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Tightly Connected Water Wires Facilitate Fast Proton Uptake at The Proton Entrance of Proton Pumping Proteins

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Proton translocation by biological membrane proton pumps is an essential part of many bioenergetic processes.[1] It involves proton uptake from the environment (often cellular solution) and proton transfer (PT) inside the pumps. Many of the proton pumping proteins transfer protons in a very efficient manner. For example, in the F_o component of F-type ATPases, the proton conductance of 6×10^3 s^{-1} significantly exceeds the delivery rate of protons (4×10^2 s^{-1}) by free diffusion from bulk solution.[2] Therefore, both uptake and internal transfer must be efficient enough to achieve such a fast proton conductance.

The internal PT inside the pumps is an example of PT in a confined space, where water molecules are restrained in a limited volume and easily form stable structures. Such well organized H-bonding networks among water molecules in a confined space are critical for the functions of many PT related proteins.[3] These networks are also responsible for the rapid proton conductance through a water-filled carbon nanotube where proton diffusion is 40 times faster than that in bulk.[4] However, most of the water molecules that are involved in the proton uptake process from the environments are located in a nonconfined, open space, in which their coordination is more dynamic. Therefore, the "supply chain" of protons from the environment would be interrupted if the proton uptake in this step was not fast enough (e.g., only relied on random diffusion). Interestingly, many proton entrances of membrane proton pumps are equipped with negatively charged amino acid residues. According to the "proton antenna" theory of Gutman,[5] these Asp and Glu residues near the proton entrance form a "Coulomb cage" that can attract protons from long distances ranging from 1 to a few nanometers. However, structural (vehicle) diffusion driven by electrostatic attraction cannot explain the efficiency of such a directed PT toward the proton entrance observed here. In this work, we therefore introduce the role of locally structured H-bond networks in mediating proton translocation toward charged acceptor residues in open space.

In this study, we investigated the proton uptake of an engineered subunit C of F_1F_o ATP synthase from *E. coli*[6] by a series of Q-HOP molecular dynamics simulations allowing for PT between titratable groups.[7] In these simulations, hydronium ions were randomly placed 1.2−1.4 nm away from the mutated loop of the subunit. One to three residues were mutated to Asp to mimic negatively charged proton entrances (details of the simulation setup can be found in the Supporting Information, SI). In 16 out of 36 simulations, the proton was transferred to the one to three exposed aspartic acid residues of the subunit within 10 to 80 ps (Figure 1a and SI). Figure 1a shows the analysis of 1 of these 16 simulations as an example. Here, the proton reached the single Asp residue within 10 ps involving 15 proton hopping events. The movement of the proton (1.38 nm in less than 10 ps) is ∼1 order of magnitude faster than it takes a hydronium ion to randomly diffuse over such a distance in bulk solution. This fast and directed PT cannot be explained by structural diffusion and electrostatic attractions only.

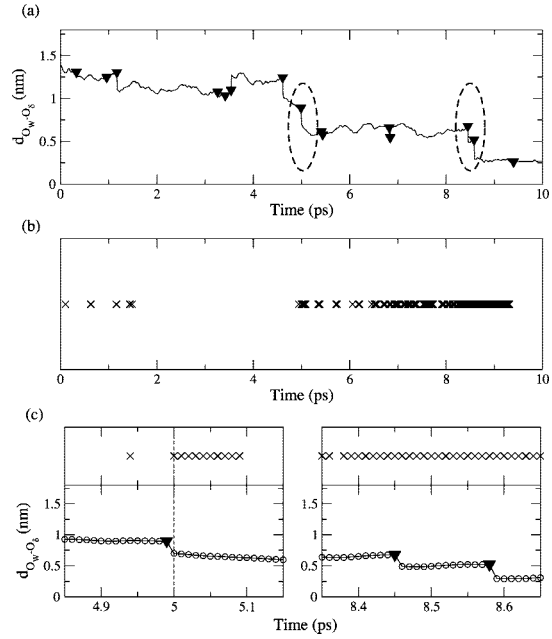

Figure 1. (a) Distance between the oxygen atom of the hydronium ion and the closest carboxyl oxygen of the aspartic acid at the proton entrance as a function of the simulation time during one simulation. Dashed ellipses indicate the parts expanded in (c). (b) Crosses indicate when tightly connected water wires (TCWs) exist that connect the hydronium ion to the aspartic acid. (c) Detailed views of the parts marked by the dashed ellipses in (a) and (b). In (a) and (c), ▼ denotes proton hopping events between a hydronium ion and a water molecule.

According to our previous study of the protonation equilibrium of solvated acetic acid,[8] PT from a hydronium ion to a water molecule in aqueous solution only occurs when the O−O distance is less than 0.275 nm. Proton transfer from a hydronium ion to the carboxylate group requires an O−O distance < 0.30 nm. Raschke and Levitt also suggested that the O−O distance of a "good quality" H-bond between two water molecules lies between 0.27 and 0.28 nm.[9] Therefore, we chose 0.275 nm as a criterion for "tight" H-bonds between water molecules and 0.30 nm for H-bonds between water and aspartic acid. Surprisingly, even with this rather short threshold, a considerable number of tightly connected water wires (TCWs) were found near the negatively charged residues at the proton entrance (Figure 1b and SI). Figure 1b shows that TCWs connecting the hydronium ion and the aspartic acid(s) are formed, especially when the O−O distance between them is shorter than 1 nm. A closer view of the coexistence of such connecting TCWs and the proton transfer toward the proton entrance (Figure 1c) illustrates that TCWs connected to the aspartic acid facilitate the proton uptake at the proton entrance. The TCWs act as a proton acceptor as well as a proton bridge that connects the hydronium ions in the environment to the proton entrance.

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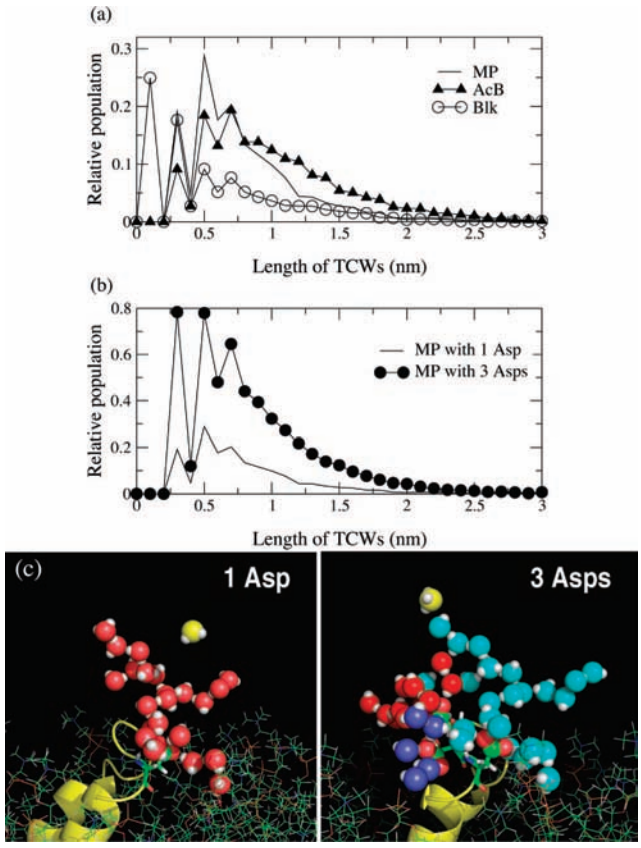

Figure 2. (a) Relative population of TCWs (number of TCWs divided by the total number of snapshots) with different lengths in the MP, AcB, and Blk simulations. (b) Relative population of TCWs with different lengths in the MP simulations with different numbers of aspartic acid residues. (c) Snapshots showing the increasing number of TCWs in the simulations with more aspartic acid residues. Different colors represent different TCWs. The H_3O^+ is colored in light yellow; the protein is shown as ribbons.

For better statistics and comparisons, additional molecular dynamics simulations without proton hopping using the GROMACS package[10] were performed on the same systems with a lipid membrane[11] and protein (MP), on systems containing only a capped negatively charged aspartic acid in bulk solution (AcB), and a pure water box (Blk). All systems were run at different ionic strengths, and the MP system was also run at two different temperatures (details are given in the SI). The results of these simulations are summarized in Figure 2. Figure 2a shows the relative population (defined as the number of TCWs divided by the total number of snapshots) of tightly connected water wires with different lengths (defined as the distance from the carboxyl group of the aspartic acid to the oxygen atom of the farthest water in the wire). In the MP and AcB simulations, a large fraction of TCWs with length > 0.7 nm (average size of the Coulomb cage of one negatively charged residue[5]) was found. The total relative populations of these long TCWs are 0.68 and 1.10 (or 43% and 64% of the total population) for the MP and AcB simulations, respectively. This indicates that, for more than two-thirds of the simulation time, there exist nanometer-sized water wires swinging near the proton entrance, like tentacles of the sea anemones, that are ready to collect protons that are close to or fall into the Coulomb cage of the negatively charged proton entrance. The larger population in the AcB simulations compared to that in the MP simulation is due to about twice as many accessible water molecules in the AcB simulation. In contrast, the relative population of long TCWs (0.32) in the control simulations Blk is less than one-half or one-third of those in the MP and AcB simulations. Even the total relative population including short water chains is lower in the Blk simulation (1.00 for Blk, 1.58 and 1.73 for MP and AcB). This shows that the increasing numbers of long TCWs in the MP and AcB simulations are induced by the negatively charged residues. Control simulations of AcB and MP with a protonated aspartic acid showing a decreased relative population of long TCWs further support this point (see SI).

In MP simulations with two to three aspartic acids in the loop (entrance region), the TCWs near the proton entrance had the same length as that for the TCWs with a single Asp (Figure 2b). However, as the proton entrance is lined by such residues, the total number of TCWs, both short and long, increases. The increasing number (or relative population) of long TCWs covers more volume near the proton entrance (see Figure 2c) and therefore increases the chances for proton capture from the environment.

In summary, tightly connected water wires also exist in systems with nonconfined water like membrane proton pump/bulk solution systems. The TCWs connected to the negatively charged proton entrance facilitate the fast proton uptake by the proton pump. They function as a direct proton bridge or/and stabilizer of protons within the Coulomb cage of the proton entrance. Negatively charged residue(s) at the proton entrance induced the large population of long TCWs. More negatively charged residues increase the population of such long TCWs, which in turn increase the possibility to capture protons from the solution.

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Supporting Information Available: Complete data for all simulations as well as the simulation setup. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Luecke, H.; Schobert, B.; Richter, H. T.; Cartailler, J. P.; Lanyi, J. K. *Science* **1999**, *286*, 255–260. (b) Iwata, S.; Ostermeier, C.; Ludwig, B.; Michel, H. *Nature* **1995**, *376*, 660–669. (c) Michel, H. *Biochemistry* **1999**, *38*, 15129–15140. (d) Abrahams, J. P.; Leslie, A. G. W.; Lutter, R.; Walker, J. E. *Nature* **1994**, *370*, 621–628.
(2) (a) Wright, C. A. *Biochim. Biophys. Acta* **2006**, *1757*, 886–912. (b) Feniouk, B. A.; Kozlova, M. A.; Knorre, D. A.; Cherepanov, D. A.; Mulkidjanian, A. Y.; Junge, W. *Biophys. J.* **2004**, *86*, 4094–4109.
(3) (a) Rhodes, M. M.; Réblová, K.; Šponer, J.; Walter, N. G. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 13380–13385. (b) Olkhova, E.; Hutter, M. C.; Lill, M. A.; Helms, V.; Michel, H. *Biophys. J.* **2004**, *86*, 1873–1889. (c) Braun-Sand, S.; Strajbl, M.; Warshel, A. *Biophys. J.* **2004**, *87*, 2221–2239. (d) Marx, D. *ChemPhysChem* **2006**, *7*, 1848–1870. (e) Swanson, J. M. J.; Maupin, C. M.; Chen, H.; Petersen, M. K.; Xu, J.; Wu, Y.; Voth, G. A. *J. Phys. Chem. B* **2007**, *111*, 4300–4314.
(4) Dellago, C.; Naor, M.; Hummer, G. *Phys. Rev. Lett.* **2003**, *90* (1−4), 105902.
(5) Gutman, M.; Nachliel, E. *Annu. Rev. Phys. Chem.* **1997**, *48*, 329–356.
(6) Girvin, M. E.; Rastogi, V. K.; Abildgaard, F.; Markley, J. L.; Fillingame, R. H. *Biochemistry* **1998**, *37*, 8817–8824.
(7) Lill, M. A; Helms, V. *J. Chem. Phys.* **2001**, *115*, 7993–8005.
(8) Gu, W.; Frigato, T.; Straatsma, T. P.; Helms, V. *Angew. Chem., Int. Ed.* **2007**, *46*, 2939–2943.
(9) Raschke, T. M.; Levitt, M. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 6777–6782.
(10) Van Der Spoel, D.; Lindahl, E.; Hess, B.; Groenhof, G.; Mark, A. E.; Berendsen, H. J. C. *J. Comput. Chem.* **2005**, *26*, 1701–1718.
(11) Siu, S. W. I.; Vacha, R.; Jungwirth, P.; Böckmann, R. A. *J. Chem. Phys.* **2008**, *128*, 125103.

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