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On the Proton Exclusion of Aquaporins: A Statistical Mechanics Study

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Received September 19, 2007; E-mail: hirata@ims.ac.jp

The proton exclusion from aquaporins (AQPs) is one of the most important questions to be solved in the fields of biochemistry, medicine, and pharmacology. Although the channels are extremely permeable for water, approximately a billion molecules per second pass through the channel, protons are strictly excluded from the permeation.[1−4] In many previous works of molecular dynamics simulation of proton exclusion, AQP1 and GlpF have been used to study this problem.[5−15] There are essentially two mechanisms conceivable for the proton transfer in the channel. One is the proton jump mechanism similar to that in bulk ice or water, in which a proton transfers from one minimum to the other in the double well potential of a hydrogen bond between two water molecules by tunneling through the barrier, which is called the Grotthuss mechanism.[16] The process requires the two water molecules to be in the right mutual orientation to form the double well potential. If water molecules are prevented from the reorientational dynamics by any reason, a proton may not transport through the channel.[6−9] The other mechanism of the proton transport is due to the translational motion of water molecules; that is, a proton may move in the channel by "riding" on a water molecule or making a hydronium ion. The mechanism is similar to the usual ion transport in the channel. Therefore, any mechanism that prevents the ion from the translational motion through the channel can be the cause of the proton insulation: steric hindrance, electrostatic barrier, and so on.[9−15] The unspecific desolvation effects proposed by Warshel is nothing but the electrostatic barrier enhanced by decreased water population or screening.[10,11] The mechanism should be readily examined if one can calculate the distribution of the hydronium ion in the channel. The information of the hydronium-ion distribution in the channel may also be useful for examining the possibility of the proton-jump mechanism, because a proton should be existing most likely in the form of the hydronium ion except for the moment of barrier crossing.

Unfortunately, it is extremely difficult task for the molecular simulation to examine the distribution of molecular ions in a channel, because it should sample the free energy surface of the ion including their orientations throughout the channel.

The three-dimensional (3D) RISM theory is a powerful tool to tackle such problems[17] (see Supporting Information). The 3D-RISM theory is a statistical-mechanics method to integrate over the entire configuration space of solution equilibrated with a solute conformation; thereby it is free from the notorious sampling problem inherent in the molecular simulation. The theory has been applied successfully to the selective ion binding by human lysozyme to reproduce experimental results.[18] Most recently, the mechanism of water permeation of open and closed channel of AQPz was also considered by the theory.[19] In the present paper, we apply the theory to AQP1 and GlpF for elucidating the proton exclusion from those channels.

In Figure 1, the contour map of the electrostatic potential due to the channel atoms, the 3D-distribution of water and of hydronium ions, and the one-dimensional profile of the distribution of the solution components are depicted along the channel axis. As can be readily seen from the figure, water in the both channels is continuously distributed through out the channel. However, the distribution of hydronium ions is intermitted by gaps both for AQP1 and GlpF, although there is some difference in the distribution between the two channels: in AQP1, the hydronium ion is excluded from large area extending from R197 (or Ar/R) to NPA, while the gap in GlpF is limited in a small area around R206 (Ar/R). Note that "gap" does not mean "nothing is there," since water molecules are distributed continuously through out the channel. It is also understood from the figure that the distribution of hydronium ions is essentially determined by the electrostatic potential inside the channel: hydronium ions are excluded primarily from the channel by the positive electrostatic atmosphere. The difference in the electrostatic potential between AQP1 and GlpF originates apparently from the additional positive field produced by the residue H182 in AQP1. From those results, we can draw an important conclusion with respect to the mechanism of proton exclusion in AQP1. It is needless to mention about the proton jump mechanism, as the proton as a positive charge cannot pass through the large electrostatic barrier inside the channel. On the other hand, the gap of the distribution is small in GlpF, which leaves a slight possibility for the proton to transfer through the proton jump mechanism. Remember water is distributed continuously even in the area where the hydronium ion is excluded. If the water molecules and hydronium ions around that area have some freedom to rotate to arrange themselves to make the double well potential for the proton, then the proton may jump through the potential barrier via tunneling. The distribution of oxygen and hydrogen of water around the area does not indicate the particular coordination that prevents the molecule from the reorientation. Can a proton, then, permeate all the way through the channel via the proton-jump mechanism? In order to answer the question, we have examined the water distribution around the NPA region of GlpF, where the mechanism is suspected to be broken because of the formation of so-called "bipolar orientation."[6−8]

Drawn in Figure 2 are the distributions of oxygen and hydrogen atoms of water at the NPA region in AQP1 and GlpF. The oxygen atom of a water molecule is coordinated by the two hydrogen atoms of the residues, N203 and N68 of GlpF, N194 and N78 of AQP1. In Figure 3, the radial distribution functions of water around the nitrogen atom of N68 and N203 for GlpF are depicted. The peaks of oxygen are about the same distance from the both residues making hydrogen-bonds. (See the illustrative picture in the insets
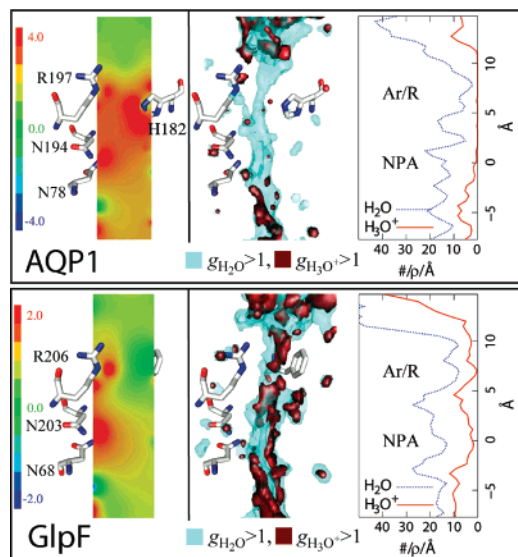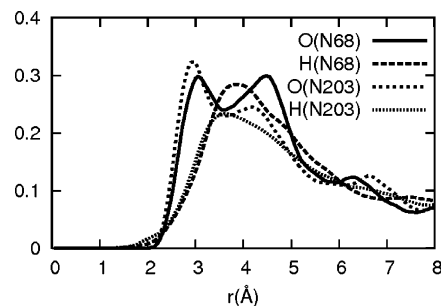
Figure 1. The distribution functions of water and hydronium ions in aquaporin channels. The distribution of water (blue transparent surface), $g > 1$, and those of hydronium ion (brown surface), $g > 1$ and distribution profiles, in AQP1 and GlpF channel, are shown in upper and lower panels, respectively. The contour colors show the electrostatic potential of protein in esu unit.
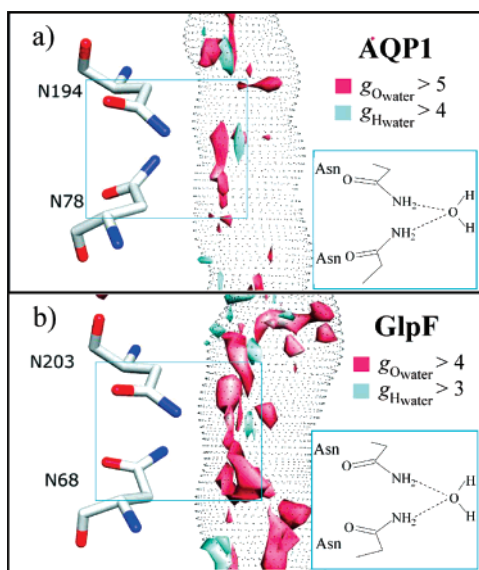
Figure 2. Panels a and b show the distribution of oxygen (pale red) and hydrogen (light blue) of water at the NPA region of AQP1 and GlpF. The dotted area denotes the surface of the channel.

of Figure 2.) Such orientation of water molecules entirely conflicts with the configuration of the hydrogen-bond network of water, thereby it excludes the possibility of the proton-jump mechanism around that area.

In both channels, water distributes continuously throughout the channel, while the distribution of hydronium ions is intermitted by gaps due to the electrostatic repulsion originated from the positive charges in the channels. The gap is very large in the case of AQP1, extending from R197 to the NPA region. From the results, we can readily conclude in the case of AQP1 that protons are excluded from permeation primarily due to the electrostatic repulsion inside

Figure 3. Radial distribution functions of oxygen and hydrogen of water around the nitrogen atoms of N68 and N203 of GlpF.

channel. On the other hand, in the case of GlpF, the results leave slight possibility for proton to permeate through the gap around R206 by the proton jump mechanism. However, the mechanism does not work entirely throughout the channel due to the formation of the bipolar orientation at the NPA region. So, a proton has small but finite conductivity in GlpF through the combined mechanism of the proton jump and the diffusion of hydronium ions in accord with experiment.[3]

Supporting Information Available: Calculation methods and detail of solvent potential parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA077087+