relatively short, they are sufficient to observe proton motion in our model system. In all three simulations, the excess proton actually left the channel so that longer runs would not provide additional information concerning proton diffusion. Fig. 1 *b* shows a snapshot of the system taken from the methyl-terminated CPMD simulation after 10 time steps.

RESULTS

Table 1 lists the time averages of the Ramachandran angles, ϕ and ψ, for the CP run at 150 K of the methyl-terminated channel. The range of values for the angles are consistent

TABLE 1 Average values for the Ramachandran angles, ϕ and ψ, and the average distances of the carbonyl oxygens from the channel axis of 13-residue polyglycine β-helix, derived from 1.0-ps CPMD run at 150 K

Residue	ϕ/deg	ψ/deg	R_{CO}/Å
1	129	−121	3.67
2	−112	143	3.83
3	121	−136	3.00
4	−104	138	3.39
5	120	−110	3.70
6	−119	114	3.32
7	142	−125	3.58
8	−108	110	3.42
9	147	−128	4.24
10	−96	140	3.50
11	110	−115	3.27
12	−124	61	2.97

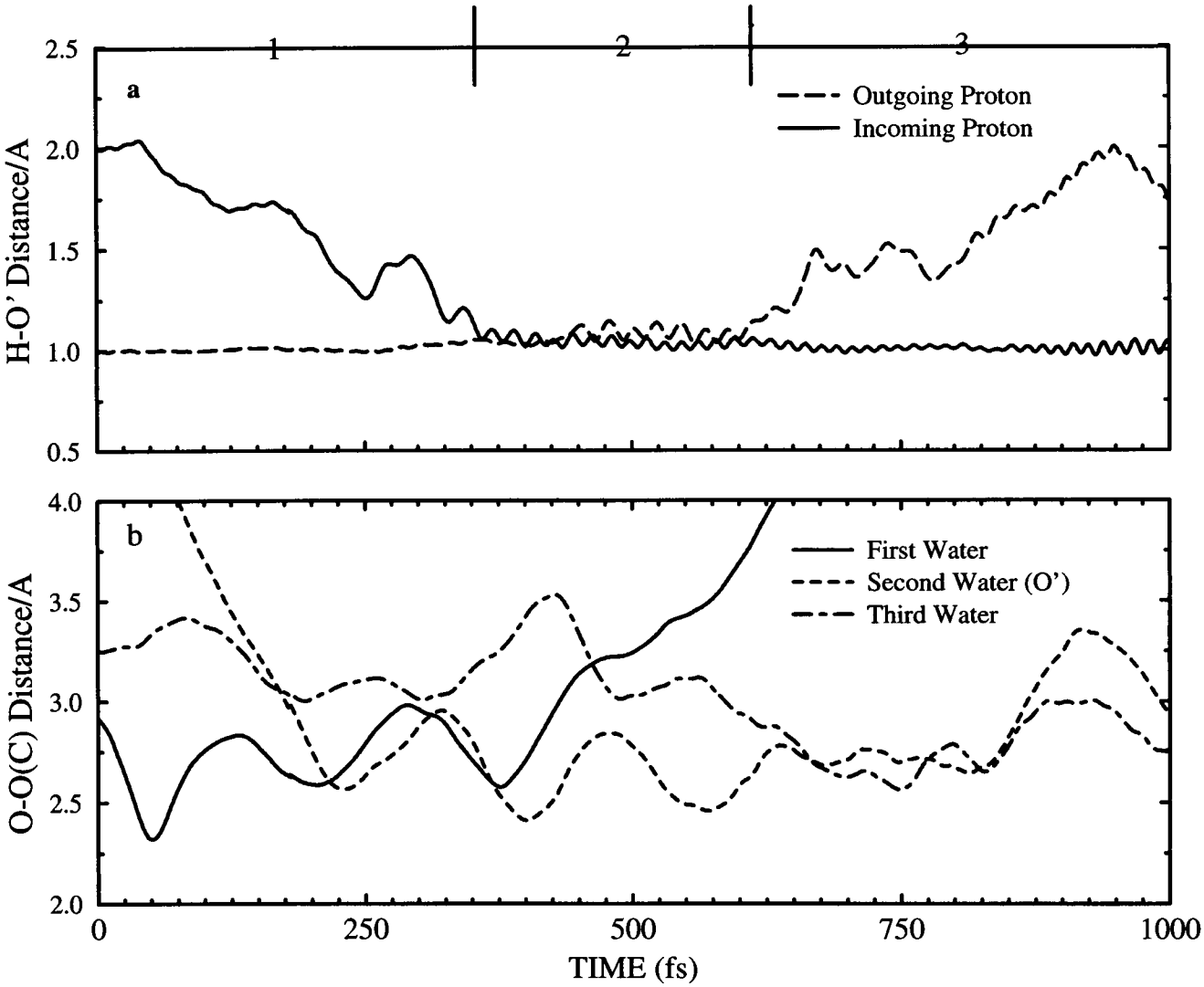

FIGURE 2 (*a*) Evolution of hydrogen atom(H)-reference oxygen (O′) separations, during a CPMD simulation at 150 K; incoming (——) and outgoing (- - -) protons are indicated. (*b*) Evolution of the carbonyl oxygen(O)-reference water oxygen (O′) distances during the CPMD simulation at 150 K.

with those found in a classical simulation for the true ion channel, gA (Roux and Karplus, 1990). Also shown in Table 1 are the average carbonyl oxygen distances from the channel axis. It is conceivable that the imposition of periodic boundary conditions, in which the box size is not much larger than the 12-residue helix itself, could force a constriction of the channel. In doing so, the carbonyl oxygens would then be artificially placed, and the solvation of the waters would be unrealistic. However, the average distances of carbonyl oxygens from the channel axis are also consistent with previous classical simulations of gA (Sagnella and Voth, 1996; Roux and Karplus, 1990). The average CO bond length obtained in our CP simulation was 1.28 Å. This value is close to the standard CO bond length of 1.24 Å (Stryer, 1988).

In all three CP simulations there was diffusion of the proton through the channel and in each instance the overall mechanism was the same. For convenience in describing the observed mechanism, we used the oxygen (O′) of one particular channel water as a reference point. The trajectory analyzed in Fig. 2 is for the methyl-terminated channel at 150 K. Similar results (not shown) were obtained for the other two runs, except that the proton eventually becomes captured at the NH_2 terminus as NH_3^+.

Fig. 2 *a* shows the 150 K trajectory of the excess proton as it approaches the reference oxygen, O′. Fig. 2 *b* contains the trajectories of three carbonyl-oxygen to water-oxygen distances. Each figure depicts a 1-ps trajectory, which divides naturally into three time slices. The first involves the approach of the excess proton to O′, the second covers the lifetime of the newly formed hydronium ion, and the third involves the departure of a proton. The solid line in Fig. 2 *b* represents the carbonyl-oxygen to water-oxygen distance for the nearest neighbor of the O′ channel water, whereas the short dashed line represents the distance between the O′ and its solvating carbonyl. On comparing Fig. 2, *a* and *b*,

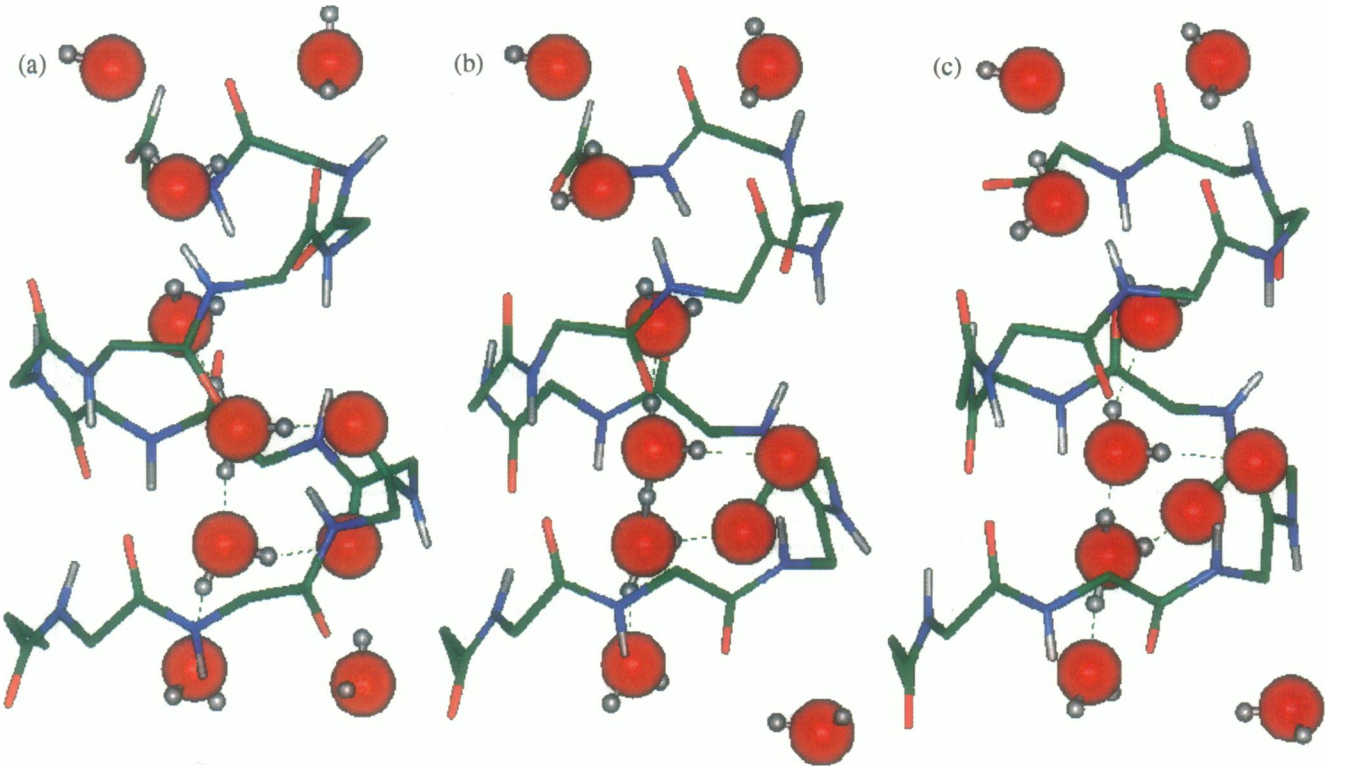

FIGURE 3 Three snapshots of the methyl-terminated polyglycine channel taken from the ab initio CPMD simulation at 150 K, to illustrate a proton transfer event. (*a*) The system before the proton transfer. (*b*) The transition state, in which the proton is shared between two water oxygens creating a transient $H_5O_2^+$ complex hydrogen bonded to the carbonyl oxygens. (*c*) The system after the proton has completely transferred.

one sees a clear correlation between the solvation of the channel water molecule O′ by a carbonyl oxygen and the transfer of the excess proton. For example, around $t \sim 220$ fs, the channel carbonyl-O′ separation becomes very short, indicating strong solvation of the channel water. Although the proton-oxygen distance also becomes shorter, it is only after the carbonyl-water distance drops below 3.0 Å that the proton actually jumps. This observation indicates the importance of a priori carbonyl solvation of the channel water for proton transfer to occur. At $t \sim 350$ fs, the transfer is complete and the previous carbonyl-water bond breaks. For proton diffusion to continue, the next water in line must be similarly solvated. This next water molecule, being at the end of the channel, forms a hydrogen bond across the periodic boundary with a carbonyl oxygen of the channel backbone, and it is this condition that stimulated the proton transfer event. To reiterate, in each instance, there existed a strong hydrogen bond between the hydronium and a neighboring water molecule, but it was not until the hydrogen bond between this nearest neighbor and the channel carbonyl was formed that the proton was observed to transfer.

Fig. 3 illustrates this point. Within Fig. 3 there are three snapshots taken of a single proton transfer event. In the first (Fig. 3 *a*), the hydronium is hydrogen bonded to its neighboring waters and a carbonyl oxygen (dashed lines). In the second snapshot (Fig. 3 *b*), it can be seen that a carbonyl of the channel backbone has moved in more closely to the receiving water (O′ in Fig. 2), which has rotated about the channel axis thereby orienting one of its protons toward the carbonyl oxygen. The net result is increased hydrogen bonding between the receiving water and a carbonyl-oxygen of the channel backbone. The proton is now shared between the two waters, creating a transient $H_5O_2^+$ species. In the third snapshot (Fig. 3 *c*), the carbonyl, which solvated the donating water, has begun to withdraw, and the proton transfer is complete.

DISCUSSION

The rate of proton transfer through gA at saturation (pH 0–2) has been measured to be on the order 10^9 H^+ per second (Akeson and Deamer, 1991). Within that pH range, the conductance was found to be limited by the actual translocation of the proton. Our results on the p-Gly model system seemingly indicate the intrinsic rate for propagation along the channel to be in the picosecond domain, i.e., two to three orders of magnitude faster. However, it must be kept in mind that several phenomena included in the measurement of the above experimental rate, by virtue of the time scales involved, are not in our simulation. For example, when a proton passes through the channel it leaves behind a chain of waters with dipoles pointing roughly in the opposite direction to the original configuration. There is a certain

energetic requirement for the necessary flipping of these waters if the next proton is to pass through and contribute to the net flux. In other words, as a proton passes through the channel it effectively closes the channel to successive proton flow in that direction. It is conceivable that the experimentally observed rate of proton transfer is controlled by the energetics required to achieve a flipping of the channel water dipoles so that a new proton can be accommodated. Computer simulations of water motion within gA (Chiu et al., 1989) have measured a flipping of water dipoles precipitated by fluctuations in the dimer stability and diffusion of water at the end of the channel. The process occurred over a 20-ps interval within a 70-ps simulation, which is a much longer time than can be accessed by the present simulation.

Another issue relates to our treatment of the channel mouth and the junction, two features present in the true system. Several kinetic models have been suggested to describe ion translocation through gA (Finkelstein and Andersen, 1981; Becker et al., 1992). In the commonly accepted 3B2SI model (Van Mau et al., 1994; Benamar et al., 1993; Urban and Hladky, 1979.) for ion translocation through gA, there exist three potential energy barriers to ion diffusion, the largest of which occurs at the middle of the bilayer (channel junction). The other two barriers are attributed to the desolvation of the ion as it enters the channel. Recent work by Hu and Cross (1995), which found that the rate-determining step to the diffusion of Na^+ occurs at the the bilayer center, further supports the 3B2SI model. However, it must be stressed that the mechanism of proton diffusion through the channel observed herein is fundamentally different from that of cation diffusion; therefore, it is difficult to make direct comparison of the energy requirements between the two systems. Nonetheless, a combination of the two effects, namely, the resetting of the channel and the barriers within, could be the determining factor to the overall rate of proton translocation in gA at saturation.

In the CH_3-terminated CPMD run, we did observe the proton leaving the channel and remaining in the region between the two ends of the channel for approximately 0.5 ps. It is possible that this delay in proton migration is a result of the alignment of the water dipoles upon reentering the channel, as well as a result of a barrier in this region. However, nothing quantitative can be deduced regarding the influence of this region on the proton transfer rate based on the present simulation due to the relatively short time scale of the trajectories. If the rate of flow through a monomer is indeed quite fast, then the overall rate is decreased considerably by the phenomena described above and simulations on a much longer time scale must be run to actually see proton diffusion continue.

The proton transfer observed in the CPMD simulations is an essentially barrierless process, and substitution of H for D renders quantum effects relatively small. The dependence of proton transfer rate on the O-O separation has been seen in a number of studies and most recently in the path-integral treatment of a protonated linear chain of waters by Pomès and Roux (1995, 1996). The result of quantum effects is a broadening and shallowing of the free energy surface. A strictly classical treatment of long-range proton transfer would, therefore, result in an underestimate of the rate.

Another important point is that the system simulated was not gA but a p-Gly analog. p-Gly, lacking the side groups of gA, is a much floppier moiety (i.e., the range of possible angles of the channel backbone is much greater than that of the true ion channel). This added flexibility enables the carbonyls to move more efficiently in response to a changing environment and to more readily solvate both the hydronium and its neighboring waters. As a matter of fact, Chiu et al. (1991) have shown that increased flexibility of the gA channel does actually result in increased mobility of the channel waters. Furthermore, the interaction of the proton with fields generated by the periodic images of the channel may also lead to the proton mobility in the channel being artificially high.

We fully realize the limitations of a simulation run of only 1 ps, but this time scale was enough to see the proton transfer through the model channel. Also, given the length of the simulation, there is likely no effect on the observed mechanism due to longer time scale backbone motion or an augmentation of the importance of the carbonyl solvation. Longer, more computationally demanding simulations would be necessary to provide more insight into the proton transfer in this system.

CONCLUSIONS

This preliminary investigation has focused on the possible role of the channel backbone in facilitating the proton diffusion in gA. The results of our CP simulation suggest a very high intrinsic rate for proton diffusion in the channel interior, thus pointing to the importance of other rate-limiting processes in determining the rate of proton diffusion through the channel. The simulation also identified a mechanism for motion in the channel, in which there exists a clear correlation between the carbonyl-oxygen solvation of the hydronium's nearest-neighbor water and the proton transfer event. This observed coupled mechanism is thus somewhat analogous to that found in bulk water, with the role of the second neighbors being played by the carbonyl group (Tuckerman et al., 1995). There has been prior speculation, concerning proton transfer in biosystems, that residues take an active role in the proton-shuttling mechanism (Nagle and Morowitz, 1978). Our results support the possibility that molecules other than water can be vital to proton transfer.

Clearly, this is not the complete story of proton transfer in gA. However, our calculations provide some insight into the mechanics of such a process. A more complete picture will require a more detailed description of the dimer junction. Unfortunately, the system size and run times required would then be prohibitively large for current ab initio methodology.

We acknowledge Gianluigi Veglia and Sandrasegaram Gnanakaran for their help and insight. We thank Tim Cross for an enlightening discussion of gA and an anonymous referee for valuable comments on the original manuscript.

This research was supported by the National Science Foundation and the National Institutes of Health.

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