Free Energy Profiles for H$^+$ Conduction along Hydrogen-Bonded Chains of Water Molecules

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ABSTRACT   The molecular mechanism for proton conduction along hydrogen-bonded chains, or "proton wires," is studied with free energy simulations. The complete transport of a charge along a proton wire requires two complementary processes: 1) translocation of an excess proton (propagation of an ionic defect), and 2) reorientation of the hydrogen-bonded chain (propagation of a bonding defect). The potential of mean force profile for these two steps is computed in model systems comprising a single-file chain of nine dissociable and polarizable water molecules represented by the PM6 model of Stillinger and co-workers. Results of molecular dynamics simulations with umbrella sampling indicate that the unprotonated chain is preferably polarized, and that the inversion of its total dipole moment involves an activation free energy of 8 kcal/mol. In contrast, the rapid translocation of an excess H$^+$ across a chain extending between two spherical solvent droplets is an activationless process. These results suggest that the propagation of a bonding defect constitutes a limiting step for the passage of several protons along single-file chains of water molecules, whereas the ionic translocation may be fast enough to occur within the lifetime of transient hydrogen-bonded water chains in biological membranes.

INTRODUCTION

Hydrogen-bonded chains of water molecules, such as those observed in the structure of the photosynthetic reaction center (Baciou and Michel, 1995), and in the lumen-side domain of cytochrome *f* (Martinez et al., 1996), are thought to be implicated in the function of energy-transducing membranes. An emerging idea is that such chains could act as proton wires by providing an effective pathway for the rapid translocation of protons over long distances. A similar mechanism, with transient hydrogen-bonded water chains, has been proposed in relation to proton leakage across lipid bilayers (Nagle, 1987; Deamer and Nichols, 1989; Marrink et al., 1996). A characterization of proton wires at the microscopic level is required for a better understanding of those complex systems.

The narrow transmembrane channel formed by gramicidin (GA) provides a remarkable model system for which the translocation of protons along a single-file hydrogen-bonded chain of water molecules can be characterized quantitatively (Akeson and Deamer, 1991). In this system, the high rate of diffusion of H$^+$ arises because the mechanism for the translocation of the excess proton does not require the net molecular displacement of an ionic solute or of water molecules along the way. Instead, protons can "hop" or transfer along each hydrogen bond of a preexisting network of hydrogen bonds, following a Grotthuss mechanism involving structural rearrangement rather than molecular diffusion (Agmon, 1995). The Grotthuss mechanism can be

described as the combination of two complementary steps (Nagle and Tristam-Nagle, 1983). The conduction of several protons in one direction under the influence of a proton electrochemical potential necessitates both 1) the translocation of proton, or propagation of an "ionic defect," and 2) the reorientation of the chain, or propagation of a "turning defect." This is because, as shown in Scheme 1, the translocation, or "hop," of an excess proton (II) along a chain of suitably oriented hydrogen bonds (I) leaves the hydrogen bonds in the opposite orientation (III). Thus the translocation of the next proton in the same direction requires the preliminary reorientation of the chain from III to I. The latter process is referred to as "turn" because it involves the reorientation of each water molecule (or other proton donor-acceptor group directly involved in the wire) in the chain. Because this process necessitates breaking and reforming hydrogen bonds along the chain, it is also called "propagation of a bonding defect."

Even though putative mechanisms of biological proton conduction by such hydrogen-bonded networks, or proton wires, were formulated two decades ago (Nagle and Morowitz, 1978), the molecular basis for the fast translocation of protons along hydrogen-bonded chains is not fully understood. Experimentally, this is due to the difficulty of detecting what is essentially a transient phenomenon. On the theoretical front, however, several recent studies have begun to provide meaningful insight into the mechanism of proton transport in hydrogen-bonded water networks. Ab initio studies of the dynamics of proton transfer in water have revealed how the migration process of H$^+$ in solution arises from the structural reorganization of the hydrogen-bonded network involving neighboring water molecules (Ando and Hynes, 1994; Tuckerman et al., 1995). Molecular dynamics studies of single-file water chains in vacuo using an empirical force field and discretized Feynman path

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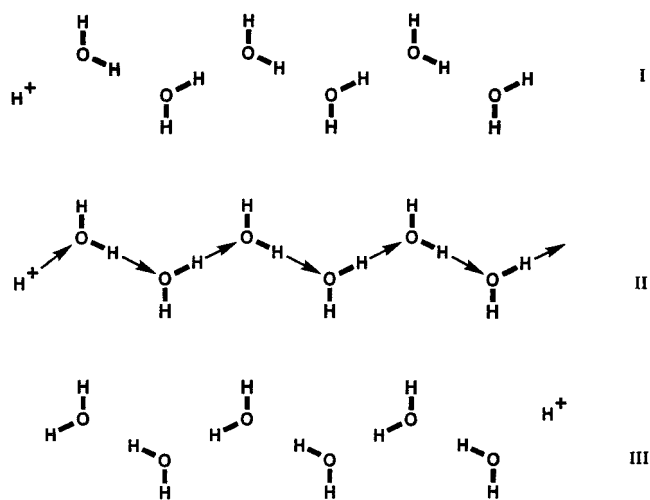
Scheme 1.

integrals have helped to characterize the nature of the fluctuations governing proton translocation and the interplay between the flexibility of the water chain and the contribution of quantum effects (Pomès and Roux, 1995, 1996a). The approach was also used to determine the influence of the GA channel on the mechanism of proton translocation (Pomès and Roux, 1996b). The latter simulations suggested that ionic translocation across much of the length of the pore can occur within the picosecond time range, a time scale supported by independent simulations of proton translocation in a polyglycin analog of the GA channel performed at the ab initio level (Sagnella et al., 1996).

Although such studies have unveiled important properties of the proton wire, the limitations of proton conduction along water chains and other hydrogen-bonded chains remain unclear (see the discussion by DeCoursey and Cherny, 1997). This arises in part because the focus has been placed primarily on proton transport itself, and not on the reorientation of the unprotonated water chain. Yet the slow rate of water rotational reorientation in model ion channels (Breed et al., 1996), the relatively rare occurrence of dipole reorientation of a water chain forced to extend across the lipid bilayer (Marrink et al., 1996), and the persistence of turning defects over several hundred picoseconds in the GA channel (Pomès and Roux, 1996b) suggest that a comparatively large activation energy may be involved in the reorientation step, in both polar and nonpolar environments. This could have important implications, particularly in the case of transient, nonpolar water pores. Because water wires extending across the lipid bilayer are thermodynamically unstable, they form infrequently, and their lifetime is probably limited to a few picoseconds (Marrink et al., 1996). Thus the possible role of such hydrogen-bonded chains in proton leakage depends on their kinetic competence in translocating protons before breaking up. The properties underlying proton conduction clearly require further characterization. As a first step, the present work extends the use of computational techniques to calculate the free energy profiles for

the Grotthuss mechanism intrinsic to a hydrogen-bonded chain of water molecules in a model nonpolar pore.

In this paper we characterize the translocation and reorientation processes separately, using molecular dynamics umbrella sampling simulations. We calculate the potential of mean force (PMF) profiles for charge migration and for the reorientation of the water chain in two model systems of proton wires, and contrast the mechanisms involved. The first model consists of an isolated linear chain of nine water molecules, whereas in the second model, spherical droplets of 25 water molecules are added to each end of the single file to mimic the influence of a polar environment at either side of the membrane. The center of charge is used as reaction coordinate for the translocation of the excess proton along the single file, whereas the reorientation is characterized by the inversion of the total dipole moment of the water chain. Results indicate that in the presence of solvent droplets, the protonic translocation proceeds spontaneously along the entire length of the chain at 300 K. In contrast, the reorientation of the unprotonated wire involves a substantial free energy barrier of 7–8 kcal/mol. The respective mechanisms are discussed, and possible implications for the mechanism of proton conduction across the biological membrane are proposed.

METHODS

Two models were constructed: a linear chain of nine water molecules in vacuo, and a "dumbbell" model of 59 water molecules. Both were used to perform classical molecular dynamics simulations, successively in the unprotonated chain and in the presence of an excess proton. The PMF for the reorientation of the dipole of the single-file region in the unprotonated chain and the potential of mean force for the displacement of the center of charge along the axis of the protonated chain were calculated for each of the two models by umbrella sampling simulations (Allen and Tildesley, 1987).

Potential energy function

The potential energy function used to model the single-file water chain, the Polarization Model, version PM6, was designed by Stillinger and coworkers to reproduce the properties of water and its ionic products (Weber and Stillinger, 1982). The PM6 model consists of O^{2-} and H^+ ions. In addition to pairwise-additive terms, the electronic polarizability is accounted for by a many-body function. Because it is dissociable and polarizable, the PM6 model offers a reasonably realistic method for the simulation of proton transfer processes in water clusters and a tractable alternative to costly ab initio simulations (Pomès and Roux, 1996a). For a complete description of the potential energy function, the reader is referred to the original papers (Stillinger and David, 1978; Stillinger, 1979; Weber and Stillinger, 1982); for a description of its use in conjunction with molecular dynamics simulations of proton wires, see our earlier papers (Pomès and Roux, 1995, 1996a,b).

The first model (Model I) of the proton wire consists of a linear chain of nine water molecules. To preserve the linearity and connectivity of the hydrogen-bonded chain, the oxygen atoms were subjected to two constraints: 1) cylindrical restoring force on the oxygen atoms beyond 0.75 Å from the chain's axis (z axis) to maintain linearity; 2) harmonic restoring force on the end points outside of the range $z = \pm 10.00$ Å to avoid evaporation of the water molecules. The force constants of both constraining potentials were chosen as 10 kcal/mol/Å^2. The above constraints mimic

the influence of a hypothetical, perfectly cylindrical hydrophobic pore accommodating a single file of water molecules.

The second model (Model II) was designed to characterize the influence of bulk water lying at either end of the single file region on the H$^+$ translocation and water reorientation processes. To construct the second model, the chain of nine PM6 water molecules was capped by spherical droplets of 25 water molecules. These spherical droplets are in tangential contact with the extrema of the linear chain, thus constituting a "dumbbell." The TIP3P potential energy function (Jorgensen et al., 1983) was used for the water droplets. The computational advantage from using unpolarizable and undissociable potential energy functions such as TIP3P for the bulk water regions arises in large systems because the PM6 model is significantly more costly in terms of computational time, because of the iterative calculation of the many-body polarization term.

To preserve the dumbbell geometry, the water droplets' O atoms were subjected to harmonic restoring forces acting beyond 5.5 Å from the droplets' centers, which were located, respectively, at $z = \pm 17.2$ Å. Because of the constraints imposed, the interfaces between the droplets and the single file are well defined spatially: the nine PM6 water molecules remain in the single-file region throughout the simulations, and the two water models cannot mix or interconvert.

The optimization of the TIP3P-PM6 water-water interactions was required for consistency at the interface between the linear chain and the spherical droplet. The dissociation energy of two water molecules in vacuo (H$_2$O-HOH) into two isolated water molecules, and the dissociation of a protonated water dimer (H$_2$O-H$^+$-OH$_2$) into H$_2$O and H$_3$O$^+$ were examined. The optimized geometries and energies are given in Table 1. The PM6 O atoms were assigned Lennard-Jones parameters identical to those of the TIP3P O atoms for the calculation of PM6-TIP3P interactions. The Lennard-Jones parameters of PM6 H atoms were $\epsilon = 0.0498$ kcal/mol and $R_{min} = 1.84$ Å. Finally, the Lennard-Jones parameter R_{min} for the O(PM6)-H(TIP3P) pairwise potential term was taken as 2.43 Å to match the water-water hydrogen-bonding energies of the two possible isomers, donor-acceptor and acceptor-donor. At −8.6 kcal/mol, this energy is comparable to the hydrogen bond energy of the TIP3P dimer, −7.0 kcal/mol. Further optimization of the system is not an easy task, as the two potential functions have radically different forms. Although discrepancies remain in both the energy and the geometry of the hybrid hydrogen bonds, the approximation is sufficient to conserve the tetrahedral coordination of PM6 water molecules in a TIP3P environment, and to allow the hydrogen bonds to reorganize without favoring one type of TIP3P-PM6 hydrogen bond over the other (donor-acceptor versus acceptor-donor). The energy of dissociation of the protonated dimer is 28% smaller in the hybrid model than in the all-PM6 O$_2$H$_5^+$ (Table 1). Because the hybrid model is not intended for the study of proton diffusion in the droplets, and because TIP3P-PM6 proton transfer is precluded by construction, the preference for proton hydration in the PM6 part of the dumbbell model should not affect the study of proton translocation in the single-file region.

Molecular dynamics simulations

After inclusion of the PM6 potential energy function, version 22 of the CHARMM program (Brooks et al., 1983; Mackerell et al., 1998) was used

TABLE 1 PM6-TIP3P interactions

Dimer		$E_{binding}$ (kcal/mol)	O⋯O (Å)	O⋯H (Å)
H$_2$O⋯⋯⋯HOH				
TIP3P	PM6	−8.56	3.01	2.04
PM6	TIP3P	−8.56	2.85	1.86
TIP3P	TIP3P	−7.04	2.75	1.76
PM6	PM6	−5.55	2.90	1.91
H$_2$O⋯⋯⋯HOH$_2^+$				
PM6	PM6	−32.78	2.47	1.24
TIP3P	PM6	−23.77	2.99	1.94

to generate the molecular dynamics trajectories from which the PMF profiles were calculated. The Langevin equations of motion were propagated at 300 K with a friction coefficient of 1 ps^{-1} and a time step of 0.5 fs. Each picosecond of dynamics required 30 s of CPU for the nonameric chain, and 6 min of CPU for the dumbbell, on a R-4000 SGI workstation. The PMF profiles were calculated as follows. The configuration of the system was recorded every 5 fs. The projection of the total dipole moment of the wire (PM6 waters only) on the z axis of the linear hydrogen-bonded chain was calculated for each configuration at time t as

$$\mu_z(t) = q_H \sum_{i=1}^{N_H} z_{H_i}(t) + q_O \sum_{j=1}^{N_O} z_{O_j}(t) \qquad (1)$$

in units of e · Å, where N_H and N_O are the total numbers of H and O nuclei, and q_H and q_O are the formal charges of H and O (respectively, 1 and −2e in the PM6 model). In the unprotonated chain (O$_9$H$_{18}$), this expression yields the z component of the total dipole moment, whereas in the protonated chain (O$_9$H$_{19}^+$), it corresponds to the projection of the center of charge along the z axis. The PMF was calculated from the equilibrium probability distributions of μ_z obtained from the simulations.

A biasing potential energy function was imposed on μ_z to force the sampling over the large free energy barrier for reorientation of the unprotonated chain. This "umbrella sampling" calculation (Allen and Tildesley, 1987) was performed by means of harmonic biasing functions of the form $V_i(\mu_z) = (1/2)k_i(\mu_z - \mu_{zi})^2$ imposed on μ_z. All k_i coefficients were set to 20 kcal · mol^{-1} · e^{-2} · Å^{-2}, whereas μ_{zi} varied in increments of 0.5, from −6.0 to 0.5 e · Å in the nonamer, and from −7.0 to 0.5 e · Å in the dumbbell. Each window of the umbrella simulation consisted of a 60-ps run with 10 ps of equilibration and 50 ps of data collection. The simulation time required to build the PMF for the wire's reorientation totaled 900 ps for the isolated nonamer, and 950 ps for the dumbbell. The construction of the PMF profiles from the data obtained from the various umbrella simulations, or "unbiasing," was performed with the WHAM algorithm (Kumar et al., 1992; Roux, 1995). The same calculation was repeated for an isolated chain of nine TIP3P water molecules to gauge the influence of polarization of the force field on the reorientation mechanism (total simulation time of 3.0 ns).

In the simulations of the protonated wire, umbrella sampling was not required, because there were no substantial free energy barriers opposing the translocation of H$^+$. Consequently, the PMF for proton mobility was computed from long unbiased simulations totaling 2.5 ns in the isolated nonameric chain and 1 ns in the dumbbell system.

RESULTS

Propagation of a bonding defect

At equilibrium, the unprotonated chain of nine PM6 water molecules is arranged preferentially in one of two "polarized" configurations for which the total dipole moment is aligned with the chain axis. Moreover, each configuration corresponds to an oriented donor-acceptor pattern in the hydrogen bonding between the adjacent water molecules of the wire (Fig. 1). This can be rationalized in simple electrostatic terms from the maximization of favorable interactions between the molecular dipoles of each pair of water molecules and from the formation of a continuous hydrogen-bonded chain. The reorientation of the chain is sequential, as illustrated in Fig. 1: intermediate configurations involve the successive reorientation of water molecules, 1, 2, 3, etc., rather than the flip of individual water molecules located randomly in the chain. Thus the interconversion between the two polarized configurations involves the mi-
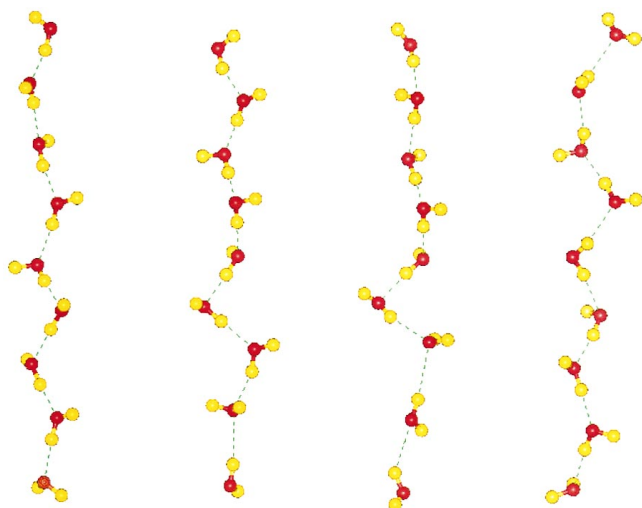
FIGURE 1   Snapshots of the PM6 water nonamer illustrating the sequential reorientation of the hydrogen-bonded chain. Hydrogen bonding is indicated by dashed lines. From left to right, $\mu_z \simeq -7.0$ (oriented chain), -3.5 (reorientation of the second water from bottom), -2.0 (reorientation of the third water from bottom), and 0.0 e $\cdot$ Å (the fifth water reorients, two oriented half-chains).

gration of a single hydrogen-bonding defect from end to end, consistently with the idealized picture of the Grotthuss mechanism.

The completely oriented chain corresponds to deep wells at $\mu_z = \pm 7.15$ e $\cdot$ Å in the PMF for the reorientation of the unprotonated nonameric chain in vacuo (Fig. 2). In contrast, the state corresponding to two oriented "half-chains" where the central water molecule projects a dipole moment of zero along the chain axis, and the dipoles of the outer four molecules on each side of the central water point either toward it or away from it (Fig. 1), corresponds to a free
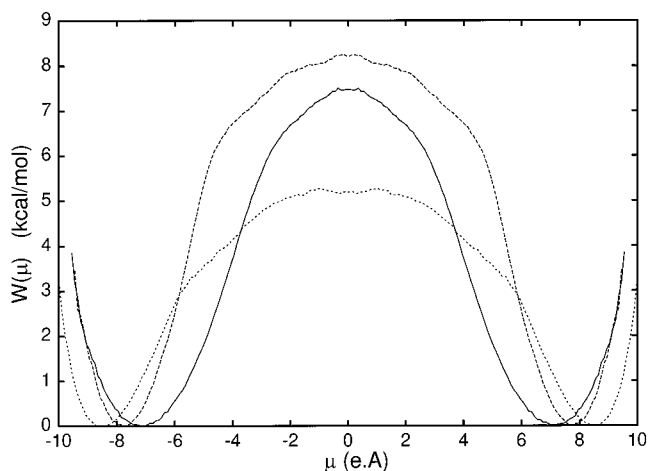

FIGURE 2   Potential of mean force for the reorientation of the unprotonated water chain: ——, PM6 water nonamer; – – –, dumbbell model; ·····, TIP3P water nonamer. Note that in the latter case, μ_z was scaled by 0.417, the fractional charge of H atoms in the TIP3P potential, for consistency with the magnitude of μ_z obtained with the PM6 model, in which the formal charge of H is 1 (see Eq. 1).

energy barrier of 7.5 kcal/mol. Dipole-dipole and hydrogen-bonding interactions of the water molecules in the linear hydrogen-bonded chain constitute the dominant forces that favor the orientation of the chain and oppose the passage from one stable configuration to the other.

The free energy profile for reorienting a TIP3P water chain is included in Fig. 2 for comparison. Although the profile is qualitatively similar to that obtained with the PM6 water model, at 5.2 kcal/mol the barrier is smaller by 30%. The inclusion of electronic polarizability has a significant effect on the reorientation process: the structure of the (unpolarizable) TIP3P water chain near $\mu_z = 0$ exhibits less order than its PM6 counterpart (not shown), and the free energy penalty for diminishing the magnitude of the chain's dipole moment is higher when polarization is included. Nevertheless, the TIP3P model, by favoring strongly the totally oriented configurations over partially oriented or random configurations, reproduces the essential features of the reorientation process obtained with the PM6 model. In particular, both water models indicate the existence of a significant free energy barrier.

The PMF profile for the reorientation of the dumbbell model, where solvent droplets were added to the extremities of the nonameric chain, is also shown in Fig. 2. The free energy for flipping the total dipole of the chain is very similar to that of the isolated chain, and involves a slightly larger barrier of 8.3 kcal/mol. Although the presence of a polar medium capping the model pore further stabilizes the polarized conformations of the chain, the influence of the bulk water droplets at either end of the single file region is not dramatic. Compared to the isolated water nonamer, the oriented chain has a larger dipole component along the channel axis (± 7.75 versus ± 7.15 e $\cdot$ Å), and the propagation of the turning defect is easier throughout the center of the chain: the slope of the barrier decreases notably after the one or two water molecules near the end of the single file have reoriented. Whereas the free energy cost for rotation of the waters at the end of the single file is largest, the relative flatness of the PMF profile near the top of the barrier reflects the somewhat collective nature of the reorientation of the central water molecules in the chain.

Propagation of an ionic defect

As described in more detail elsewhere (Pomès and Roux, 1995, 1996a), the structure of linear chains of water molecules is strongly affected by the inclusion of an excess proton. The excess charge of H^+ induces short, strong water-water hydrogen bonds, along which the potential energy profile for proton transfer involves small or nonexistent barriers. Fluctuations in the polar environment of the proton modulates the asymmetry of the well(s) and result in variations in the O-O separations, thereby leading to the propagation of the proton along the system. The proton transfer itself is thus determined by thermal fluctuations of the geometry of the hydrogen bonds in the water chain. An

analogous process involving the rearrangement of water molecules around $(OH_3)^+$ and $(O_2H_5)^+$ ionic species has been proposed as a mechanism for proton diffusion in bulk water that may be called "structural diffusion" (Tuckerman et al., 1995).

In the nonameric water chain in vacuo, the excess proton is located on average near the center of the system, and thermal fluctuations cause its diffusion along the four central hydrogen bonds of the chain. The excess proton does not wander off to the extremities of the hydrogen-bonded chain, where the charge would be more poorly solvated than in the center. The PMF for proton mobility in the isolated nonameric water chain is shown in Fig. 3. The free energy profile for the motion of the center of charge along the chain's axis is a single well centered about the origin, which corresponds on average to a central hydronium ion and to two oriented half-chains whose dipoles point away from the excess charge. This free energy well is fit well by a quadratic function $f(\mu_z) = (1/2)k\mu_z^2$, where $k = 0.55$ kcal $\cdot$ e^{-2} $\cdot$ Å^{-2}.

After the addition of solvent droplets, the free energy profile changes to a broad, flat-bottomed well (Fig. 3). This is because the presence of the excess proton near the extremities of the single file is now electrostatically stabilized by the presence of the polar medium provided by the water droplets. As a result, the translocation of H$^+$ takes place spontaneously over the entire length of the wire under the influence of thermal fluctuations. This process is very fast and occurs within the time scale of a picosecond. It should be noted that because the Langevin equation was used to propagate the equations of motion, however, it is not possible to extract more precise temporal estimates from these simulations.

As a collective reaction coordinate, the location of the center of charge (μ_z) reflects not only the location of the excess proton in the $(O_9H_{19})^+$ chain, but also the configuration of all of the water molecules in the single file, thereby taki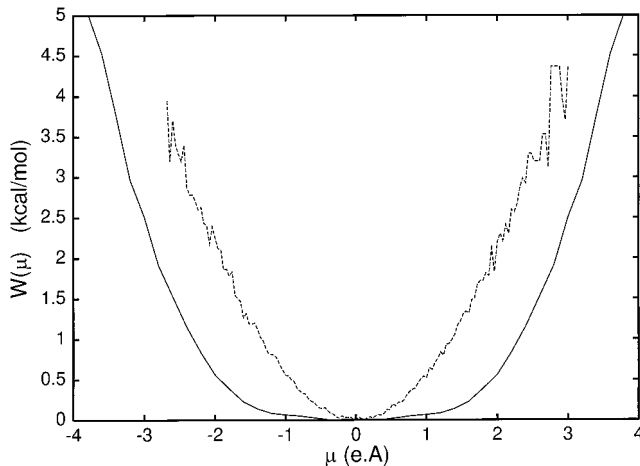ng into account changes in the orientation of individual water molecules as the charge moves along the chain. It is useful to correlate that reaction coordinate to the location of the excess proton along the hydrogen-bonded chain. The latter is approximated by the following function:

$$\xi_{OO} = \left(\sum_{i=1}^{8} w_i\right)^{-1} \sum_{i=1}^{8} w_i(i - 4.5) \qquad (2)$$

where $w_i = e^{-\alpha(r_i - \beta)}/(1 + e^{-\alpha(r_i - \beta)})$, $\alpha = 30$ Å^{-1}, $\beta = 2.58$ Å, and r_i is the distance separating O atoms i and $i + 1$ in the linear chain. w_i is a smooth step-like function that gives most weight to strong hydrogen bonds ($r_i \leq 2.5$ Å), less to intermediate-strength hydrogen bonds ($2.5 \leq r_i \leq 2.7$ Å), and least to weaker hydrogen bonds ($r_i \geq 2.7$ Å). Thus the function ξ_{OO} follows the location of the strongest bond(s) in the chain. Such a reaction coordinate makes use of the strong coupling between short O-O separations and the presence of H$^+$: for each configuration, the shortest O-O "bond" in the wire is assigned the strongest weight because it is the one most likely to host the excess proton. This is equivalent to following the midpoint of a hypothetical $O_2H_5^+$ cluster over the course of the simulation. A more general formulation extendable to two- and three-dimensional hydrogen-bonded networks would be obtained by replacing the dimensionless index i in the above expression for ξ_{OO} by the Cartesian coordinates of the midpoint of each hydrogen bond. In simulations of the GA channel, this approach was shown to constitute a good approximation of the location of the excess charge in the proton wire (Pomès and Roux, 1996b). As seen from Fig. 4, the two reaction coordinates μ_z and ξ_{OO} are strongly correlated throughout the course of the simulation, and the excess proton does indeed travel from end to end of the hydrogen-bonded chain: at $\xi_{OO} = \pm3.5$, water molecules 1–2 and 8–9, respectively, have the properties of a protonated water

FIGURE 3 Potential of mean force for the translocation of an excess proton. – – –, Isolated $(O_9H_{19})^+$; ——, dumbbell model.
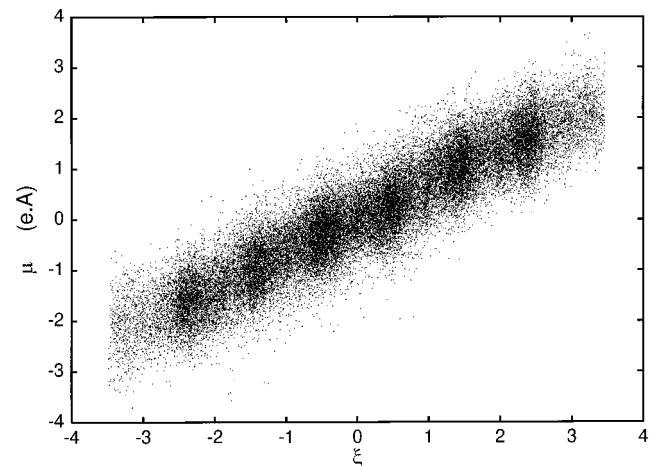

FIGURE 4 Comparison of the two collective reaction coordinates for proton translocation, μ_z and ξ_{OO}, obtained from the dumbbell simulation. There is a linear relationship between the location of the center of charge on the z axis, μ_z, and the index of the strongest hydrogen bond, given by ξ_{OO} (see text).

dimer, $O_2H_5^+$. Because it incorporates the overall configuration of the hydrogen-bonded chain, μ_z is consistent with the reaction coordinate chosen in the study of the unprotonated chain and constitutes an appropriate collective reaction coordinate to follow proton translocation.

DISCUSSION

The mechanism of proton translocation is a semicollective process involving thermal fluctuations of water-water hydrogen bond lengths. As described in detail elsewhere (Pomès and Roux, 1995, 1996a), these fluctuations govern the migration of the excess proton along a tight core of water molecules in the hydrogen-bonded chain. These studies also indicated that because the thermal fluctuations governing the transport of H^+ along hydrogen-bonded water chains involve predominantly classical degrees of freedom, the classical limit is appropriate. In an isolated linear water cluster, the tight protonated core is confined to the center of the chain. However, under the influence of a polar medium at the ends of the single-file chain, at 300 K the migration of an excess proton in a nonpolar pore can occur spontaneously from end to end of the wire with thermal fluctuations. This result is in striking contrast to the large activation energy required to reorient the unprotonated chain.

In the absence of an excess proton, the thermodynamic preference for oriented hydrogen-bonded chains is an intrinsic property of water in a nonpolar pore. There are, in principle, many ways to orient the individual water molecules so that the net dipole projection is identically zero. For instance, any configuration in which the z components of adjacent pairs of water molecules cancel each other results in $\mu_z = 0$. This would tend to favor such configurations entropically. Yet in all such cases with a net dipole of zero, the sum of all unfavorable molecular dipole-dipole interactions is maximal compared to the oriented configurations. In addition, the enthalpic cost of breaking a hydrogen bond in the single file is large enough that only one defect is observed at a time. The presence of a free energy barrier for the chain reorientation is thus due primarily to enthalpic factors. In previous computer simulations of single-file water chains in GA (Chiu et al., 1991; Roux and Karplus, 1994, and references therein), in the lipid bilayer (Marrink et al., 1996), and in model pores (Breed et al., 1996), the hydrogen-bonded chain was often observed to be oriented rather than disordered. The slow rotational relaxation of water in model hydrophobic channels has been studied in detail (Breed et al., 1996). The collective nature of the reorientation process, after its initiation by turning of the water molecules at the end points, was noted by Marrink et al. (1996). Furthermore, the reduced barrier obtained with the TIP3P model indicates that electronic polarizability is important in modeling of the dynamics of single-file water chains, in agreement with other studies (Duca and Jordan, 1997).

A motivation for this study arose from relatively long (100–400 ps) molecular dynamics simulations of the GA channel (Pomès and Roux, 1996b), which suggested that the overall translocation of protons is limited by slow rearrangements of the hydrogen-bond defects in the hydrogen-bonded network of the proton wire. These rearrangements involve changes in the relative orientation of individual water molecules in the chain and constitute the primary event in the propagation of a bonding defect. Because they occur on a typical time scale of 100 ps in the GA channel, they appear to be essentially independent of the motion of the proton itself, which takes place on a much shorter time scale (less than 1 ps) (Pomès and Roux, 1996b; Sagnella et al., 1996). Thus the time scales of the physical events responsible for the propagation of the ionic and bonding defects differ very significantly from each other. If the water molecules in the pore do not form a continuous hydrogen-bonded chain, the limitation for proton translocation is given by the making and breaking of hydrogen bonds. Yet the present simulations suggest that it is costly to interrupt the hydrogen-bonded water chain in a nonpolar environment. The scarcity of hydrogen-bonding partners (at most two per water molecule) favors the presence of hydrogen bonds between adjacent water molecules. This factor, together with the preference for an oriented hydrogen-bonded chain, contributes to a fast ionic translocation while hindering the propagation of the bonding defect.

This separation of time scales has direct implications for the Grotthuss mechanism of proton transport in transient, nonpolar water pores. To explain proton leakage across biological membranes, a plausible mechanism has been proposed that necessitates a very fast translocation of protons through transient water chains extending across the lipid bilayer (Nagle, 1987; Deamer and Nichols, 1989; Marrink et al., 1996, and references therein). This is because the free energy of the formation of a single file of water molecules in the adverse, hydrophobic core of the lipid bilayer is largely unfavorable. Accordingly, computer simulations of water in an explicit lipid bilayer suggest that such chains form rarely and have a very short lifetime, estimated at a few picoseconds (Marrink et al., 1996). Based on their observation of a single occurrence of turning defect translocation during their 320-ps simulation of a water chain constrained to extend across the lipid bilayer, Marrink et al. (1996) conclude that the reorientation step is likely to be too infrequent to occur during the lifetime of the water pore, which rules out the passage of several protons. This argument is supported by the magnitude of the energy barrier opposing the propagation of a turning defect, i.e., 10 times the thermal energy, obtained here. Furthermore, provided that the diffusion of H^+ to the pore entrance is not rate limiting, the concentration of these water pores would be sufficient to explain measured conductance rates of protons by the membrane only if it is assumed that a proton is translocated through each of them before their collapse. Assuming an activation control for the ionic translocation, Marrink et al. (1996) estimate that the rate of passage of

protons is on the order of one per nanosecond, which leads them to question the validity of such a mechanism. However, the activationless nature of the PMF for proton translocation and the high rate of ionic diffusion observed in this work suggest that transient water chains would be kinetically competent to permit proton leakage. Recent measurements of the permeability of the phospholipid bilayer to protons were consistent with the transient pore mechanism for proton translocation in lipids with acyl chains shorter than 20–22 carbon atoms, corresponding to an ~30-Å-thick hydrophobic region (Paula et al., 1996). The present study provides elements to reconcile the transient hydrogen-bonded chain mechanism with a detailed molecular picture of water wires in nonpolar channels.

The model systems studied here represent proton wires that are not subjected to any electrostatic interactions with the wall of the pore. As such, they constitute an idealized model of hydrophobic water channels. In the presence of local electrostatic interactions between the water wire and hydrogen-bonding or polar groups in a biological channel, one would expect particular configurations of the water chain to be stabilized, thus modifying the free energy profile. For example, similar to the results obtained for other cations (Woolf and Roux, 1997), PMF profiles may reveal "hydrated-proton binding sites" in the GA channel. Likewise, polar interactions involving the single-file region, while favoring pore hydration, are also likely to affect the free energy profile for the wire's reorientation after the passage of a proton. For instance, for an efficient proton transport mechanism it is possible that polar transmembrane proton conductors such as the GA channel facilitate the reorientation process relative to nonpolar water pores through the stabilization of intermediate configurations. Thus it may be expected that the balance of water-water and water-channel interactions modulates the relative ease of ionic and bonding translocations.

CONCLUSIONS

In the above study we investigated the dynamics and PMF profiles for the translocation of an excess proton and for the reorientation of a linear chain of nine water molecules, successively in vacuo and with the addition of two droplets of water (dumbbell) to gauge the influence of bulk solvent at either end of the single file. The translocation of an excess proton along the single file region is confined to the center of the isolated chain for energetic reasons, whereas in the dumbbell model the stabilization provided by the water droplets allows the proton to readily migrate from one end of the chain to the other. This process involves no significant free energy barrier and thus occurs spontaneously with thermal energy.

In contrast, the PMF for the reorientation of the dipoles of the unprotonated hydrogen-bonded water chain involves a free energy barrier of ~8 kcal/mol. This barrier does not change significantly upon the inclusion of solvent droplets

and can be attributed to the intrinsic enthalpy for unfavorable dipole-dipole interactions in the chain. Thus the present study suggests that the reorientation process, not the passage of a proton itself, constitutes the limiting step for the passage of several protons through water chains in nonpolar pores.

The results support kinetic competence of a hopping mechanism for the proton conductance by nonpolar water pores such as those proposed to form transiently in lipid bilayers (Nagle, 1987; Marrink et al., 1996; Paula et al., 1996). In such systems, the lifetime of the oriented hydrogen-bonded chain would be long enough to allow the rapid passage of one proton, though too short for the reorientation to take place. Although more detailed studies are needed to further our understanding of the factors governing proton translocation across biological membranes, this study demonstrates the feasibility of free energy simulations with collective reaction coordinates and their usefulness in the study of the detailed molecular properties of hydrogen-bonded chains relevant to both steps of the Grotthuss mechanism.

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