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Molecular Mechanism of Biological Proton Transport

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Abstract. Proton transport across lipid membranes is a fundamental aspect of biological energy transduction (metabolism). This function is mediated by a Grotthuss mechanism involving proton hopping along hydrogen-bonded networks embedded in membrane-spanning proteins. Using molecular simulations, we have explored the structural, dynamic, and thermodynamic properties giving rise to long-range proton translocation in hydrogen-bonded networks involving water molecules, or 'water wires,' which are emerging as ubiquitous H^+-transport devices in biological systems.

THE GROTTHUSS MECHANISM

Hydrogen-bonded networks possess a very special property: they can mediate the long-range translocation of an excess H^+ via chemical exchange of hydrogen nuclei. This process was first imagined by De Grotthuss in 1806 to explain the electrochemical dissociation of water in galvanic cells [1],and was first formulated in the context of biological systems by Nagle and Morowitz in 1978 [2]. The elementary exchange step of the Grotthuss mechanism consists of proton transfer between adjacent hydrogen-bonded groups in the network. The repetition of this step along a suitably-oriented chain results in the net transport of one proton from end to end (see Fig. 1), without the need for a proton-carrying molecule to diffuse throughout the system. This hopping is known as the transport of an *ionic defect*. In order for a second proton to be translocated in the same direction, the inversion of the chain must first take place, because hopping leaves the chain in the opposite orientation. Because the reorientation of each H-bearing group in the chain creates a defect in the continuity of the hydrogen-bonded chain (HBC), the overall reorientation process is described a the translocation of a *bonding defect*. Both of these 'proton-hop' and subsequent 'turn' steps are thus required in the directional transport of protons. In this paper, we summarize recent advances in the detailed description of both hop and turn steps of the Grotthuss mechanism in biologically-relevant systems at the atomic level.

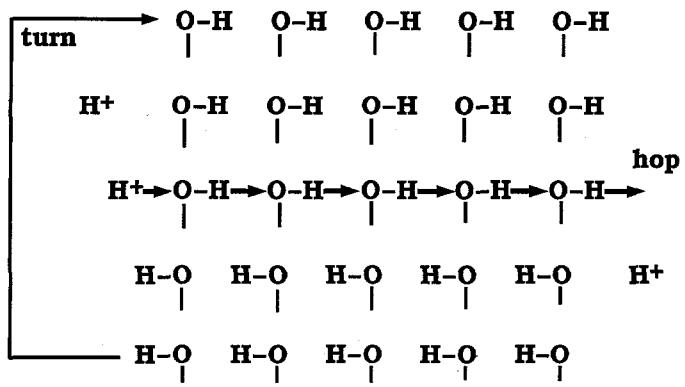

FIGURE 1. Hop and turn steps of the Grotthuss mechanism.

BIOLOGICAL RELEVANCE

Pure lipid bilayers contain nonpolar cores made up of aliphatic chains. For this reason, they constitute microscopic capacitors that are largely impermeable to ions. Nature has harnessed that property by using the build-up of electrochemical proton gradients, $\Delta\mu_{H+}$, across the membrane, to perform energy transduction. These gradients consist both of transmembrane electric fields and of pH gradients.

To transport H^+ across the membrane, special molecular assemblies are required [3]. In *active transport*, the energy required for (or liberated by) proton transport against (in the direction of) $\Delta\mu_{H+}$ is provided (used) by coupling to exoenergetic (endoenergetic) chemical reactions. Thus, cytochrome *c* oxidase, the terminal link of the proton-pumping electron-transport-chain in mitochondrial and bacterial respiration, utilizes electrons released by the oxidation of cytochrome *c* to reduce O_2 to water, and the energy liberated by this reaction is used to pump four protons against an electrochemical H^+ gradient. Subsequently, the energy liberated by the downfield translocation of H^+ is harnessed by another complex transmembrane assembly, ATP synthase, to phophorylate adenosine diphosphate (ADP).

By contrast, in *passive transport* the rapid equilibration of protons across the membrane is not coupled to chemical reactions. Instead, passive transport is mediated either by channels or transporters, which use different mechanisms. Valinomycin, an example of a transporter, is a titratable macrocycle that diffuses across the bilayer alternately in its protonated and unprotonated form, thus physically shuttling protons. Channels, on the other hand, are membrane-spanning proteins forming water-filled pores. Gramicidin A (GA) is a well-characterized membrane channel. In its active form, it assembles in phospholipid bilayers to form a cylindrical pore 0.5 nm in diameter which accommodates a single-file of eight or nine water molecules and mediates the translocation of monovalent cations such as H^+, K^+, and Na^+. Because the diffusion rate of H^+ in GA is faster than that of water molecules themselves, this channel provides a flagrant example of proton hopping along a *water wire* via a Grotthuss mechanism [4].

The idealized picture of the Grotthuss mechanism depicted in Fig. 1 leaves many questions unanswered. What are the structural properties of proton wires relevant to ionic translocation? What is the importance of quantum effects arising from the light mass of H^+? What is the role of thermal fluctuations in long-range transport? Is the hopping process totally concerted, or does it involve intermediates? What is the influence of the protein environment? What is the nature and importance of the turn, or reorientation step, relative to proton-hopping? Finally, how are these various properties used by energy-transducing biomolecules in the active conduction of protons? To address these questions at the molecular level, we have considered molecular models of increasing complexity, from simple water chains, to the water wire of GA, to proton-pumping proteins. In the following section, we first briefly review the fundamental properties affecting proton transfer between two water molecules. In the subsequent sections, we summarize the main results obtained our studies, and how these have led us to identify a number of dominant factors at play in biological proton transport.

PROTON TRANSFER IN $O_2H_5^+$

The smallest molecular system of wide relevance to proton transport both in chemistry and biology is a protonated water dimer, $H_2O\cdots H^+\cdots OH_2$, or $O_2H_5^+$. In that system, three important properties control the exchange of H^+:

(i) *Hydrogen-bond length.* The presence of an excess proton induces a strong, short hydrogen bond (0.24 nm) in which H^+ is shared by the two water molecules. Whenever the OO separation becomes larger under the influence of thermal motions, the potential energy profile for the motion of the excess proton changes from a broad, flat well to a symmetric bistable well. The barrier separating these two wells rises with increasing OO separation, eventually forcing H^+ to form a covalent bond with one of the O atoms ($H_2OH^+\cdots OH_2$).

(ii) *Quantum Effects.* Zero-point energy and nuclear quantum tunneling effects arise from the light mass of H^+. Both result in the delocalization (sharing) of H^+ between the two water molecules, even in the presence of a potential energy barrier.

(iii) *Fluctuating Polar Environment.* The presence of polar species flanking $O_2H_5^+$ creates an asymmetry in the potential energy profile of H^+. If this asymmetry is strong enough, H^+ is confined to the potential energy well which is lower in energy. Large fluctuations in the polar environment may be required to invert this asymmetry and thereby transfer H^+ to the other water molecule.

All of these effects have to be considered at once for a realistic description of the hopping process. In an extended HB network, the complexity is compounded by the fact that not just one, but many HB become potential sites for transfer. To address this problem, we performed molecular dynamic simulations using the polarizable, dissociable PM6 model of water [5]. In some simulations, we treated all the exchangeable H nuclei with discretized Feynman path integrals so as to account for nuclear quantum dispersion [6].

STRUCTURE AND DYNAMICS OF WATER WIRES

In one-dimensional HB networks consisting of protonated water chains of a few water molecules, the excess proton is often shared by two water molecules in an $O_2H_5^+$-like cluster. While quantum effects (primarily those due to the proton's zero-point energy) were found to be significant, they are not required for H^+ transfer, as most of the time the potential energy barrier is either nonexistant or smaller than the zero-point energy of H^+ [7,8].

Exchange of H^+ between adjacent hydrogen bonds, which governs the translocation, involves structural fluctuations in the HB chain that take place spontaneously at 300 K. In this process, thermal motion of the heavy (O) atoms

of the chain modulate the potential energy profiles of individual hydrogen-bonding H nuclei, which exchanges the location of the shared proton and give rise to the net transport of the ionic defect in the chain. In chains of nine water molecules extending over 2 nm, such motions are neither totally concerted nor Markovian. Rather, they exhibit a strong semi-collective character [8].

PROTON HOPPING IN GRAMICIDIN

The next level of complexity considered consists in embedding the water wire in the cylindrical tube of GA. Compared to that of simple linear water chains, in GA the hydrogen-bonded network gains dimensionality. This is because in addition to the possibility of forming up to two HB with adjacent water molecules in the single file, each water molecule can also donate hydrogen atoms to peptide O atoms lining the pore. As a result, the HBC is not always continuous, but rather, bonding defects can occur, particularly whenever a single-file water molecule donates both of its H nuclei to the pore (see Fig.2).

In the presence of an excess H^+, the HB network formed by the single-file water molecules comprises two parts: near the excess charge, the water molecules are strongly polarized and form a well-connected HB cluster of up to seven water molecules extending over much of the pore's length, whereas bonding defects limit the extension of this polarized cluster across the entire length (2.3 nm) of the pore.

The hopping of H^+ in the protonated cluster, as in simpler protonated water chains (i.e. in the absence of the channel), takes place spontaneously with thermal fluctuations in the picosecond time-range. Analysis of the proton-hopping coordinate reveals that the protonated species is best described as $O_nH_{2n+1}^+$, where n fluctuates between 1 (hydronium ion) or two (protonated water dimer) up to six, pointing to the importance of cooperative dynamic fluctuations in the motion of heavy atoms.

By contrast, bonding defects confining the extension of the polarized water cluster block further hopping of H^+ [9]. Migration of these defects occurs via the reorientation of water molecules. In the interior of the GA channel, water reorientation takes place infrequently compared to the ps timescale for proton hopping

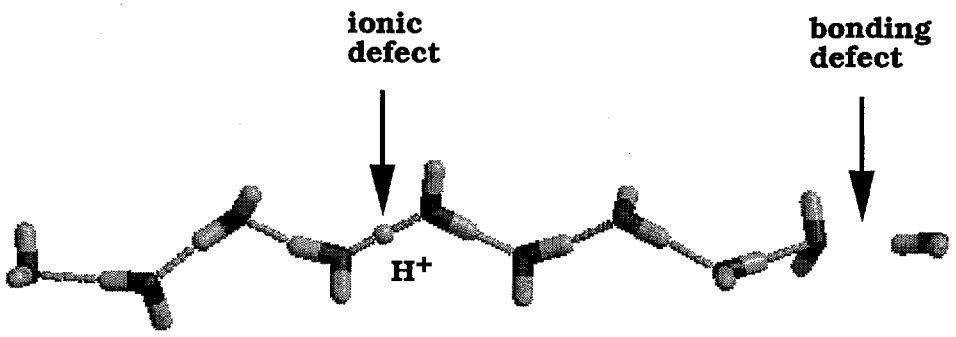

FIGURE 2. Water wire in the GA channel in the presence of H^+. The channel was omitted for clarity. Proton hopping takes place spontaneously along the continuous hydrogen-bonded chain. A water molecule donating both H atoms to peptide groups lining the cylindrical pore creates a bonding defect.

along the continuous segment of the water chain: according to the simulations, the lifetime of bonding defects is of the order of 0.1 ns. This prediction is consistent with recent FTIR spectroscopic results which suggest that (1) strong "proton polarizability" (rapid hopping induced by thermal fluctuations) exists in the single-file water chain of GA, but that (2) not all the water molecules of the single file are involved in the process due to hydrogen-bonding with the backbone [10].

Thus there is a separation of time scales arising from the structure (connectivity) of the HB network in the GA channel: the migration of bonding defects, a step complementary to proton hopping in the Grotthuss mechanism, constitutes the rate limiting step to the fast translocation of protons.

THE TURN STEP OF GROTTHUSS

The previous results suggest that reorientation of water molecules in the water wire in GA is kinetically important to the overall process of proton conduction. To further characterize the turn step of the Grotthuss mechanism, we performed potential of mean-force (PMF) calculations for the inversion of the total dipole moment of water wires, successively in GA and in an inert cylindrical pore. This latter model constitutes an idealized "hydrophobic channel" in which, as indicated earlier, each water molecule can form at most two hydrogen bonds, one with each adjacent water in the single file arrangement. Comparison of the results obtained with this hydrophobic pore to those obtained with the GA channel allows to characterize the influence of the protein on the reorientation process.

Both in GA and in the hydrophobic pore, the HB chain is preferentially fully-oriented, in agreement with the idealized picture of the Grotthuss mechanism (Fig.1). Furthermore, in both systems the reorientation is sequential (i.e. it involves the sequential migration of a bonding defect throughout the chain), and it

is a strongly activated process. In the model, inert pore, the activation free energy for the inversion of a chain of nine water molecules is 7 to 8 kcal/mol [11], whereas in GA it is only about half as large [Pomès, R., and Roux, B., manuscript in preparation]. This effect is due to hydrogen-bonding between single-file water molecules and the interior of the GA channel, which result in the relative stabilization of partly-oriented chains by enabling water molecules to adopt intermediate configurations, perpendicular to the channel axis (see Fig.2).

Thus the polar environment of GA has a dual and opposite effect on the hop and turn steps of the Grotthuss mechanism: on the one hand, it slows down the rapid, unactivated translocation of H^+ by inducing bonding defects, but on the other hand, it facilitates the slow, activated, rate-limiting turn process.

H^+ SHUTTLING IN A PROTON PUMP

The systems studied above comprise water channels involved in passive proton conduction. Importantly, there is growing evidence for the implication of water chains in the mediation of long-range, active proton transport by energy-transducing proteins. Thus, chains of water molecules revealed by crystallographic studies have been proposed to function as proton wires in the photosynthetic reaction center [12] and in the lumen-side domain of cytochrome *f* [13]. Furthermore, computational and spectroscopic studies lend support to the presence of internal water wires in two proton pumps for which water is not structurally resolved, bacteriorhodopsin [14] and cytochrome *c* oxidase [15,16].

In the latter system, mutagenesis studies [15] also implicate a conserved glutamic acid residue in the pathway for the uptake of pumped protons into the pocket of the binuclear center, where the reduction of O_2 takes place. The side chain of this residue (residue 242 in the numbering of the bovine heart enzyme) lies between two chains of water molecules in such a way as to interrupt the continuity of the hydrogen-bonded chain, which led to the proposal that it constitutes a shuttle for proton relay to the active site [15,16]. Using free energy calculations, we have studied the conformational isomerization of this side chain, with its carboxylic acid group successively in protonated and in unprotonated states [17]. The results indicate that Glu242 can adopt three or four conformational states which are all significantly populated. Importantly, it was determined that this isomerization exposes the carboxylic acid group alternatively to hydrogen-bonding with the two water channels, in support of a hop-turn-hop sequence of events in a Grotthuss mechanism for the relay of H^+. Furthermore, the isomerization was found to involve relatively small activation energy barriers (1 to 3 kcal/mol), which suggests that the this proton-relay mechanism is kinetically competent.

Further studies of the Grotthuss mechanism in cytochrome *c* oxidase are under way in an effort to determine whether the water-glu-water chain is a passive proton wire, or whether the process is coupled to the redox state of the enzyme, thereby acting as a ratchet in the directionality required for proton pumping.

SUMMARY

We have characterized the molecular mechanism of passive proton transport in water-filled pores. In particular, we have gained meaningful insight into the importance of quantum effects, into the nature and structure of transient intermediates involved into the ionic translocation, and on the dynamic properties governing H^+-hopping along extended HB chains of water molecules. Furthermore, from a study of both dynamic and thermodynamic properties of water chains, we have identified the reorientation of H-bearing groups as the physical process limiting the net transport of H^+ in a realistic protein environment. This insight has led us to address more complex proton-pumping systems in which both water wires and titratable amino acid side chains are involved in the mediation of proton translocation.

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