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Enhancement of Proton Conductance by Mutations of the Selectivity Filter of Aquaporin-1

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Prevention of cation permeation in wild-type aquaporin-1 (AQP1) is believed to be associated with the Asn-Pro-Ala (NPA) region and the aromatic/arginine selectivity filter (SF) domain. Previous work has suggested that the NPA region helps to impede proton permeation due to the protein backbone collective macrodipoles that create an environment favoring a directionally discontinuous channel hydrogen-bonded water chain and a large electrostatic barrier. The SF domain contributes to the proton permeation barrier by a spatial restriction mechanism and direct electrostatic interactions. To further explore these various effects, the free-energy barriers and the maximum cation conductance for the permeation of various cations through the AQP1-R195V and AQP1-R195S mutants are predicted computationally. The cations studied included the hydrated excess proton that utilizes the Grotthuss shuttling mechanism, a model "classical" charge localized hydronium cation that exhibits no Grotthuss shuttling, and a sodium cation. The hydrated excess proton was simulated using a specialized multi-state molecular dynamics method including a proper physical treatment of the proton shuttling and charge defect delocalization. Both AQP1 mutants exhibit a surprising cooperative effect leading to a reduction in the free-energy barrier for proton permeation around the NPA region due to altered water configurations in the SF region, with AQP1-R195S having a higher conductance than AQP1-R195V. The theoretical predictions are experimentally confirmed in wild-type AQP1 and the mutants expressed in *Xenopus* oocytes. The combined results suggest that the SF domain is a specialized structure that has evolved to impede proton permeation in aquaporins.

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Abbreviations used: AQP1, aquaporin-1; ar/R, aromatic/arginine; MD, molecular dynamics; MS-EVB, multi-state empirical valence bond; PMF, potential of mean force; PT, proton transport; RDF, radial distribution function; SF, selectivity filter.

Introduction

Aquaporins are a family of small pore-forming membrane proteins that facilitate the transport of water and small hydrophilic molecules.[1] Thus far, 13 mammalian aquaporins have been discovered (AQP0–AQP12).[1,2] Among them, AQP0, AQP1, AQP2, AQP4, and AQP5 are exclusively permeable to water, while AQP 3, AQP7, AQP9, and AQP10 are also permeable to small molecules such as glycerol, urea, and ammonia.[3,4] The functions of AQP11 and AQP12 have not been fully elucidated, but they probably belong to the aquaglyceroporin group.[5] AQP8 constitutes a special example in that it is impermeable to glycerol and urea but allows the passage of ammonia molecules.[6] These aquaporins are of considerable biological importance in the metabolism of animals and plants.[7] Genetic defects in aquaporins can lead to various diseases such as an impaired urinary concentrating ability.[8] Among the aquaporin family members, aquaporin-1 (AQP1) has been studied extensively in both experiments[9–18] and molecular dynamics (MD) simulations.[18–32] AQP1 effectively blocks cation flux including that from excess protons, thus helping maintain the osmotic pressure of the cell membrane and the cytosolic pH. In the present study, AQP1 is employed to explore the cation filter properties of aquaporins.

Cation permeability in the aquaporin family is believed to be regulated by two filter structures residing along the water channel. A pair of oppositely oriented Asn-Pro-Ala (NPA) structures constitutes a narrow filter region at the center of the channel, where the half-membrane spanning α-helix B (HB) and helix E (HE) meet (see Fig. 1). This double NPA motif has been identified in most aquaporins and has been adopted as a fingerprint for their identification. Previous specialized MD studies[22,29,30] have been used to calculate the potential of mean force (PMF; i.e., the free-energy profile) for hydrated excess proton permeation in wild-type AQP1, including a computational model system that did not include backbone atom charges on the HB and HE helices.[29,30] These results indicate that the collective macrodipoles of the backbone atoms help to create an unfavorable electrostatic environment for proton transport (PT), in agreement with a suggestion from a previous computational analysis using a hybrid MD and continuum electrostatics approach.[24] However, this feature alone cannot completely explain the proton blocking behavior of AQP1,[29,30] nor can the cation dehydration penalty.[25,26]

The second filter, the aromatic/arginine (ar/R) constriction selectivity filter (SF) domain, is located about 7 Å toward the extracellular face of the protein and has a maximum diameter of 2 Å, which confines the channel water molecules to be a single-file water chain (Fig. 1). The SF domain has been associated with the exclusion of substrates based mainly on size

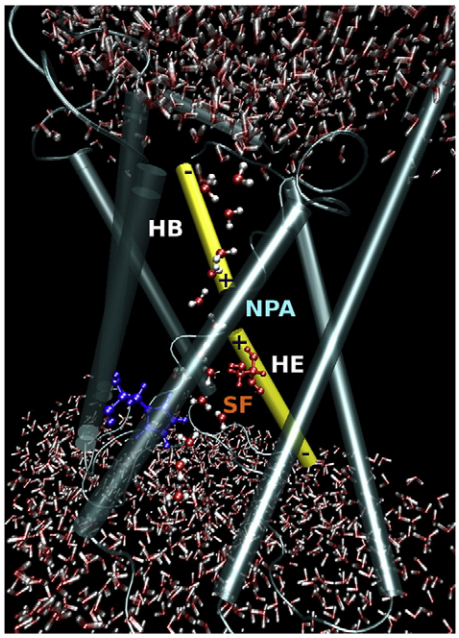

Fig. 1. A depiction of the AQP1-R195S mutant system from the MS-EVB simulations. The AQP1-R195S protein is colored cyan. The top of the figure is toward the cytoplasm side. Ser195 and His180 are colored in red and blue, respectively. The HB and HE helices are highlighted in yellow with the dipole field indicated. The SF domain and the NPA motif are indicated by orange and blue, respectively.

constraints.[27] In relevant experimental research,[11] site-directed mutagenesis was employed to study proton permeability in the rAQP1 (rat isoform AQP1) SF domain mutants. Replacing His180 with alanine increases the channel diameter and reduces the dehydration penalty for the proton. However, single mutant AQP1-H180A does not show an obvious enhancement in proton conductance. However, also mutating Arg195 to valine increases the channel diameter at the restriction region and eliminates the repulsive interaction between arginine and the hydrated proton. Both the AQP1-R195V single mutant and AQP1-H180A/R195V double mutant become permeable to protons, with the double mutant AQP1-H180A/R195V having four times higher conductance than the single mutant.[11] Subsequent computer simulations were able to reproduce and explain this behavior at the molecular level.[29] Experimental data from malarial PfAQP[12] also indicate that the permeation of proton and ammonium cannot be prevented by the NPA motif alone.

To further examine the specific role of the SF domain in controlling aquaporin proton permeation, an SF domain mutant, AQP1-R195S, is the focus of the present study. Compared with the previously investigated AQP1-R195V mutant, the serine mutation is both simple and contains a polar hydroxide group on the side chain that should be