

Water and Deuterium Oxide Permeability through Aquaporin 1: MD Predictions and Experimental Verification

Artem B. Mamonov,¹ Rob D. Coalson,¹ Mark L. Zeidel,² and John C. Mathai²

¹Department of Chemistry and ²Department of Medicine, University of Pittsburgh, Pittsburgh, PA 15260

Determining the mechanisms of flux through protein channels requires a combination of structural data, permeability measurement, and molecular dynamics (MD) simulations. To further clarify the mechanism of flux through aquaporin 1 (AQP1), osmotic p_f (cm³/s/pore) and diffusion p_d (cm³/s/pore) permeability coefficients per pore of H₂O and D₂O in AQP1 were calculated using MD simulations. We then compared the simulation results with experimental measurements of the osmotic AQP1 permeabilities of H₂O and D₂O. In this manner we evaluated the ability of MD simulations to predict actual flux results. For the MD simulations, the force field parameters of the D₂O model were reparameterized from the TIP3P water model to reproduce the experimentally observed difference in the bulk self diffusion constants of H₂O vs. D₂O. Two MD systems (one for each solvent) were constructed, each containing explicit palmitoyl-oleoyl-phosphatidyl-ethanolamine (POPE) phospholipid molecules, solvent, and AQP1. It was found that the calculated value of p_f for D₂O is ~15% smaller than for H₂O. Bovine AQP1 was reconstituted into palmitoyl-oleoyl-phosphatidylcholine (POPC) liposomes, and it was found that the measured macroscopic osmotic permeability coefficient P_f (cm/s) of D₂O is ~21% lower than for H₂O. The combined computational and experimental results suggest that deuterium oxide permeability through AQP1 is similar to that of water. The slightly lower observed osmotic permeability of D₂O compared to H₂O in AQP1 is most likely due to the lower self diffusion constant of D₂O.

INTRODUCTION

Aquaporins belong to a family of membrane proteins that passively transport water, glycerol, and other small molecules (Nielsen et al., 2002). The archetypical water channel AQP1 has been shown to be highly specific for water and excludes protons, ammonia, and sugars such as glycerol (Zeidel et al., 1992, 1994). The specificity of the channel arises from the presence of two highly conserved NPA motifs (Asn-Pro-Ala residues) that form the narrow constriction of the pore region, which acts as a molecular sieve. Crystallographic studies suggest that the pore is narrow at the constriction and lets only water diffuse through it (Murata et al., 2000; Sui et al., 2001). Molecular dynamics (MD) simulation studies suggest the presence of another energy barrier that offers both steric and electrostatic selectivity, named the aromatic (ar/R) constriction, which is formed by the four amino acids Phe-56, His-180, Cys-189, and Arg-195 (rat AQP1) in water-selective aquaporins (de Groot et al., 2003; Chakrabarti et al., 2004; Chen et al., 2006). In aquaglyceroporins this constriction region is wider, as His-180 is usually replaced by Gly, which allows for water and glycerol to pass through as shown in GlpF (Fu et al., 2000).

Although general geometry of aquaporin channels differs only slightly between aquaporin family members, its water permeability may differ by several orders of magnitude. Water is thought to hydrogen bond to arginine in the NPA region (Miloshevsky and Jordan, 2004;

Vidossich et al., 2004) and the degree of hydrogen bonding or the energy barrier caused by hydrogen bonding is thought to dictate the rate of water flow through aquaporins. Furthermore, in a study of model channel desformylgramicidin, which shows a 100-fold increase in water permeability compared with gramicidin A, the lowered hydrogen bond-mediated energy barrier is thought to be responsible for large differences observed in water permeability (de Groot et al., 2002).

To determine whether such a finely tuned water channel allows the passage of deuterium oxide (D₂O), a close analogue of water, we measured the permeability of D₂O in AQP1-reconstituted proteoliposomes. D₂O differs from H₂O in the following respects: D₂O has a lower zero-point vibrational energy leading to a stronger O-D bond and a stronger deuterium bond compared with the H-bond (Scheiner and Cuma, 1996). The effect of the stronger deuterium bond plus the heavier mass of D is believed to result in a self diffusion coefficient of D₂O that is 18.6% lower than that of water (Mills, 1973; Grigera, 2001). Since water is thought to interact with specific residues Phe-56 and Arg-195 in the pore via hydrogen bonding (Miloshevsky and Jordan, 2004), it would be interesting to study the passage of D₂O, which has a stronger deuterium bond. To test if kinetics of D₂O permeability is different from H₂O we

Correspondence to John Mathai: jmathai@bidmc.harvard.edu

Abbreviations used in this paper: AQP1, aquaporin 1; MD, molecular dynamics.

simulated D₂O flux and then measured it, allowing us to determine the predictive power of MD simulations. Both MD simulations and experimental results indicate that D₂O and H₂O permeate AQP1 at similar rates.

MATERIALS AND METHODS

MD Simulations

Molecular dynamics (MD) simulations were used to calculate the diffusion and osmotic permeability of H₂O and D₂O through AQP1. The X-ray structure of bovine AQP1 was downloaded from the Protein Data Bank (www.rcsb.org) with pdb code 1J4N (Sui et al., 2001). The tetrameric structure was assembled from the coordinates of the monomer using transformation matrices provided in the pdb file. The tetramer was then embedded in a pre-equilibrated patch of 177 palmitoyl-oleoyl-phosphatidyl-ethanolamine (POPE) molecules and solvated by 15,079 CHARMM modified TIP3P water molecules (Jorgensen et al., 1983; Neria et al., 1996) (referred to here as TIP3P), so that the total system contained 80,434 atoms. This system was energy minimized and equilibrated for 3 ns at 298 K and 1 atm. To construct the AQP1/D₂O system, the TIP3P water model was replaced with a TIP3P-HW model (described below) and equilibrated for 1 ns at 298 K and 1 atm. The system equilibrium was checked by monitoring potential energy and volume over the course of the simulation. The standard deviation of potential energy from the mean was $\sim 0.13\%$ (~ 210 kcal/mol) and for volume was $\sim 0.15\%$ (~ 1150 Å³), which suggests that the system is in equilibrium.

Diffusion permeabilities were calculated for both AQP1/H₂O and AQP1/D₂O systems from equilibrium MD simulations at constant volume and constant temperature of 298 K from 31 and 26 ns trajectories, respectively. A weak harmonic restraining potential of 0.12 kcal/mol/Å² was applied in all three dimensions to AQP1 backbone α carbons to prevent the protein from drifting. Diffusion permeability was calculated using the following equation (Zhu et al., 2004):

$$p_d = \frac{V_W}{N_A} q_0 = v_w q_0, \quad (1)$$

where p_d (cm³/s/pore) is the diffusion permeability coefficient per pore, V_W is the molar volume of the solvent, N_A is Avogadro's number, q_0 is the unidirectional permeation rate, and v_w is the volume of a single solvent molecule. q_0 was calculated by counting the number of H₂O or D₂O molecules passing from one side of the channel to the other per unit time. The pore region was chosen to be the narrow central section of the channel, 22 Å in length, where water molecules pass in single file or nearly single file fashion (see Fig. 3, below).

Osmotic permeabilities were calculated by following the non-equilibrium MD method described by Zhu et al. (2004). A hydrostatic pressure gradient was established across the membrane by applying extra force to a layer of solvent molecules parallel to the membrane interface. Applied pressure MD simulations of different time length were performed at 25, 50, 100, and 200 MPa for both AQP1/H₂O and AQP1/D₂O systems. A force of 0.013445, 0.02689, 0.05378, and 0.10756 kcal/mol/Å was applied to oxygen atoms of H₂O/D₂O within an 8-Å solvent layer to generate 25, 50, 100, and 200 MPa pressure, respectively.

To prevent the protein and the lipid bilayer from moving under the influence of this external force, a harmonic restraint of 0.12 kcal/mol/Å² was applied in all three dimensions to the backbone α carbons of the protein and a harmonic restraint of 0.8 kcal/mol/Å² to the phosphorus atoms of the POPE molecules. This procedure differs somewhat from the 1D restraining procedure

used by Zhu et al. (2004). However, our calculated osmotic permeability for H₂O turned out to be similar to Zhu et al. (2004) (see below), implying that small differences in the restraining procedure do not have a major effect on the calculated osmotic permeabilities.

The net flux of H₂O/D₂O through AQP1 was calculated by averaging the number of molecules that crossed three planes located 5 Å away from each other inside the channel per pore per unit time. Osmotic permeabilities were calculated from the slope of the best (linear least squares) fit line of H₂O/D₂O flux versus applied hydrostatic pressure according to the following equation (Zhu et al., 2004):

$$p_j = \frac{j}{\Delta P} k_B T, \quad (2)$$

where p_j (cm³/s/pore) is the osmotic permeability coefficient per pore, j (molecules/s) is the net solvent flux through a single water channel, ΔP is the applied hydrostatic pressure, k_B is the Boltzmann constant, and T is the absolute temperature.

For our MD simulations we used the CHARMM 27 force field (MacKerell et al., 1998), which was derived to be consistent with the TIP3P model (Neria et al., 1996). For the parameterization of deuterium oxide we started with the TIP3P model as a reference, doubled the mass of the hydrogen atoms, and adjusted the partial charges of both the deuterium and oxygen atoms. The physical motivation for modifying these partial charges is to take into account the quantum effect that liquid D₂O is characterized by a stronger deuterium bond than the H bond found in liquid H₂O. This quantum effect arises because D₂O has a lower zero-point vibrational energy than H₂O (Scheiner and Cuma, 1996). It is well known that TIP3P water considerably overestimates (by more than a factor of 2) the self diffusion constant of water (van der Spoel et al., 1998; Mark and Nilsson, 2001). For this reason, we chose as a target for parameterization to reproduce the experimentally measured 18.6% difference in the self diffusion constants of H₂O vs. D₂O (Mills, 1973) rather than the experimental self diffusion constant of heavy water. A similar reparameterization of the SPC/E model for deuterium oxide has been reported recently in which only the mass and partial charges of the hydrogen atoms were changed in order to reproduce the experimental self diffusion constant, the molar volume and the potential energy of heavy water (Grigera, 2001). For our reparameterization, a simulation box of 3921 TIP3P water molecules was constructed, energy minimized, and equilibrated at 298 K and 1 atm. The mass of hydrogen atoms was set to 2 amu and atomic partial charges were incremented by fractions of a percent. The system was equilibrated for 0.5 ns followed by 1 ns production runs performed at a constant temperature of 298 K and a constant pressure of 1 atm. The self diffusion constant was calculated from the collected coordinate time series using the mean-square-displacement method (Allen and Tildesley, 1987). Adjustment of D₂O atomic partial charges along with recalculation of the self diffusion constant was repeated until we generated a diffusion coefficient $\sim 18\%$ smaller than the value obtained with TIP3P under the same conditions.

All MD simulations were performed using the NAMD package (Kale et al., 1999) version 2.5. For long range electrostatics, the Particle Mesh Ewald method (Essmann et al., 1995) was used as implemented in the NAMD program along with periodic boundary conditions. The MD time step was set to 2 fs and the bonds between each hydrogen atom and its mother atom were fixed via the SHAKEH algorithm (Kale et al., 1999) to the nominal bond length given in the parameter file. MD coordinates of AQP1 simulations were saved every 1 ps and for the parameterization of TIP3P-HW water model every 100 fs. Most simulations were performed on Lemieux supercomputer at the Pittsburgh Supercomputing Center (www.psc.edu). It took ~ 4.6 h to run 1 ns of AQP1 simulations on 160 CPUs of Lemieux. All visualizations

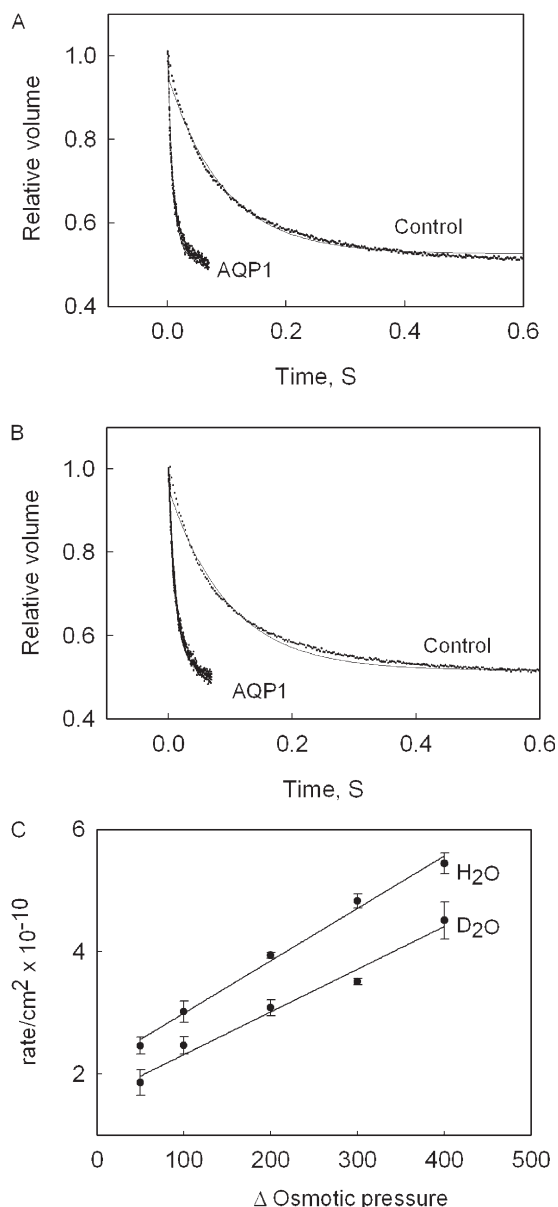


Figure 1. Osmotic permeability of H₂O and D₂O. Shown is the time course of volume change of AQP1-containing vesicles and control lipid vesicles on abrupt exposure to a doubling of external osmotic pressure in H₂O (A) and D₂O (B). C shows the effect of varying osmotic pressure (mOsm/kg) on permeability of H₂O and D₂O through AQP1.

and analysis of MD trajectories were made with the VMD program (Humphrey et al., 1996).

Reconstitution of AQP1 into Liposomes

Bovine AQP1 was purified from bovine red blood cells using methods similar to that used for purifying human AQP1 (Zeidel et al., 1992). The AQP1 reconstitution procedure was similar to that described earlier (Zeidel et al., 1994). In brief, 6–8 mg of palmitoyl-oleoyl-phosphatidylcholine (POPC) lipids (Avanti Lipids) was bath sonicated for three cycles of 3 min duration at 4 mW setting in 20 mM MOPS, pH 7.4. *N*-octylglucoside (OG) was added to the sonicated lipids to achieve a final concentration of 1.2% (vol/vol). To this, 50–60 μg of AQP1 in 1.5% OG was added and

incubated for 30 min on ice. The mixture was diluted 25-fold into reconstitution buffer (150 mM NaCl, 20 mM MOPS, pH 7.4) containing 10 mM carboxyfluorescein (CF). The proteoliposomes formed were collected by centrifugation at 100,000 *g* for 1 h. The external CF was removed by two additional centrifugal washes. The final pellet was resuspended in 300 μl of reconstitution buffer and used for permeability studies.

Measurement of Osmotic Permeabilities

Macroscopic osmotic permeabilities (P_f) of H₂O and D₂O were measured as described earlier (Zeidel et al., 1994). In brief, the proteoliposomes were subjected to a doubling of external osmotic pressure in a stopped-flow fluorimeter (Applied Photophysics, SX.17 MV), and the fluorescence decrease of carboxyfluorescein due to self quenching caused by shrinkage of the vesicle was measured as a function of time. The data were fit using a single exponential function. P_f was calculated by comparing the single-exponential time constants fitted to a family of simulated curves generated using the water permeability equation in which P_f was varied to that obtained experimentally. MathCad was used to numerically integrate the water permeability equation:

$$\frac{dV_r(t)}{dt} = P_f \times r_{sv} \times V_w \times \left(\frac{C_{in}}{V_r(t)} - C_{out} \right). \quad (3)$$

In Eq. 3, $V_r(t)$ is the relative volume of the vesicle at time t (i.e., volume at time t , divided by the initial volume), P_f (cm/s) is the macroscopic osmotic water permeability coefficient, r_{sv} is the surface area to volume ratio of a vesicle, and C_{in} and C_{out} are initial solute concentrations inside and outside the vesicle, respectively. The size of the vesicle was measured by laser light scatter using a DynaPro particle sizer. All other chemicals were obtained from Sigma-Aldrich.

RESULTS AND DISCUSSION

Earlier studies before discovery of aquaporins showed that permeation of D₂O across erythrocytes and red cell ghosts was inhibited by mercury (Karan and Macey, 1980; Ye and Verkman, 1989). However, mercurial compounds are nonspecific and do not inhibit the AQP1 channel completely (Zeidel et al., 1992). In this study we have performed a quantitative permeability measurement using purified AQP1, reconstituted into proteoliposomes, which eliminates the background permeability due to other proteins (Roudier et al., 1998; Yang et al., 2001). Fig. 1 shows the time course of relative volume change of liposomes on application of an osmotic gradient, causing the efflux of H₂O (Fig. 1 A) or D₂O (Fig. 1 B). It was found that the measured osmotic permeability of D₂O ($1.8 \times 10^{-2} \pm 0.42 \times 10^{-2}$ cm/s) is ~21% lower than that for H₂O ($2.3 \times 10^{-2} \pm 0.310^{-2}$ cm/s). This difference in permeability can be attributed to the higher viscosity of D₂O vs. H₂O and an 18.6% lower self diffusion coefficient of D₂O compared with that of

TABLE I
Force Field Parameters of TIP3P-HW Model

r_{min}^{OO}	r_{min}^{DD}	ϵ^{OO}	ϵ^{DD}	q^O	q^D	b^{OD}	θ^{DOD}
Å	Å	kcal/mol	kcal/mol	<i>e</i> units	<i>e</i> units	Å	deg
3.5365	0.449	0.1521	0.046	-0.842	-0.421	0.9572	104.52

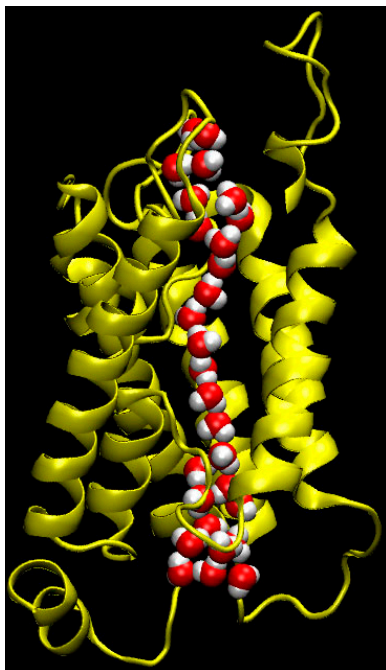


Figure 2. A snapshot from MD simulation of AQP1/H₂O system. Only one monomer of the water channel is shown for clarity. Note the single file arrangement of water molecules inside the channel.

water (Mills, 1973). In red blood cells, before discovery of aquaporins, the decreased permeability of D₂O was attributed to higher viscosity of D₂O (Karan and Macey, 1980). A similar decrease of conductance of D₂O through gramicidin was also attributed to higher viscosity of D₂O (Tredgold and Jones, 1979). The background osmotic permeabilities of H₂O and D₂O in the absence of a water channel through POPC lipid membranes are 0.0032 cm/s and 0.0034 cm/s respectively. To confirm that the observed difference in permeability is not an isotope effect, we measured the osmotic permeability of AQP1 at various osmotic pressure values. Fig. 1 C shows that there is a linear increase in rate of efflux of H₂O and D₂O with increasing osmotic pressure. Furthermore the efflux of H₂O and D₂O shows a parallel increase with increasing osmotic pressure, suggesting that there are no additional interactions between the permeant and the channel as flux increases at higher osmotic pressures.

The force field parameters of our TIP3P-HW (D₂O) model are summarized in Table I. Partial atomic charges are 0.96% higher compared with the TIP3P model.

TABLE II
Results of Equilibrium MD Simulations

Time	No. of permeations	q_0	$p_d \times 10^{14}$	
<i>ns</i>		<i>molecules/ns/pore</i>	<i>cm³/s/pore</i>	
H ₂ O	31.1	71	0.29 ± 0.026	0.85 ± 0.078
D ₂ O	26.3	65	0.31 ± 0.085	1.0 ± 0.28

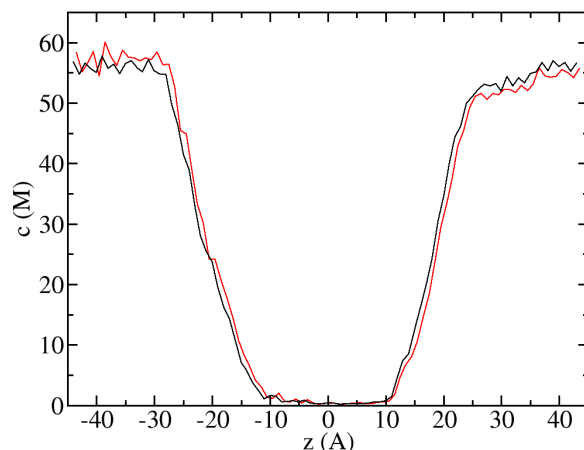


Figure 3. Concentration profile of H₂O along z axis (channel axis) at equilibrium conditions (black line) and 100 MPa applied hydrostatic pressure (red line). Note the low concentration region between approximately $z = -11 \text{ \AA}$ and $z = +11 \text{ \AA}$; this is the “channel pore region” discussed in the text. Left side corresponds to cytoplasmic side.

This implies a larger dipole moment of 7.904×10^{-30} Cm which is also 0.96% larger than for the TIP3P model. The dimer potential energy in the most favorable configuration is -6.759 kcal/mol, which is 2.4% larger in magnitude than for the TIP3P model. The heat of vaporization for TIP3P-HW is 10.68 kcal/mol, which is 2.9% larger than for the TIP3P model and compares well with the experimentally determined value of 11.11 kcal/mol for D₂O (Nemethy and Scheraga, 1964). In our MD simulations, the TIP3P-HW model was characterized by a bulk diffusion coefficient of $4.00 \times 10^{-5} \text{ cm}^2/\text{s}$, which is 18.7% lower than that of the TIP3P model of water ($4.92 \times 10^{-5} \text{ cm}^2/\text{s}$). This difference is in good agreement with the experimentally measured difference of 18.6% between H₂O and D₂O (Mills, 1973). The densities of TIP3P and TIP3P-HW models were found to be 1.00 g/cm³ and 1.13 g/cm³, respectively. This is also in good agreement with experimental densities of 0.997 g/cm³ for H₂O and 1.104 g/cm³ for D₂O (Weast, 1977). These solvent models were subsequently used to calculate the osmotic and diffusion permeabilities of H₂O and D₂O in AQP1.

A snapshot of the AQP1/H₂O system from the equilibrium MD trajectory is presented in Fig. 2, with only one

TABLE III
Results of Applied Pressure MD Simulations for H₂O

Applied pressure	Time	No. of H ₂ O permeations	Flux	$p_f \times 10^{14}$
<i>MPa</i>	<i>ns</i>		<i>H₂O/ns/pore</i>	<i>cm³/s/pore</i>
25	8	21	0.65	
50	26.8	107	1.0	
100	21.9	171	1.9	10.0 ± 0.56
200	22	455	5.2	

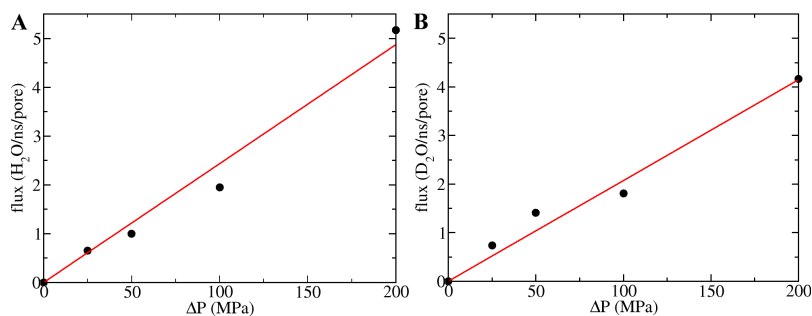


Figure 4. Computed flux versus applied hydrostatic pressure for H₂O (A) and D₂O (B). Circles (black) represent the calculated (average) data point values, which are shown along with the best-fit line (red). Standard error for the best fit line for H₂O is 5.6% and for D₂O 5.3%.

monomer and channel shown for the sake of clarity. The single file arrangement and rotation of the orientation of the water dipole, which are unique properties of the AQP family (de Groot and Grubmuller, 2001; Tajkhorshid et al., 2002), are correctly reproduced in our simulations. The single file channel occupancy for H₂O and D₂O are 9.7 and 9.5 molecules, respectively. The concentration profile of H₂O across the channel system at equilibrium conditions is compared in Fig. 3 with that obtained at 100 MPa applied hydrostatic pressure. When hydrostatic pressure was applied, the concentration of water became slightly higher on the left side and lower on the right side of the membrane due to the compressibility of water. The main results of our equilibrium MD simulations for H₂O and D₂O are summarized in Table II. The unidirectional permeation rate (q_0) of H₂O was found to be 0.29, which compares well to the value of 0.2 predicted by another AQP study (de Groot and Grubmuller, 2001). This difference can be attributed to the fact that the authors used a different (lower resolution) structure of AQP1 and a different water model. We found that q_0 for D₂O is $\sim 7\%$ higher than for H₂O, albeit with relatively large error bars (Table II).

Results of our osmotic pressure simulations are reported in Table III and Fig. 4 A for H₂O, and in Table IV and Fig. 4 B for D₂O. We found that the osmotic permeability of D₂O ($8.5 \times 10^{-14} \text{ cm}^3/\text{s}/\text{pore}$) is 15% lower than that of H₂O ($10.0 \times 10^{-14} \text{ cm}^3/\text{s}/\text{pore}$). This difference compares well with the experimentally measured difference of $\sim 21\%$. We could not compare the individual values of the osmotic permeabilities because of the experimental uncertainty in the number of the AQP1

channels per proteoliposome. The osmotic permeability of H₂O $10.0 \times 10^{-14} \text{ cm}^3/\text{s}/\text{pore}$ obtained in our MD simulations is similar to that predicted by another MD study where a value of $7.1 \times 10^{-14} \text{ cm}^3/\text{s}/\text{pore}$ was reported (Zhu et al., 2004). In addition it also compares well with the experimentally measured unit osmotic conductance (p_f) of $11.7 \times 10^{-14} \pm 1.8 \times 10^{-14} \text{ cm}^3/\text{s}/\text{pore}$ reported by Zeidel et al. (1994). Since water moves in a single file fashion through the AQP1 channel, the p_f/p_d ratio should give the number of solute molecules lining the pore (Finkelstein, 1987). The calculated p_f/p_d ratios for H₂O and D₂O are 11.8 and 8.4, respectively. The p_f/p_d ratio of H₂O is in good agreement with the value 11.9 predicted in Zhu et al. (2004) and 13.2 measured experimentally (Mathai et al., 1996).

In conclusion, a new TIP3P-HW model for D₂O was developed for simulation of D₂O transport through AQP1. This model reproduces the experimental differences observed in the density and the self diffusion coefficient of H₂O vs. D₂O. Both MD simulations and experimental measurements confirm that D₂O and H₂O permeate the AQP1 channel. The observed lower permeability of D₂O is attributed to the lower self diffusion coefficient and higher viscosity of D₂O compared with H₂O. Our study showed that MD could accurately predict the decreased permeability of D₂O in AQP1 in advance of experimental measurements. This study will be helpful for designing MD simulations to study permeation of solutes through AQP1 that are not easily amenable to experiments such as carbon dioxide, hydrogen sulfide, and oxygen.

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TABLE IV

Results of applied pressure MD simulations for D₂O

Applied pressure	Time	No. of D ₂ O permeations	Flux	$p_f \times 10^{14}$
MPa	ns		D ₂ O/ ns/ pore	cm ³ /s/pore
25	7	21	0.74	
50	22	126	1.4	
100	34	239	1.8	8.5 ± 0.45
200	20	333	4.2	

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