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RELAY AND BLOCKAGE OF PROTONS IN WATER CHAINS

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1. ABSTRACT

The movement of H^+ is one of the most important and ubiquitous reactions to take place in biological systems. The gramicidin A (gA) dimer, which forms a water-filled channel selective to small monovalent cations in biological membranes, is used as a model system to study the molecular determinants of biological proton transport with computer simulations. The single-file chain of water molecules, or water wire, embedded in the channel interior mediates the translocation of H^+ via a hop-and-turn Grotthuss relay mechanism. Earlier work showing how the mobility of the excess proton in gA is essentially determined by the fine structure and the dynamic fluctuations (structural diffusion) of the hydrogen-bonded network is summarized. The structure and fluctuations of a methanol-containing water chain in the channel lumen suggest a molecular mechanism for the experimentally-measured attenuation of proton conductance by methanol.

2. INTRODUCTION

The transfer and long-range transport of H^+ are among the most important phenomena in chemistry and biology. These reactions are central to acid-base equilibria in aqueous solutions and to energy transduction in living systems. The translocation of protons across biological membranes is a fundamental requirement of bioenergetics. The production of ATP is driven by an electrochemical gradient resulting from the pumping of H^+ across the bioenergetic membrane, a process known as chemiosmotic coupling (1). For that reason, the control of proton translocation across protein assemblies spanning energy-transducing membranes is essential to life.

The transport of an excess proton is thought to involve a Grotthuss relay mechanism consisting of successive transfers of hydrogen nuclei between water molecules forming hydrogen-bonded networks. In such relay processes, the molecular mechanism governing the long-range transport of H^+ is determined both by the nature of the hydrated proton and by structural fluctuations of the hydrogen-bonded network (2,3). In bulk water, the elementary step for the transfer of H^+ between two water molecules involves the interconversion between the Eigen and Zundel forms of the hydrated proton, respectively $OH_3^+(OH_2)_3$ and $H^+(OH_2)_2$. In the first of these two limiting forms, the excess proton is hosted by one water molecule and in the second, it is shared by two water molecules in a strong hydrogen bond. Theoretical studies have shown that this elementary exchange is driven by the rearrangement of hydrogen bonds in the second hydration shell of the excess proton, a process that has been called *structural diffusion* (2,4).

Likewise, the translocation of H^+ across channels and proteins embedded in biological membranes can also occur by way of a Grotthuss mechanism. The long-range relay of H^+ involves water molecules and titratable amino-acid side chains forming hydrogen-bonded networks of low dimensionality, or proton wires (5,6). In linear hydrogen-bonded arrays, this process may be described as a hop-and-

turn mechanism, in which the hop steps consist of successive H^+ transfers along a polarized hydrogen-bonded chain. Because hops invert the donor-acceptor pattern of the chain, the subsequent rearrangement (turn) of proton relay groups is required to restore the original polarization of the wire. Structural evidence for the involvement of water molecules in the relay of protons through the interior of energy-transducing proteins includes bacteriorhodopsin, a light-activated proton pump from the purple membrane of *Halobacterium salinarum* (7). The complete elucidation of proton translocation mechanisms in complex energy-transducing enzymes is a formidable challenge due to the coupling of long-range proton transfer to photochemical or redox reactions and to protein conformational changes (8). Because of its comparative simplicity, gramicidin A (gA), a peptide that forms ion channels in various bilayers, constitutes a good model for the study of biological proton translocation. gA is a pentadecapeptide whose alternating sequence of D- and L- amino acids adopts a right-handed beta$^{6.3}$ helix fold in lipid bilayers (9-11). Head-to-head association of two gA monomers results in the formation of a pore permeable to water and to small monovalent cations (12,13). The functional form of the dimer defines a cylindrical cavity lined with the peptide backbone, exposing its predominantly hydrophobic amino-acid side chains to the core of the bilayer. The lumen, which is approximately 25Å in length and 4Å in diameter, accomodates a single-file chain of water molecules that mediates passive proton translocation.

In the remainder of this paper, we first summarize the molecular determinants of proton transport that have emerged from previous studies of gA, emphasizing the concepts and properties likely to be of general relevance to other biological water wires. Among other factors, the balance of molecular interactions leading simultaneously to the solvation and the mobility of protons is highlighted. As an extension and an illustration of these concepts, we then examine the molecular mechanism of attenuation of proton transport by methanol in a dioxolane-linked gA dimer.

3. MOLECULAR DETERMINANTS OF PROTON CONDUCTION

For the effective permeation of any molecular species to occur, a channel must satisfy a dual requirement: (i) it must provide a local environment suitable to the proper *solvation* of the permeating species, and (ii) this stabilization must not be so strong as to hinder permeant *mobility*. In narrow pores, the permeant is at least partly stripped of surrounding water molecules, and the channel itself must provide the stabilizing interactions resulting in partition from bulk solvent into the channel. In turn, interactions with specific groups on the inner walls of narrow channels, which have been called *surrogate waters*, offer the possibility of controlling the selectivity of the channel. In a membrane channel, this may be achieved locally, by a selectivity filter, or throughout a more extended region in which the permeant and water molecules proceed as a single file. Permeant mobility is achieved as long as the permeant does not bind too strongly relative to the fully-hydrated state, which could result in channel blockage. In the case of a single-file environment, the repetition of the channel-solvation motif can help attain this balance both in a thermodynamic sense and kinetically (14).

The molecular forces leading to the selectivity and transport of monoatomic ions are emerging from studies of the gramicidin channel (15,16), of the potassium channel KcsA (17), and of the chloride channel ClC (18). In addition, the molecular determinants of water permeation through aquaporins have recently been investigated by spectroscopic (19,20) and computational (21,22) studies. Both in gA and in KcsA, the surrogate solvation of alkali metal ions such as potassium is provided by carbonyl groups of the channel backbone in narrow single-file regions. Gramicidin mediates the permeation of small monovalent cations but hardly discriminates between various alkali ions (11,13). In contrast, the coordination of K^+ by KcsA is snug enough to confer specificity and to allow the close proximity of several ions despite the strong coulombic repulsion between like charges (23-25). In channels from the aquaporin family, permeating water molecules also proceed in single-file and form hydrogen bonds both with backbone carbonyl groups and with amide groups, but the molecular origin for the blockage of ions, including H^+, is still a matter of debate.

Protons constitute a very special case of permeating cations because they can form covalent interactions with relay groups or molecules such as water. The molecular basis of the hop-and-turn or Grotthuss relay mechanism for the conduction of an excess proton by a wire is shown schematically in figure 1. In this scheme, the successive transfer or *hop* of hydrogen nuclei between adjacent relay groups forming a polarized hydrogen-bonded chain is complemented by the reorganization (*turn*) of the hydrgogen-bonded network so as to restore the polarity of the wire prior to the passage of another proton in the same direction (5). The propagation of hop and turn steps may be described respectively in terms of the mobility of an ionic defect and of a bonding defect. In water wires, the ionic defect corresponds to the hydrated proton, whereas a bonding defect is defined by the interruption of the polarized chain due to the reorientation of a water molecule. Before illustrating the conduction and blockage of protons in gA channels, we first summarize some of the governing principles and mechanistic aspects implied by the hop-and-turn mechanism in proton wires. The interplay of equilibrium properties and non-equilibrium effects is emphasized. Dynamic fluctuations are paramount to any transport mechanism; in hop-and-turn processes, each of the requirements listed below is modulated by thermal motions giving rise to the transport of ionic and bonding defects.

3.1. Wire Nucleation

A preliminary requirement to the conduction of protons by a relay mechanism is the presence of relay groups in a channel or channel-like cavity such as those found in the interior of proton pumps bacteriorhodopsin and cytochrome *c* oxidase (26). In the case of water wires,

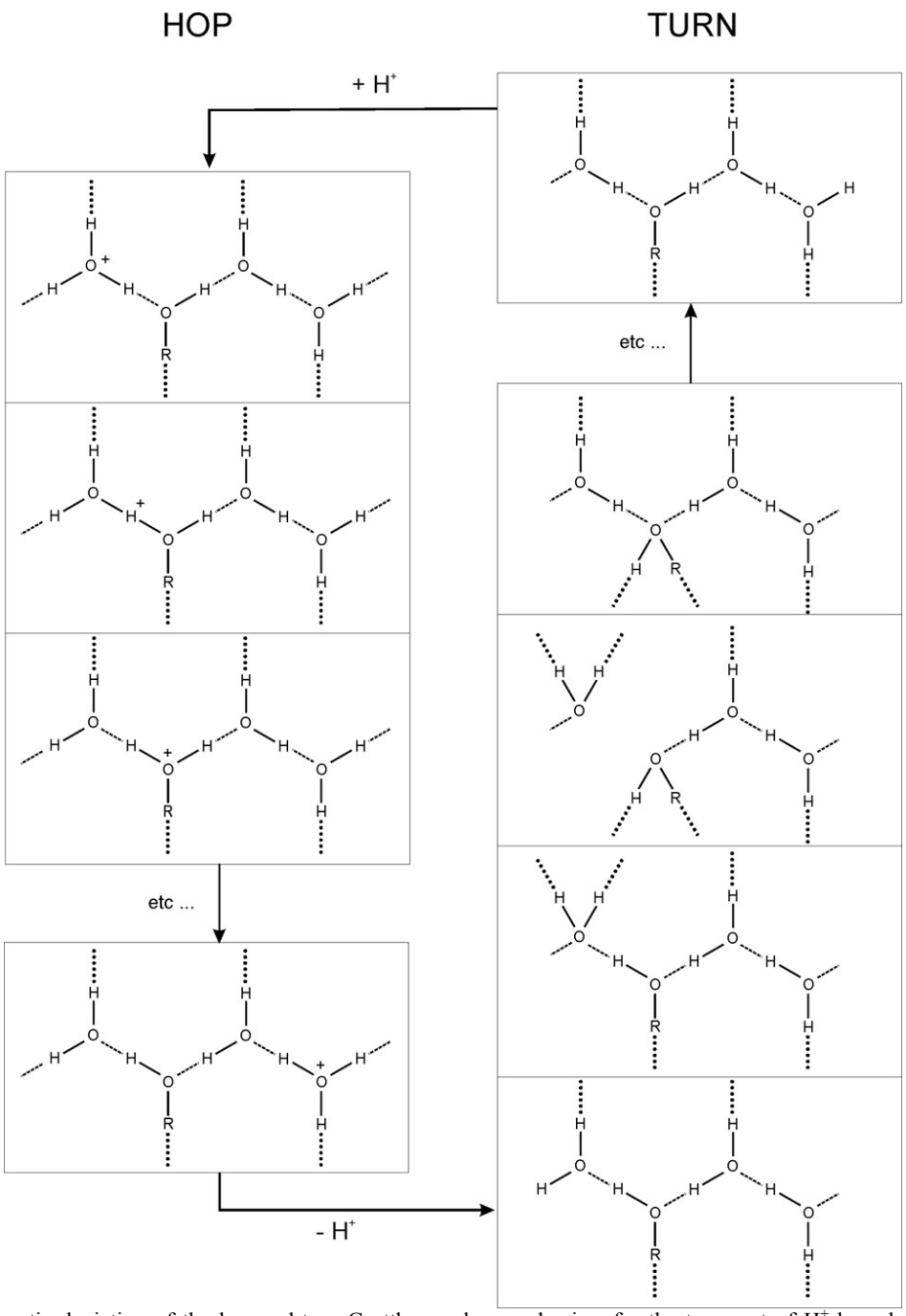

Figure 1. Schematic depiction of the hop-and-turn Grotthuss relay mechanism for the transport of H^+ by a hydrogen-bonded chain (proton wire). Dashed lines represent hydrogen bonds in the pseudo-unidimensional proton wire whereas dotted lines indicate stabilizing hydrogen bonds between the wire and the channel lumen. Cases in which all the relay groups are water molecules (R = H) and where one of these water molecules has been replaced by a molecule of methanol (R = CH_3) are considered in the text. In the latter case, there is no hydrogen bonding between R and the channel.

the relay groups may not be present at all times but could instead nucleate transiently, as has been proposed for the leakage of H^+ across pure lipid bilayers by transient hydrogen-bonded water chains (see Ref. 27 and references therein) or the protonation of the Schiff base in bacteriorhodopsin (7).

3.2. Hydrogen Bonding

For proton hopping to occur, two consecutive relay groups must be hydrogen-bonded to each other. This is because the transfer of H^+ cannot happen through distances longer than that of a hydrogen bond. Not only are protons too reactive to be found in free form in biological

systems, but also, contrary to that of electrons, their de Broglie wavelength (approximately 0.05nm) is too short to enable their quantum tunneling through extended regions of space.

Hydrogen transfer between two water molecules strongly depends on the length of the hydrogen bond. The potential energy profile for the transfer of a H atom in a (moderately strong) water-water hydrogen bond is a bistable well with a substantial activation energy barrier. The height of that activation energy barrier increases with the length of the hydrogen bond. In the presence of an excess proton, the hydrogen bond is much shorter (and stronger); the potential energy barrier opposing H^+ transfer is either absent or considerably reduced, thereby facilitating the transfer (28,29). Thus, the interchange between hydronium and Zundel cations, the two predominant forms of the hydrated proton in a water wire, is characterized by changes in hydrogen bond length.

Inversely, the reorganization of the hydrogen-bonded chain required for the translocation of a bonding defect implies that each hydrogen bond be broken in turn. A good proton duct is one that assists the proton-relay chain in tackling the dual requirement of a proton wire: to enable strong hydrogen bonds between relaying groups for the efficient transfer and relay of H^+; and to help weaken these hydrogen bonds so as to facilitate the reorientation of proton-relaying groups (30).

3.3. Hydrogen-Bond Polarization

Since the translocation of both ionic and bonding defects inverts the polarity of each hydrogen bond in the chain, thermally-accessible conformations of the wire must include alternate polarization of each hydrogen bond (whereby the donor-acceptor pair is arranged alternately left and right in the process depicted in Fig.1) suitable to directional proton transport. It has been proposed that the adverse polarization of the water chain induced by two alpha helices lining the single-file region of aquaporins is what prevents the intrusion of protons in these channels (22).

3.4. Proton Solvation

As emphasized above, the channel must lead to the stabilization of the hydrated proton (ionic defect). In general, this may be achieved by long-range electrostatic forces or locally, by hydrogen-bonding interactions. In a single-file environment, the stable states of the hydrated proton are dominated by hydronium and Zundel forms, and proton hopping occurs via successive exchanges between these two forms (3,28,29,31). Because water molecules hosting the excess proton are coordinated to exactly three hydrogen-bond acceptors (2,4), an environment favoring such a coordination is suitable to the solvation of the hydrated H^+, as depicted schematically in figure 1.

3.5. Stabilization of Bonding Defects

Likewise, molecular interactions favoring the presence of a bonding defect in the chain are important for the reorganization of the hydrogen-bonded network. Thus, the permeation of an excess proton along a chain of water molecules requires adequate solvation both of a hydrated proton and of unprotonated water molecules. In model non-polar pores devoid of hydrogen-bonding partners to the water chain, the turn step of the Grotthuss mechanism was shown to be a thermally-activated process opposed by a significant free energy barrier (27,32).

The above considerations provide guidelines to gauge the possibility of proton conduction by a relay mechanism and the performance of a hydrogen-bonded network in that process. In the next section, we discuss how these concepts are met for rapid relay of H^+ in a good proton duct, gramicidin; inversely, in section 5 we show how their partial violation may lead to the attenuation of proton currents by methanol.

4. PROTON RELAY IN GRAMICIDIN

Evidence for the relay of protons by the single-file water chain embedded in the lumen of gA has been provided by experimental measurements (12,33). The molecular basis of the hop-and-turn mechanism in gA has been the object of detailed computational studies (3,30), which have opened the way to kinetic models of H^+ permeation linking atomistic simulations to the large body of conductance data (34-37). The simulations revealed how the equilibrium structure and the dynamic fluctuations of the entire hydrogen-bonded network involving the water chain give rise to H^+ conduction in a narrow biological pore. In particular, the effect of hydrogen bonding interactions between channel and water molecules on the hop-and-turn conduction mechanism was analyzed by comparison to the results obtained in studies of model non-polar channels, in which such interactions were missing (3,27-32).

In preformed and pre-polarized hydrogen-bonded water chains found in gA and in non-polar pores, spontaneous, small-amplitude oscillations in the length of water-water hydrogen bonds are sufficient to drive the exchange between hydronium and Zundel cations within picosecond time scales (28,29,31). As depicted schematically in figure 1, hydrogen bond donation from water molecules to the gA channel backbone carbonyl groups completes water-water hydrogen bonds to provide a coordination well suited for proton solvation and proton mobility in the proton wire by stabilizing both elementary forms of the hydrated proton, hydronium and Zundel ions. The translocation of the ionic defect is thus facilitated by the fact that water molecules in the chain are primed for hosting the excess proton by adequate tri-coordination; in other words, the single-file region of the channel provides *presolvation* of the hydrated proton throughout most of the single-file region. The repetition of hops along the polarized section of the wire results in the long-range displacement of H^+ without necessitating disruption of the hydrogen-bonded network. In addition, because the periodicity of the gA channel backbone provides approximately the same local environment to each single-file water molecule, the relay groups have comparable proton affinities, which favors high mobility of the ionic defect (30).

By virtue of the symmetry inherent to the head-to-head dimer organization, gA possesses no net dipole moment on average. Thus, the channel does not impose a preferential polarization to the water molecules in the single-file region, which, in the absence of an excess proton and of an electric field, are equally likely to point towards one mouth or the other. Accordingly, in the channel lumen, as in model non-polar pores, the water chain adopts either one of two polarized conformations due to strong water-water dipole interactions. Likewise, the inversion of the chain's polarity is an activated process involving the sequential reorientation of water molecules. However, hydrogen-bond donation to the channel wall stabilizes intermediate conformations of water molecules in the process of reorienting between their two prefered polarized states (see the right-hand side of figure 1), reducing the magnitude of the activation free energy that opposes reorganization of the chain in non-polar pores. Thus the "solvation" of bonding defects by the channel results in catalysis of the turn step of the Grotthuss mechanism (30).

In summary, molecular simulation studies of proton transport in gA have shown how structural diffusion in biological pores arises from subtle local fluctuations of the hydrogen-bonded network and how these properties are themselves determined by the hydrogen-bonding coordination, arrangement, and topology of proton-relay groups. Water chains form highly modulable hydrogen-bonded networks, whose hydrogen-bonding properties are harnessed by carbonyl groups lining the lumen of gA to assist both hop and turn steps of the Grotthuss mechanism. In that sense, the single-file region of the gA channel provides a blueprint for the rapid (~ns), passive translocation of protons across biomembranes.

5. BLOCKAGE BY METHANOL

Together with native gA, linked gramicidin channels offer an avenue to refine the emerging understanding of biological proton relay mechanisms. Covalent linkage of the N-termini of two gA monomers by a dioxolane group results in two diasteroisomeric forms, SS and RR, of the channel (38). Experimental measurements of single channel conductance in planar bilayers showed that methanol attenuates proton currents significantly in the SS dioxolane-linked gA channel, leading to the proposal that methanol partitions inside the pore and delays the passage of protons through the single file (39). How does methanol affect H^+ transport? When inside the channel, a molecule of MeOH could either block proton relay completely or slow it down relative to all-water wires. The unambiguous discrimination between these two mechanisms depends, among other things, on the fraction of time that the channel is occupied by methanol. Two limiting cases can be considered: 1) if methanol resides in the lumen for a fraction of the time, complete blockage would result in the attenuation of proton currents by the same fraction, whereas 2) if methanol occupies the channel permanently, proton permeation would require relay between water and methanol molecules, as shown in figure 1. Since the fractional occupancy of gA by methanol is not known, it is necessary to determine whether or not protons are transferred between water and methanol molecules in order to distinguish between blockage and slow-down of proton transport in gA channels. Here we investigate the structural basis for a relay mechanism using molecular simulations.

Support for a relay mechanism is found in the anomalously fast transport of proton in pure alcohol solutions. Structural diffusion of H^+ in liquid methanol was recently investigated with computer simulations, revealing a chain-like arrangement of methanol hydroxyl groups which is reminiscent of the single-file chain of water molecules found in the interior of gA (40). In the interior of proteins, the direct participation of hydroxyl groups in proton relay is supported by the role of serine residues in enzymes such as green fluorescent proteins (41) and photosynthetic reaction centers (42). In addition, a structural model of a continuous and polarized proton wire with the hydroxyl group of a Tyr side chain joining two chains of water molecules was proposed to explain functional uptake of protons in mutant forms of bacterial cytochrome *c* oxidase (43). For relay mechanisms to be competent, the properties of the hydrogen-bonded network must be compatible with both hop and turn steps of the Grotthuss mechanism (see above), suggesting that dynamic studies of hydrogen-bonded networks can provide clues as to their ability to function as proton wires. Thus, the role of hydrogen-bonded network fluctuations in shuttling of protons by the carboxylic moiety of a glutamic acid residue was investigated in the proton uptake D-pathway of bovine cytochrome *c* oxidase (44).

In order to explore the physical basis for the attenuation of proton currents in gramicidin channels, we present molecular simulations in which the lumen contains water molecules as well as one molecule of methanol. Both protonated and unprotonated states of methanol are considered in turn. Analysis of the structure of the hydrogen-bonded network and its fluctuations at 300K indicates that the hydrogen-bonded chain is interrupted by intercalation of the methyl moiety of methanol, suggesting that a hop-and-turn mechanism is precluded when methanol is present in the single-file region of the channel.

5.1. Methods

The model system consisted of the SS dioxolane-linked channel embedded in a hydrated glyceryl-monooleate (GMO) bilayer (figure 2). The CHARMM program (45), version 27, was used in all molecular dynamics (MD) simulations. Water molecules were described by the TIP3P model (46). The CHARMM22 force field (47,48) was used to model MeOH, the 18-carbon-chain GMO molecules and the peptidic moiety of the gA channel. The force field for the dioxolane linker was taken from a previous study (49). The internal geometry and non-bonded parameters for $MeOH_2^+$ were obtained from *ab initio* calculations; non-bonded interaction parameters were optimized to reproduce the structure and energy of the two hydrogen bonds in a single-file $MeOH_2^+(OH_2)_2$ cluster. Further details of the calculations will be given elsewhere (Yu and Pomès, in preparation).

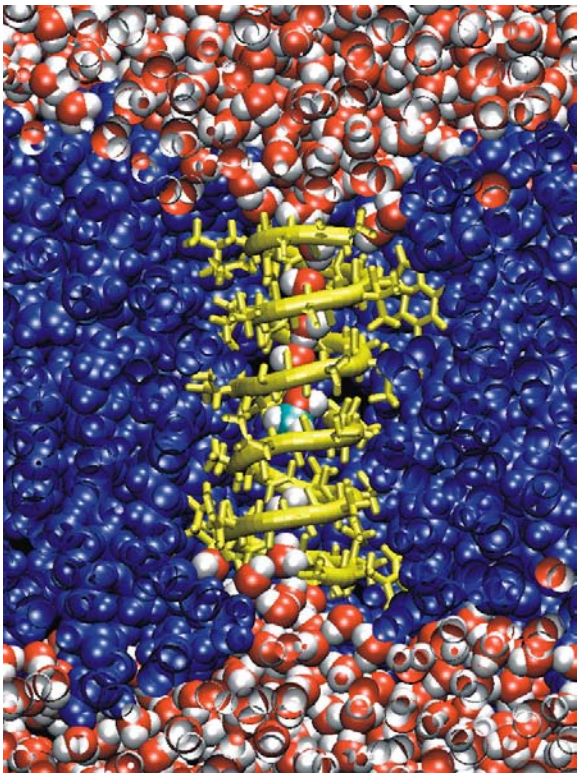

Figure 2. Overview of the system. GMO molecules are shown in dark blue, the gA channel is shown in yellow. A molecule of methanol is visible in the middle of the single-file region of the channel.

In both simulations reported here, the Langevin piston algorithm (50) was used to maintain a pressure of 1.0 atm and zero surface tension (51). A friction coefficient of 5 ps^{-1} was imposed on all heavy atoms. The SHAKE algorithm (52) was employed to fix the length of all covalent bonds involving hydrogen. The Hoover thermostat (53) was used to maintain the temperature at 300K. Periodic boundary conditions were applied together with a Particle Mesh Ewald treatment of long-range electrostatic interactions. Lennard-Jones interactions were cut off at 12Å. The time step for the integration of the equations of motion was 2fs.

The system comprised 122 GMO and 3210 water molecules, yielding a water:GMO ratio of 26:1. The initial surface area of a GMO molecule was set to the experimental value of 37.9$Å^2$ (54,55). Following the prepartion, equilibration, and a 5.8ns simulation of the water-filled SS channel, a molecule of methanol was inserted into the lumen. Two insertion modes differing by the orientation of the CO axis were considered successively. In conformation **1**, the methyl group was placed in the way of the water chain, with the CO bond approximately parallel to the channel axis. In conformation **2**, the CO bond was oriented perpendicularly to the channel axis (see figure 1). Both conformations were subjected to 500 steps of steepest-descent energy minimization, first with channel atoms fixed, then again with all atoms moving; both systems ended up in conformation **1** (figure 3). The system was then equilibrated for 200ps followed by a 7ns production MD run. The starting structure for simulations of protonated methanol was taken from the methanol run at 3.8ns. The protonated methanol was moved to the center of the channel and the system was equilibrated for 200ps before sampling for 2ns.

5.2. Results

In this subsection, we describe the hydrogen-bond network and the dynamics of a methanol-containing water column in the lumen of SS dioxolane-linked gA. In light of the analysis of relay mechanisms presented above, these results offer clues as to the molecular origin for the attenuation of proton conduction by methanol.

5.2.1. Unprotonated methanol

Although MeOH fits and remains in the lumen, it undergoes significant displacements along the channel axis during the course of the simulation. In general, the contents of the lumen fluctuate between seven and eight water molecules. The dynamic behavior of the chain alternates between small-amplitude oscillations around a mean position and rapid burst-like events displacing the column by up to half of the length of the lumen. The time evolution of the interatomic separation between the O atom of methanol and those of its two water neighbors in the single-file region is shown in figure 4. Only one of these two hydrogen bonds is present at any given time. MeOH accepts a hydrogen from a neighboring water and donates its hydrogen to a carbonyl O atom of the channel. As a result, the only stable state observed in the relatively long (7ns) simulation of the $MeOH/H_2O$ column involves interruption of the hydrogen-bonded chain by methanol. Significant gaps of up to 12Å arise occasionally in the column due to this interruption and to the non-polar nature of the methyl group of MeOH, which repels water in the confined channel lumen (figures 2,3). Rapid tumbling of methanol, which occurs eight times during the course of the simulation, exchanges the direction of the C-O bond with respect to the channel axis and the connectivity to water molecules located above and below (figure 3). These transitions are completed within 1ps. Conformations in which MeOH bridges the water chain may exist at best as a transient species in the reorientation process. Because both bridging and interrupting conformations involve MeOH forming two hydrogen bonds, the hydrogen-bond energy of the bridging conformation is comparable to that of the interrupted conformer. Thus, the strong preference for the latter is essentially due to steric repulsion between the methyl group of MeOH and the channel wall.

5.2.2. Protonated methanol

Contrary to the results reported above for methanol, $MeOH_2^+$ is expelled from the channel; this happens relatively quickly (within 0.8ns) despite its initial equilibration near the dimer junction. The ion then spends the rest of a 2ns simulation hovering at the channel/water interface (near z = 12Å). While inside the lumen, $MeOH_2^+$ remains in an orientation that interrupts the hydrogen-bonded chain (figure 5), similarly to the results obtained for MeOH. No tumbling is observed during that time; instead, the ion proceeds to exit the lumen with its polar OH_2^+

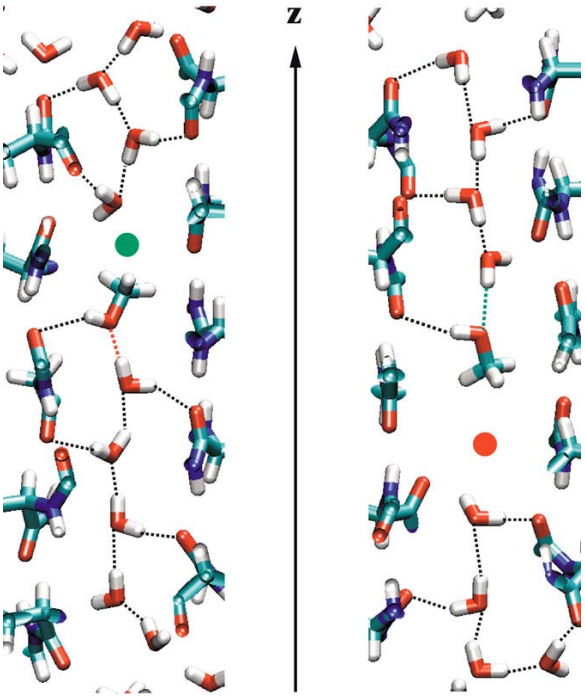

Figure 3. Representative conformations of methanol and water molecules in the lumen of the gA channel. With its CO bond approximately colinear with the channel axis, methanol donates its H atom to a backbone carbonyl O atom of the channel and accepts one from an adjacent water molecule; the methyl group interrupts the hydrogen-bonded chain. Tumbling of methanol exchanges the two conformations, exposing the hydroxyl group alternatively to polarized water chains above and below.

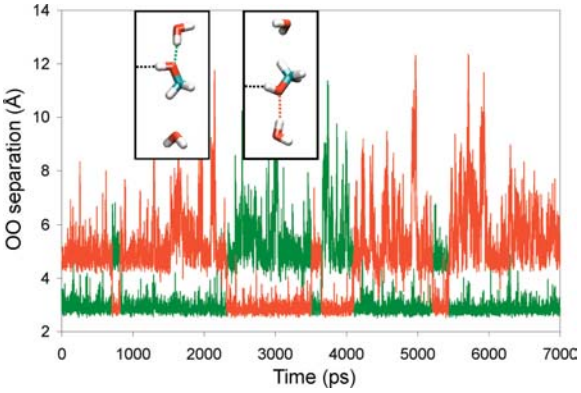

Figure 4. Structural fluctuations of the hydrogen-bonded network around unprotonated methanol, MeOH. The time evolution of the two OO distances separating the O atom of methanol from those of the two closest water molecules located respectively above and below it are shown respectively in green and red. Insets depict hydrogen bonding by methanol.

group first while maintaining its hydrogen bond with the water molecule above and its molecular orientation relative to the channel axis. The absence of bridging conformations suggests that proton relay cannot take place while methanol is present in the channel. In stark contrast, $MeOH_2^+$ is able to form two hydrogen bonds with water as soon as its methyl group pops out of the singe file.

5.3. Discussion

The above results underline properties of hydrogen-bonded networks that are important for structural diffusion. From the point of view of a relay mechanism, methanol is analogous to a water molecule with one of its (H) arms in a bulky (Me) cast. Thus, the perturbation induced in the water wire by the introduction of one molecule of methanol is the disappearance of a hydrogen donor. While most water molecules form exactly three hydrogen bonds in the single-file region of gramicidin (30), both MeOH and $MeOH_2^+$ form two hydrogen bonds while in the channel interior, one with water and one with the channel backbone. Thus, the coordination of the protonated form of methanol, which donates its two H atoms, is consistent with the hydrogen-bonded structure in liquid methanol (40). In that sense, $MeOH_2^+$, like OH_3^+ and $O_2H_5^+$, is adequately solvated in the lumen of the gA channel. However, *unlike* protonated water, protonated methanol does not permit the formation of a continuous hydrogen-bonded chain with two water neighbors unless it is located at the channel mouth, with the methyl group outside the channel.

While the relay of H^+ by MeOH could in principle still be achieved by the rotation or reorientation of $MeOH_2^+$, such events, which are not observed in the simulation, are relatively unlikely. The tumbling or reorientation of a charged protonated species, which necessitates breaking hydrogen bonds, is hampered by the strength of the hydrogen bonds that it forms with its neighbors (56). Such a property is consistent with structural diffusion in bulk water, where reorientation takes place in the second hydration shell of H^+ but not closer (2). In pure water wires such as that found in the gA channel, proton transport can occur over a primed chain of as many as six or seven water molecules without any bond making or breaking (3), consistently with the idealized picture of the hop-and-turn mechanism (figure 1). The lesser strength of the MeO(H)—HOH and H_2O—HOH hydrogen bonds makes the unprotonated forms of water and methanol better suited to turns than their protonated counterparts. Accordingly, the present results suggest that MeOH is indeed well suited as a bonding defect, since it alternatively connects with two polarized water chains (figures 3, 4).

6. CONCLUSIONS

In the above overview, we have outlined some of the general aspects of structural diffusion of relevance to the study of biological channels, membrane proteins, and enzymes. In recent years, molecular simulations of proton transport have begun to provide meaningful insight into the balance of forces underlying the Grotthuss relay mechanism in biological systems. Studies of single-file water chains embedded in narrow pore environments have helped to clarify the role of quantum effects and have shown how the long-range movement of H^+ via a hop-and-turn mechanism is controlled by low-amplitude fluctuations

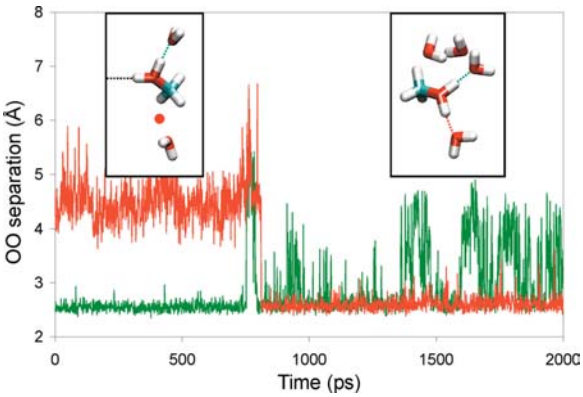

Figure 5. Structural fluctuations of the hydrogen-bonded network around protonated methanol, $MeOH_2^+$. The time evolution of the two OO distances separating the O atom of methanol from those of the two closest water molecules located respectively above and below it are shown respectively in green and red. The methyl group of methanol proceeds to exit the single file at t = 753ps and dwells at the channel/water interface for the remainder of the simulation. Insets depict hydrogen bonding before (*left*) and after (*right*) extrusion of the methyl group from the single-file region.

in the fine structure and the topology of hydrogen-bonded networks (3,27-31,56,57). Furthermore, comparative studies of non-polar pores and of gramicidin have highlighted the interplay between the solvation of ionic and bonding defects and their mobility in a proteinaceous environment (3,30). By extension, the detailed analysis of fluctuations in the hydrogen-bond coordination and the polarization of putative proton relay groups should provide useful insight into the factors leading to the modulation and the blockage of proton movement in protein interiors.

We examined the structure and fluctuations of a methanol-containing water chain in a gA channel analog. A priori, full determination of the molecular origin for the attenuation of proton conductance by methanol is a challenging problem combining the movement of H^+ with that of MeOH through the pore. In addition to possible gating or blockage of proton movement by a stationary molecule of methanol, the fractional channel occupancy and the rate of transport of methanol itself must be taken into account. Our preliminary results indicate that both unprotonated and protonated forms of methanol fit in the channel interior and are stabilized (solvated) by hydrogen bonds with water and with the channel wall. However, the present results also suggest that whereas MeOH is a suitable bonding defect, its protonated counterpart would not qualify as an ionic defect because it fails to form a continuous hydrogen-bonded chain while in the single-file region. The methyl group of permeating methanol molecules would constitute a plug blocking the relay of protons.

7. ACKNOWLEDGMENTS

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