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Molecular dynamics insights into human aquaporin 2 water channel

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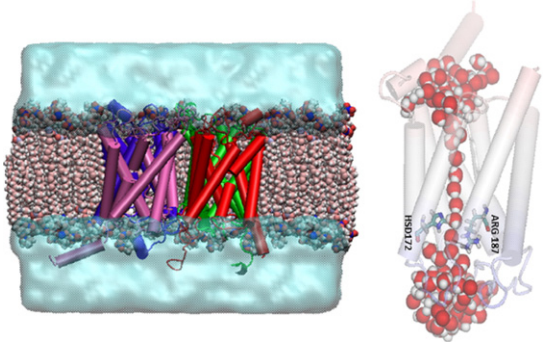
HIGHLIGHTS

- The first molecular dynamics simulation of the human aquaporin 2 is performed.
- The osmotic and diffusive permeability constants of AQP2 are provided.
- Water permeation in human AQP2 occurs in ideal single-file fashion.
- The MD results of AQP2 give insights into kidney urine concentration process.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, the first molecular dynamics simulation of the human aquaporin 2 is performed and for a better understanding of the aquaporin 2 permeability performance, the characteristics of water transport in this protein channel and key biophysical parameters of AQP2 tetramer including osmotic and diffusive permeability constants and the pore radius are investigated. For this purpose, recently recovered high resolution X-ray crystal structure of the human aquaporin 2 is used to perform twenty nanosecond molecular dynamics simulation of fully hydrated tetramer of this protein embedded in a lipid bilayer. The resulting water permeability characteristics of this protein channel showed that the water permeability of the human AQP2 is in a mean range in comparison with other human aquaporins family. Finally, the results reported in this research demonstrate that molecular dynamics simulation of human AQP2 provided useful insights into the mechanisms of water permeation and urine concentration in the human kidney.

1. Introduction

In biomembrane cells, water is transported across the membrane through protein channels. Aquaporins are a family of membrane channel proteins that exist in all live organisms such as mammals, plants, amphibia and bacteria and they play an important role in the osmotic regulation of cells by efficient and passive permeation of water across the biological membranes.

Sometimes cell membranes must exchange large volumes of water. Aquaporins usually conduct water at a rate of 10^9 molecules/s. This highly efficient water transportation of protein channels that is comparable with the free diffusion of water, can explain osmotic water regulation of many biological processes such as urine concentration in kidneys, spinal fluids maintaining and water homeostasis in brain, maintenance of lens transparency in the eye, secretion of tears, sweat and saliva. Therefore, malfunction of any aquaporins is related to several diseases.

The main responsibility of the kidney in the human body is the regulation of water homeostasis while toxic wastes are eliminated. About

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one million nephrons are located in each of the two kidneys in body that act as functional units. Different segments of a nephron have certain tasks in water homeostasis regulation and urine generation. Blood is filtered in the glomerulus. The glomerular filtration rate is 100–125 ml/min which results approximately 150–180 l/day. The remaining filtrate is concentrated along the tubular system to generate final urine, starting with the proximal tubule and the descending limb of Henle. In the proximal tubule and descending loop of Henle, approximately 90% of the total water reabsorption occurs [1], which is mediated by the water channel aquaporin-1 (AQP1). In contrast, the ascending limb of Henle and the distal convoluted tubule are water impermeable. In these segments, sodium and chloride are reabsorbed to maintain the osmotic gradient, being the driving force for water molecules. The collecting duct is the last part of the nephron and depending on the body's needs, remaining water can be reabsorbed in this segment and the final urine is generated before it is collected in the bladder. The water channels AQP2, AQP3 and AQP4 are expressed in collecting duct principal cells [2–4] and water reabsorption is carried out by these protein channels.

Nephrogenic diabetes insipidus disease is characterized by the production of large quantities of dilute urine and is caused by failure of the kidney to concentrate urine. Four different types of nephrogenic diabetes insipidus are observed until now [5–8] and gene mutations in the aquaporin 2 are the reason of two types of this disease. Therefore, human aquaporin 2 plays an essential role in kidney cellular processes and the molecular dynamics simulation of water transport in this protein channel and is an important part concerning the understanding process of nephrogenic diabetes insipidus.

So far, there is no experimental method to detect and analyze permeation of water through biological transporters such as aquaporins on a molecular level. Therefore, computational methods have been increasingly used to study behaviors of biological channels. Main computational tool is the molecular dynamics simulations which can be used for study of biological processes such as water permeation through aquaporins.

Zhu et al. [9] studied the difference between osmotic and diffusion permeabilities molecular dynamics investigation of AQP1 and their results were in agreement with observation. Also they demonstrated that the osmotic and diffusion permeabilities ratio corresponds to the number of effective steps a water molecule needs to take to permeate a channel. The single-channel water permeability of four members of the aquaporin family, including AQP1, AQPZ, AQP0 and GlpF, was evaluated by Hashido et al. using molecular dynamics simulations [10]. Their results indicated that the order of the permeation rate from largest to smallest was GlpF, AQPZ, AQP1 and AQP0 respectively.

Jensen and Mouritsen [11] determined single-channel water permeabilities for aquaglyceroporin GlpF and aquaporin Z, from equilibrium molecular dynamics simulations and they showed that osmotic water permeability of GlpF exceeds by 2–3 times that of AqpZ. Twenty nanosecond molecular dynamics simulation of the archaeal aquaporin AqpM was performed by Araya-Secchi et al. [12]. They reported the osmotic water permeability constant of AqpM tetramer.

Water self-diffusion within human AQP4 was investigated by Garate et al. [13]. In this research, the comparison of osmotic permeability results of MD simulation within experimental values was done. This comparison showed that their theoretical and the experimental osmotic permeability values differed by a factor of around 2. They explained that this difference is acceptable given the experimental difficulties in determining the channel density of the liposomes. Water permeation and movement through aquaporin Z using molecular dynamics simulations was studied by Xin et al. [14]. They related the open and closed states of AqpZ to water permeation dynamics through the channel. The MD results disclosed the gating mechanism for water transportation in this type of protein channels. Janosi and Ceccarelli [15] analyzed the structural dynamics and water permeability of the human aquaporin 5 using molecular dynamics simulations. It has been shown that the calculated permeation rate of a fully open channel was in good agreement with the reported experimental value.

In this paper, the results of the first molecular dynamics simulation of human aquaporin 2 embedded in a lipid biological membrane were reported and the most important biophysical parameters of water permeation through this type of aquaporin were estimated from the MD simulation.

2. Methodology

2.1. System preparation

In this research, recently presented high resolution (2.75 Å) X-ray crystal structure of the human aquaporin 2 tetramer was used [16]. This crystal structure was obtained from the Protein Data Bank (PDB ID: 4NEF). The tetramer structure of AQP2 was embedded into a palmitoyl-oleoyl-phosphatidyl-ethanolamine (POPE) lipid bilayer with 120 Å length in X and Y directions that was provided by Membrane Builder plugin of VMD software v1.91 [17]. Then the alignment of aquaporin and POPE membrane was performed and the lipid segments and water molecules in the range of 0.8 Å of the protein were removed to achieve a system without any overlapping. In order to fully hydrate the system, two layers of water molecules with 18 Å thickness were added below and above the protein and membrane complex using the solvate plugin of VMD software. Also, VMD's autoionize plugin was used to create 0.5 mol/L ionic concentration and neutralize the system by adding Na and Cl ions. The entire system, consisting of 111,188 atoms is presented in Fig. 1.

2.2. Molecular dynamics setup

The NAMD2.10 simulation package [18] was used to perform equilibrium molecular dynamics investigation of water permeation through the human AQP2. The CHARMM27 force field was employed for proteins [19] and phospholipids [20] and water molecules were represented with the TIP3P model [21]. In the basic form of molecular dynamics, trajectories and configurations are produced in a constant particle number, volume and energy (NVE) ensemble. However, with evolvement of molecular dynamics, it has become essential to simulate systems in other thermodynamics ensembles. For example, experimental investigations of biological membranes are often performed at constant particle number, pressure and temperature (NPT) conditions and molecular dynamics simulation of these systems must also be performed in this ensemble. In this molecular dynamics simulation the Langevin dynamics thermostat with a Langevin damping coefficient $\gamma = 5\ ps^{-1}$ was used to maintain the temperature of the system at 310 K and in order to pressure control at 1 bar the Langevin piston algorithm [22] was employed.

Periodic boundary conditions and Particle Mesh Ewald (PME) method [23] were applied to take into account finite system size and full long-range electrostatic interactions, respectively. A cut-off distance of 12 Å with a switching distance of 10 Å was applied for the Van der Waals interactions.

2.3. Molecular dynamics simulation

Three phases were considered for equilibrium molecular dynamics simulation of the system. In the first phase, water, ions, protein and lipid head groups were fixed and lipid tails were minimized and equilibrated for 0.5 ns to achieve a melting lipid tail fluid-like bilayer. In order to guide the system to the nearest local energy minimum in configuration space, minimization and equilibration of the system with protein constraint were performed in the second phase by applying harmonic constraints on the protein. Finally, in the third phase 20 ns of isobaric and isothermal molecular dynamics simulation was performed without any restraints to equilibrate the whole system.