Biochimica et Biophysica Acta 1828 (2013) 1786–1793

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Glycerol modulates water permeation through *Escherichia coli* aquaglyceroporin GlpF

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ARTICLE INFO

Article history:
Received 18 October 2012
Received in revised form 5 March 2013
Accepted 8 March 2013
Available online 16 March 2013

Keywords:
Aquaglyceroporin
Chemical potential
Glycerol
Transport
Activation barrier

ABSTRACT

Among aquaglyceroporins that transport both water and glycerol across the cell membrane, *Escherichia coli* glycerol uptake facilitator (GlpF) is the most thoroughly studied. However, one question remains: Does glycerol modulate water permeation? This study answers this fundamental question by determining the three-dimensional potential of mean force of glycerol along the permeation path through GlpF's conducting pore. There is a deep well near the Asn-Pro-Ala (NPA) motifs (6.5 kcal/mol below the bulk level) and a barrier near the selectivity filter (10.1 kcal/mol above the well bottom). This profile owes its existence to GlpF's perfect steric arrangement: The glycerol–protein van der Waals interactions are attractive near the NPA but repulsive elsewhere in the conducting pore. In light of the single-file nature of waters and glycerols lining up in GlpF's amphipathic pore, it leads to the following conclusion: Glycerol modulates water permeation in the μM range. At mM concentrations, GlpF is glycerol-saturated and a glycerol residing in the well occludes the conducting pore. Therefore, water permeation is fully correlated to glycerol dissociation that has an Arrhenius activation barrier of 6.5 kcal/mol. Validation of this theory is based on the existent *in vitro* data, some of which have not been given the proper attention they deserved: The Arrhenius activation barriers were found to be 7 kcal/mol for water permeation and 9.6 kcal/mol for glycerol permeation; The presence of up to 100 mM glycerol did not affect the kinetics of water transport with very low permeability, in apparent contradiction with the existent theories that predicted high permeability (0 M glycerol).

1. Introduction

Escherichia coli aquaglyceroporin GlpF is a member of the membrane proteins responsible for water and solute transport across the cell membrane [1–6]. Among the aquaglyceroporin sub-family of proteins that conduct both water and glycerol, GlpF is the most thoroughly studied, both *in vitro* [7–19] and *in silico* [18,20–29]. There is no controversy over the science that GlpF conducts both water and glycerol and how the amphipathic pore of GlpF selectively facilitates the passage of waters and glycerols lining up in a single file through the conducting channel [17,18,20,21]. However, one fundamental question remains: Does glycerol modulate water permeation through GlpF? And, related to this question, there are some unsolved issues about water permeation through this protein's conducting pore: The *in vitro* data indicate that GlpF is much less permeable to water than *E. coli* aquaporin Z (AQPZ) and other water-selective aquaporins are [13,14,30], but theoretical studies predict that GlpF is more permeable than AQPZ *etc.* [23,31]; The *in vitro* experiments show that water permeation has an Arrhenius activation barrier that is about 7 kcal/mol [13], but the theoretical studies all give a rather flat free-energy profile throughout the permeation channel of GlpF [20,27]. While the *in vitro* experiments indicate that the presence of up to 100 mM glycerol does not affect the kinetics of water transport [13], all *in silico* studies are limited to 0 M glycerol concentration. All these problems can be resolved once we have an accurate determination of the three-dimensional (3D) potential of mean force (PMF)[32–34] of glycerol as a function of its center-of-mass (COM) coordinates along a path leading from the periplasm to the entry vestibule of GlpF, through the channel, to the cytoplasm. This chemical-potential profile in terms of the 3D PMF, considered on the basis of the structure information available in the literature [17,18], can ascertain the conclusion that glycerol strongly modulates water permeation through GlpF.

Inside the GlpF channel, waters and glycerols line up in a single file, occluding one another from occupying the same z-coordinate. (The z-axis is chosen as normal to the membrane-water interface, pointing from the periplasm to the cytoplasm.) Therefore, waters and glycerols permeate through the amphipathic pore of GlpF in a concerted, collective diffusion, driven or not driven by the osmotic pressure. If a deep enough chemical-potential well exists inside the channel (where the chemical potential is lower than the periplasm/cytoplasm bulk level), a glycerol molecule will be bound there, with a probability determined by the glycerol concentration and the dissociation constant. The bound glycerol will occlude permeation of waters and other glycerols through the channel. GlpF will switch between being open and closed to water permeation as a glycerol is

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http://dx.doi.org/10.1016/j.bbamem.2013.03.008

dissociated from and bound to the binding site inside the protein's conducting pore. Therefore, such a chemical-potential profile means that glycerol modulates water permeation through GlpF in the glycerol concentration range around the dissociation constant.

In order to produce an accurate chemical-potential profile of glycerol, I conducted a total of 899 ns equilibrium and non-equilibrium molecular dynamics (MD) simulations, which amounts to about 10 times the computing efforts invested on GlpF in a published work of the current literature. The non-equilibrium MD simulations include three sets of steered molecular dynamics (SMD) [35–37] runs and two MD runs under pressure gradients. The accuracy of the PMF estimation was ascertained by the agreement between the non-equilibrium SMD approach and the equilibrium adaptive biasing force (ABF) approach [24,38].

Glycerol is found to have a deep chemical-potential well in the GlpF channel near the Asn-Pro-Ala (NPA) motifs that is 6.5 kcal/mol below its chemical potential in the bulk of periplasm/cytoplasm. Glycerol binding to or dissociating from this binding site strongly modulates water permeation through the GlpF pore. There are two chemical-potential barriers separating the glycerol binding site from the periplasm and cytoplasm bulk regions; The barrier at the selectivity filter (SF) between the binding site and the periplasm is 10.1 kcal/mol, and the barrier between the NPA and the cytoplasm stands at 4.6 kcal/mol above the bottom of the chemical-potential well. This profile, considered in the context of the structural characteristics of GlpF, leads to a new theory of glycerol modulated water permeation through GlpF that is in full agreement with the *in vitro* results in the current literature. It also harmonizes the existent theoretical results at 0 M glycerol concentration with the *in vitro* experiments at up to 100 mM concentrations of glycerol. Furthermore, it could be fully validated by future *in vitro* experiments measuring the glycerol-GlpF dissociation constant and the water permeability in the μM range of glycerol concentration.

2. Methods

2.1. System setup

This study was based on the following all-atom model of GlpF in the cell membrane (Fig. 1): The GlpF tetramer, formed from the crystal structure (PDB code: 1FX8) with 12 glycerols, was embedded in a patch of fully hydrated palmitoyloleylphosphatidyl-ethanolamine (POPE) bilayer. The GlpF-POPE complex is sandwiched by two layers of water, each of which is approximately 30 Å in thickness. The system is neutralized and ionized with Na^+ and Cl^- ions at a concentration of 111 mM. The entire system, consisting of 150,855 atoms, is 114 Å × 115 Å × 112 Å in dimension when fully equilibrated. This system (SysI) has a glycerol concentration of 14 mM. A second system (SysII), for 0 M glycerol, was derived from SysI by deleting all 12 glycerols. It has 150,687 atoms in all and dimensions approximately equal to those of SysI. The Cartesian coordinates are chosen such that the origin is at the geometric center of the GlpF tetramer. The *xy*-plane is parallel to the lipid–water interface and the *z*-axis is pointing from the periplasm to the cytoplasm.

All the simulations of this work were performed using NAMD 2.8 [39]. The all-atom CHARMM36 parameters [40,41] were adopted for all the inter- and intra-molecular interactions. Water was represented explicitly with the TIP3 model. The pressure and the temperature were maintained at 1 bar and 293.15 K, respectively. The Langevin damping coefficient was chosen to be 5/ps. The periodic boundary conditions were applied to all three dimensions, and the particle mesh Ewald was used for the long-range electrostatic interactions. Covalent bonds of hydrogens were fixed to their equilibrium length. The time step of 2 fs was used for short-range interactions in equilibrium simulations, but 1 fs was used for nonequilibrium runs. The same time step of 4 fs was used for long-range forces in both equilibrium and nonequilibrium simulations. The cut-off for long-range interactions was set to 12 Å with a switching distance of 10 Å. In all simulations, the alpha carbons on the trans-membrane helices of GlpF within the range of -10 Å $< z <$ 10 Å were fixed to fully respect the crystal structure.

2.2. Equilibrium MD

Two runs of 100 ns each in length were conducted for SysI and SysII respectively. Using the theoretical formulation of [42], I computed the mean square displacements (MSD) of the water molecules in the conducting pore. (The MSD curves are shown in supplemental Fig. S1.) The slope of the MSD curve gives an estimate of the osmotic permeabilities of both SysI and SysII that will be presented in the next section.

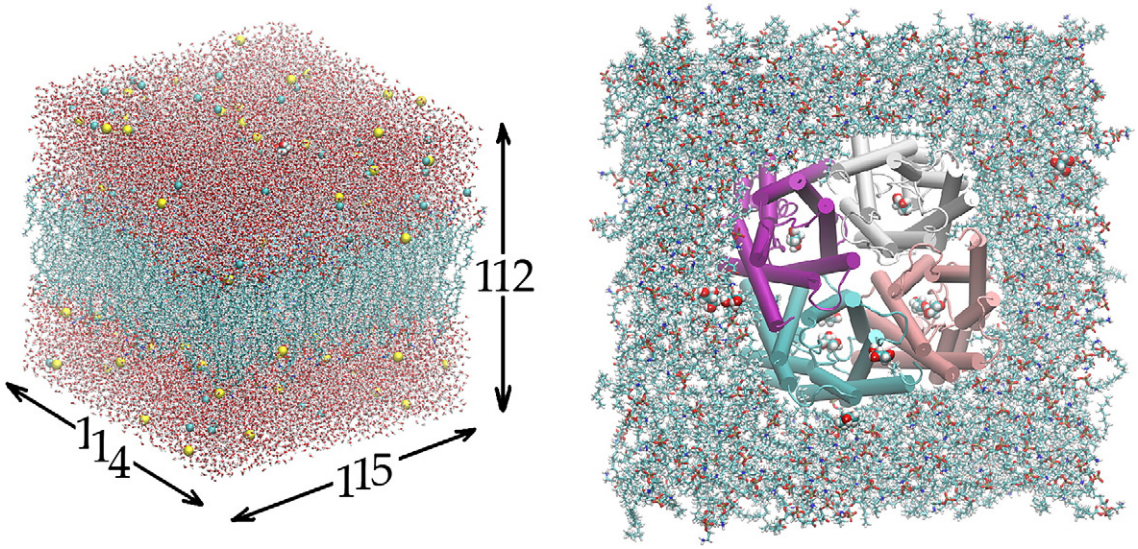

Fig. 1. All-atom model of GlpF in the cell membrane. The system is 114 Å × 115 Å × 112 Å in dimension. Visible in the left panel are waters (in licorice representation), lipids (licorice), ions (vdw), and one glycerol (vdw). Shown in the right panel are the GlpF tetramer (in cartoon representation, colored by segname), lipids (licorice), and glycerols (vdw). All except GlpF are colored by element name. Graphics rendered with VMD [46].