Origins of Proton Transport Behavior from Selectivity Domain Mutations of the Aquaporin-1 Channel

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ABSTRACT The permeation free-energy profile and maximum ion conductance of proton transport along the channel of three aquaporin-1 (AQP1) mutants (H180A/R195V, H180A, and R195V) are calculated via molecular dynamics simulations and Poisson-Nernst-Planck theory. The proton dynamics was described by the multistate empirical valence bond (MS-EVB) model. The results reveal three major contributions to the overall free-energy barrier for proton transport in AQP1: 1), the bipolar field, 2), the electrostatic repulsion due to the Arg-195 residue, and 3), the dehydration penalty due to the narrow channel pore. The double mutation (H180A/R195V) drastically drops the overall free-energy barrier by roughly 20 kcal/mol via simultaneously relaxing the direct electrostatic interaction (by R195V) and dehydration effect (by H180A).

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As the first identified and characterized integral transmembrane water channel (1), the aquaporin-1 (AQP1) channel has been studied extensively. An important property of this channel is its exceptionally high water permeability ($\sim$0.02 cm s^{-1}) in liposomes (2,3), with efficient blockage of other neutral solutes and ions, including protons. The proton-blockage property of AQP1, and of the aquaporin superfamily in general, is especially interesting given the fact that the proton is the smallest and most mobile cation in aqueous solutions. Proton mobility is three times higher than that of water and is attributed mainly to its capability to shuttle rapidly along the hydrogen bond network of water molecules according to the Grotthuss mechanism (4). This interesting property of aquaporins has attracted considerable research effort in recent years, especially in the molecular simulation field (5–11). One focus of attention has been the electrostatic effects around the fingerprint Asn-Pro-Ala (NPA) motif where two half-membrane-spanning α-helices HB (at the cytoplasm side) and HE (at the extracellular side) (Fig. 1) meet with opposite orientations (12). It has been proposed that the opposite α-helical orientations might generate a bipolar field, preventing protons to move through the channel (5–9). This hypothesis has been partially supported by the finding that the main free-energy barrier is located at the NPA motif (5–9). A molecular simulation study has also revealed a clear secondary free-energy barrier located at the extracellular side roughly 8 Å from the main barrier (8). This barrier corresponds to a constriction region with a diameter of $\sim$2 Å (12–13). The constriction region allows only one water molecule to pass through and is therefore believed to correspond to the selectivity filter (SF), which is formed by the following four residues: Phe-56, His-180, Cys-189, and Arg-195 (Fig. 1). The His-180 residue, whereas highly conserved in water-selective orthodox aquaporins, is usually replaced by Gly in aquaglyceroporins (14) to relax steric interaction with the alkyl chain of glycerol. Within the water permeation pathway, the Cys-189 residue is believed to be responsible for blocking Hg^{2+} by binding the ion to the backbone carbonyl oxygen that flanks the pore (15). The function of the aromatic hydrophobic Phe-56 is believed to help the water molecules to form stronger hydrogen bonds with the positively charged Arg-195 (16), which, if artificially made uncharged, would favor the entry of cations from the extracellular side (17).

Very recently, the conductance of water, neutral solute, and cations for several AQP1 mutants (H180A, R195V, H180A/R195V, F56A/H180A) was reported by Beitz et al. (18). Their results indicated that cations can pass through the channel, without affecting the water passage, simply by mutagenically removing the Arg-195 residue. Though the H180A mutation alone does not result in cation leakage, it does increase the cation conductance when combined with the R195V mutation (Fig. 1). These results indicate a very important role of the SF domain in cation blockage. Specifically, they suggest that the electrostatic repulsion due to the Arg-195 residue and the dehydration penalty (10,11) due to the narrow channel are also very important effects for cation blockage.

Inspired by this, we report here potential of mean force (PMF) results for the H180A/R195V, H180A, and R195V mutants (Fig. 2). The PMF is the free-energy profile for the cation (in this case excess proton) through the channel. To quantify the bipolarity contribution to the free-energy barrier, we also include the result for a wild-type (WT) channel that has the partial charges on the backbone of the HB and HE helices set to zero. This channel is hereinafter referred to as the no-backbone-charge (NBC) mutant. Such a computational

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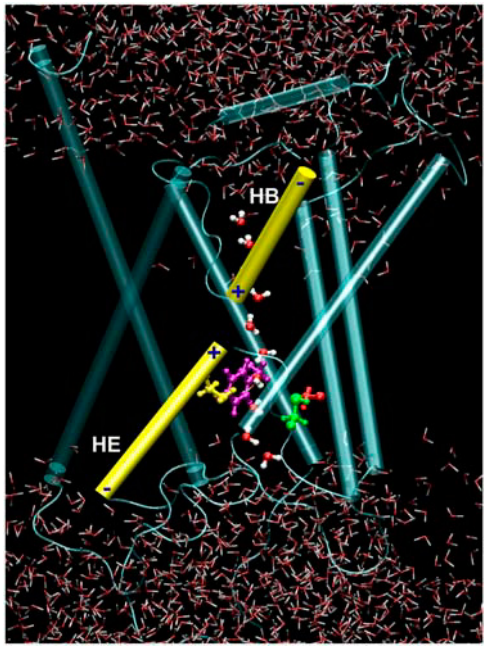

FIGURE 1 A snapshot of the AQP1 double mutant (H180A/R195V). The Phe-56, Ala-180, Val-195, and Cys-189 are colored purple, red, yellow, and green, respectively. The HB and HE helices are modeled as highlighted with the indicated dipole field. The cytoplasm side is at the top.

mutant has also been studied for the GlpF aquaporin channel with a hybrid MD and continuum electrostatics approach (7). These calculations were molecular dynamics simulations as in our previous study (8,9,19). In particular, the dynamics of the Grotthuss shuttling excess proton was reliably described by the MS-EVB2 model (20,21), and the PMFs were calculated using the umbrella sampling and weighted histogram analysis methods. The initial structure of AQP1 was based on a crystal structure (Protein Data Bank entry code, 1J4N) (13), which had been equilibrated with a fully solvated lipid bilayer for 3 ns in a MD simulation.

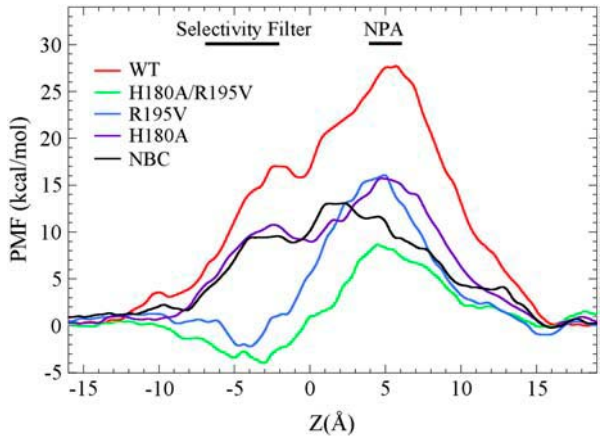

FIGURE 2 The PMF of proton transport along the channel for the WT AQP1 and the H180A/R195V, R195V, H180A, and NBC mutants. (WT and NBC results from Chen et al. (9).) Average error bars for these curves are ±0.4 kcal/mol.

The PMF profile indicates that all of the four AQP1 variants have substantially lower free-energy barriers than that of the WT channel. The primary PMF peak at the NPA domain was lowered from 28 kcal/mol to 12 kcal/mol by solely turning off the dipoles of the HB and HE helices, and the bipolar orientation of the water file as observed in the WT and in the other mutants also disappeared. This indicates a clear contribution to the overall free-energy barrier from the NPA bipolarity whose origin can be attributed to the backbone dipoles of the two opposing helices. Compared to the other mutants, this computational mutation also results in a 4.0-Å shift of the primary barrier toward the center of the lipid bilayer, where the dehydration penalty is presumed to be maximal. For the H180A/R195V and R195V mutants, however, the removal of the Arg-195 residue at the narrow SF domain causes the secondary PMF peak of 17 kcal/mol to drop, which can further facilitate the proton to entry from the bulk water into the narrow AQP1 channel. By reducing the dehydration effect, the H180A mutation lowers the overall barrier by 13 kcal/mol, but it maintains an 11 kcal/mol barrier at the SF domain. Although the SF domain has been speculated for a long time to act as a solute filter because of its small pore radius, our study provides clear evidence that the SF domain is also essential in blocking proton transport mainly by direct electrostatic interaction between its positively charged Arg-195 and the excess proton and by forming a narrow pore.

To compare with the experimental proton conductance (18), the maximum ion conductance g_{max} for the H180A/R195V mutant was calculated based on the Poisson-Nernst-Planck theory (22). The g_{max} turns out to be 8×10^{-3} pS for the H180A/R195V mutant, in reasonable agreement with the experimental lower limit value $\sim 10^{-4}$ pS (18).

Our results, taken together, suggest that the direct electrostatics, dehydration, and bipolar field are the three major contributing effects to the free-energy barrier to PT in AQP1.

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