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Molecular Dynamics Simulation of Water Transportation through Aquaporin-4 in Rat Brain Cells

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ABSTRACT

This paper investigates the mechanism of water transportation through aquaporin-4(AQP4) of rat brain cells by means of molecular dynamics simulation with CHARMM software. The AQP4 was embedded into a bilayer made of Dimystroilphosphatylcholine (DMPC). The results illustrate that water molecules move through AQP4's channel with change of orientation of oxygen of each water molecule.

Keywords: Aquaporin-4; Brain edema; Molecular dynamics; CHARMM

INTRODUCTION

Of all proteins in the cell, 20-30% is associated with biological membranes and most drug targets are membrane –associated receptors. These proteins perform a multitude of important biological functions, such as transmembrane proteins specially water channel proteins [1].

The aquaporins (AQPs) are a family of internal membrane proteins that control the movement of water molecules across lipid membranes [2-6] with 13 different mammalian AQPs proteins (AQP0 – AQP12) [7]. They are widely distributed in various organs in the human body – kidneys, eyes and the brain. Disruption of the function of aquaporins (AQPs) can cause such diseases as diabetes insipidus, congential cataracts, and hearing important [7-10]. Many studies have been done on the structure and function of aquaporins. The first structure of water channel, aquaporins-1 (AQP1) which was determined by electron crystallography [11] revealed the unusual AQP fold consisting of two tandem repeats, each with three transmembrane helices (helices 1-3 and helices 4-6) and short pore helix in loops [12].

It was known that side-chain dynamics of AQPs are critical for permeation of water through the channel [13]. In addition, sequence analysis of the aquaporins family reveals highly homology among human series (HS) members [14]. The aquaporins possess an NPA (Asn- Pro- Ala) motif that appears twice in the sequence near the center of normal axes of the molecules [12]. The sequence can be divided in half with one motif per half. The lowest energy water pathway through aquaporins is between the NPA and selectively filter (SF) sites [15]. NPAs play an essential role to conduction of water molecules through the aquaporins [12-16]. Therefore when Asn in NPAs replaced with near-isoelectric hydrophobic residues in the simulation, the aqueous pathways were broken completely [12]. The dipolar momenta of water molecules are changed in NPAs region [12- 16]. Principally, penetration of water molecules through the

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AQP1 is due to osmotic pressure; however, NPA motifs facilitate the conduction [2, 16, and 17]. In this study we find out that NPA motifs in AQP4 have the same role.

COMPUTATIONAL

Molecular dynamics (MD) and Monte Carlo (MC) methods are two main simulation methods especially for macromolecules such proteins as. Molecular dynamics is based on Newton's equations of motion and MC is a statistical thermodynamics method, in principle. All of computational steps carried out by CHARMM software.

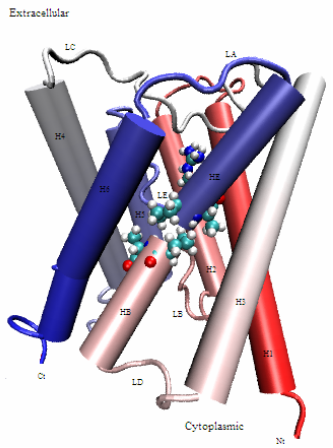
The system was equilibrated for 150 Pico-seconds (ps) by molecular dynamics. To converge to an equilibrium state, the system was coupled for the first 125 ps to a heat bath at 330 K by the use of Langevin dynamics. During the last 25 ps of equilibration the velocities were periodically rescaled to stabilize the temperature. The list of nonbonded interactions was truncated at 12 Å by the use of a group-based cutoff. The nonbonded van der Waals and electrostatic interactions were smoothly switched off over a distance of 3.0 Å, the values being maximum for a radius of less than 8 Å and zero at 11 Å. The SHAKE algorithm [18] was used to fix the length of all bonds involving hydrogen atoms. Harmonic potentials were applied to the aquporin-4 backbone to prevent large spurious motions, the center of mass of the lipid polar heads was kept around z = ± 17 Å by planner harmonic constraints to maintain the planarity of the membrane, and the penetration of water in bilayer region was prevented within z by the use of planar potentials.

Two boxes of water which were made by Monte Carlo assigned on two sides of the system. For the first 1700 ps, the AQP4 was assigned in system and minimized and the trajectory was continued for 400 ps. As a control for complete penetration of water trough the channel of AQP4, the trajectory of the system with an unprotonated N-terminus was also continued for 2000 ps.

RESULTS AND DISCUTION

Normally AQP4 is seen in monomer form but sometimes it is in tetramer from in its high concentration [19]. Data structure of AQP4 has achieved from RCSB (a famous site for proteins data structure) and after minimization with CHARMM for AQP4 the structure was used for studying of water penetration through the channel (Fig.1).

(a)



(b)

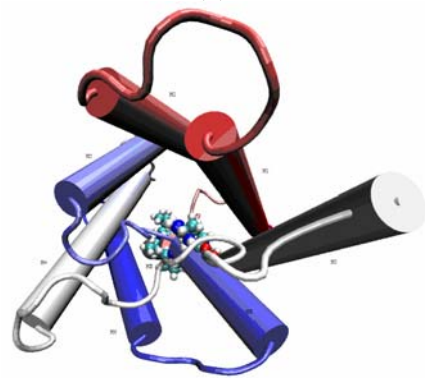


Fig.1. Normal shape of AQP4 after minimization with CHARMM. Schematic representation of the structure of an AQP4 monomer. Side view (a) and top view from the extra cellular side (b). Key secondary structural elements are labeled. The regions containing the two NPA motifs (Asn- 67- Pro- 68- Ala- 69 and Asn- 183- Pro- 184- Ala- 185) are LB-HB and LE- HE, respectively.

Then the system has constructed and minimization steps were done for it. This system contains a unit cell of prehydrated phospholipid bilayer of dimensions 72 × 58 × 200 $Å^3$ and two boxes of water in top and bottom side of the unit cell each of 72 × 58 × 16.317 $Å^3$ and AQP4 which has constrained parallel to the normal axes (z Axes) of the bilayer. Data have shown that after 15000 steps, the system achieved a stable position, Table 1 and (Fig.2 and 3).

Table 1. Data of optimization for the primary system after 15000 steps with CHARMM

N step	E(kcal/mol)	N step	E(kcal/mol)	N step	E(kcal/mol)	N step	E(kcal/mol)
0	-535.41	5000	-536.01	10000	-546.59	15000	-546.66
1000	-535.47	6000	-539.75	11000	-546.66		
2000	-538.6	7000	-541.23	12000	-546.65		
3000	-538.91	8000	-546.95	13000	-546.66		
4000	-538.26	9000	-546.4	14000	-546.66		

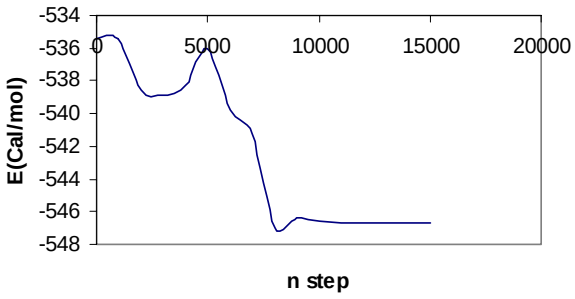


Fig.2. Change of energy of constitution for the system after 15000 steps with CHARMM.

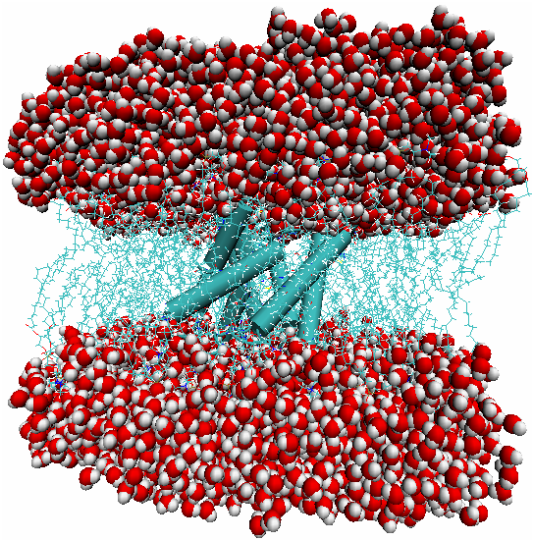


Fig.3. The AQP4 in fully hydrated DMPC membrane. The extracellular side is at the top and the intracellular side at the bottom. This picture was created with VMD software.

The NPA motifs residues in the two loops are considered the most important for function. The AQP4 in brain cells of rat, like other aquaporins has two helices dividing into the protein midway through the membrane and back to the aqueous phase in a distinct inverted helix fashion that exposes backbone atoms to the interior of the pore [19]. The two helices are held in place at the channel center through two highly inward pointed helices and approximate inversion symmetry brings about strong electrical fields along the channel axis that are inverted at the NPA motifs in a span of a few Å[20-21].

Water conduction through the AQp4 is accompanied by a rotation of the water molecules at the center of the channel where the two highly conserved NPA motifs meet to maintain the bipolar orientation ordering of the single file water molecules. The rotation of water dipole, enforced by electrical forces of the protein in span of a few Å, is facilitated through hydrogen bond exchange between the two carbonyl arrays and the Asparagines residues of the NPA motifs [20-22].

This simulation revealed a bipolar orientation ordering of the channel water that matches the pesudosymmetric aquaporins architecture from both ends of the channel, the water oxygen's point into the channel, with two central water molecules pointing their oxygen's sideways toward the two NPA motifs with which they strongly interact (Fig.4).

The curve of electrical potential of water molecules through the channel confirms this result (Fig.5). The curve illustrates two main maximum near the center of channel where NPA motifs are located.

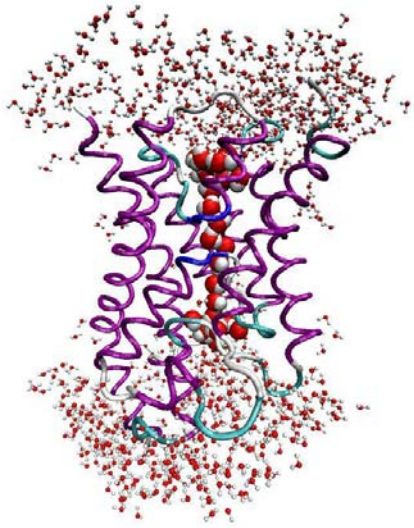


Fig.4. Water conduction through AQP4 is accompanied by a rotation of the water molecule at the center of the channel, where the two highly conserved NPA motifs meet to maintain the bipolar orientation ordering of the single file water molecules. This picture was created with VMD software.

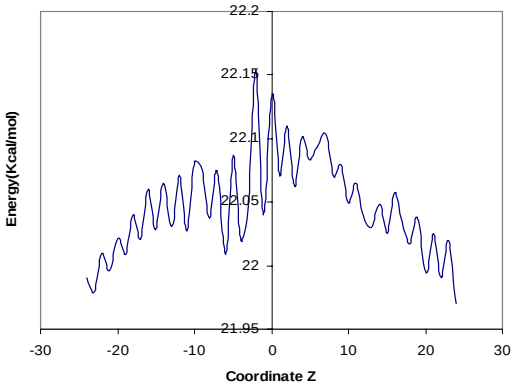


Fig.5. Change of electrical potential energy through the channel of AQP4

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