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Review

Insights into structural mechanisms of gating induced regulation of aquaporins

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ABSTRACT

Aquaporin family comprises of transmembrane channels that are specialized in conducting water and certain small, uncharged molecules across cell membranes. Essential roles of aquaporins in various physiological and pathophysiological conditions have attracted great scientific interest. Pioneering structural studies on aquaporins have almost solved the basic question of mechanism of selective water transport through these channels. Another important structural aspect of aquaporins which seeks attention is that how the flow of water through the channel is regulated by the mechanism of gating. Aquaporins are also regulated at the protein level, i.e. by trafficking which includes changes in their expression levels in the membrane. Availability of high resolution structures along with numerous molecular dynamics simulation studies have helped to gain an understanding of the structural mechanisms by which water flux through aquaporins is controlled. This review will summarize the highlights regarding structural features of aquaporins, mechanisms governing water permeation, proton exclusion and substrate specificity, and describe the structural insights into the mechanisms of aquaporin gating whereby water conduction is regulated by post translational modifications, such as phosphorylation.

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1. Introduction

Aquaporins are ubiquitous family of membrane proteins that facilitate the rapid transport of water across cell membranes and thus play a critical role in maintaining water homeostasis in all living cells. In cell membranes, aquaporins exist as homotetramers with each monomer forming an independent water channel. Aquaporin family members mediate the bidirectional water flow driven by an osmotic gradient. They enable highly efficient water permeation with flow rates in order of 10^9 s^{-1} (Zeidel et al., 1992). In addition to water, some aquaporin family members, called aquaglyceroporins transport certain small, neutral solutes such as glycerol (Heller et al., 1980; Borgnia and Agre, 2001), ammonia (Saparov et al., 2007), urea (Borgnia et al., 1999), arsenite (Liu et al., 2002). Aquaporins have a remarkable property of effective water conductance while blocking the flow of protons and thus maintain

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the electrochemical gradient across cell membranes (de Groot et al., 2003). In eukaryotic organisms, aquaporins perform a wide variety of physiological functions, such as concentrating urine in kidneys (Chen et al., 2005), maintaining lens transparency in eyes (Verkman, 2003) maintaining water homoeostasis in brain (Amiry-Moghaddam and Ottersen, 2003), cell migration during tumor growth (Saadoun et al., 2005), facilitating a rapid response to shock in yeast (Tamas et al., 1999), regulation of cell osmolarity in plants (Maurel et al., 2002), driving the opening and closing of flower petals (Azad et al., 2008). Physiological significance of aquaporins is further highlighted by the fact that a number of diseases including brain edema (Manley et al., 2000), tumor (Vacca et al., 2001), obesity (Kuriyama et al., 2002) manifest abnormal functioning of these water channels.

A lot of structural information has been deduced from primary sequence of aquaporins. Sequence analysis of AQP1 has revealed two tandem repeats each formed from three transmembrane domains with two highly conserved loops (B and E) containing the signature motif, asparagines-proline-alanine (NPA). The repeats have been predicted to be oriented 180° with respect to each other. Based on biochemical and site-directed mutagenesis studies, an 'hourglass model' has been proposed according to which loops B and E fold back into the bilayer from the opposite sides of the membrane thereby forming the aqueous pore (Jung et al., 1994a).

Aquaporin is emerging to be the richest family of membrane channels with regard to the abundance of high resolution structural data. Three dimensional crystal structures of several members of the family have been solved. The structures of 2 bacterial AQP: GlpF, glycerol channel (Fu et al., 2000) and AqpZ (Savage et al., 2003); 7 mammalian AQP: bovine and sheep AQP0 (Gonen et al., 2004; Harries et al., 2004), human AQP1 (Murata et al., 2000), bovine AQP1 (Sui et al., 2001), rat AQP4 (Hiroaki et al., 2006), human AQP4 (Ho et al., 2009), human AQP5 (Horsefield et al., 2008); SoPIP2;1 (spinach) aquaporin (Tornroth-Horsefield et al., 2006); archaeal AQP (Lee et al., 2005), PfAQP from malarial parasite *Plasmodium falciparum* (Newby et al., 2008) and yeast AQP (Fischer et al., 2009) are available in PDB. Elucidation of three dimensional structures of aquaporin family members has confirmed the hourglass fold that was previously suggested by sequence analysis. As shown in Fig. 1a, the hourglass fold consists of six transmembrane α-helices surrounding a single, narrow aqueous pore (Sui et al., 2001). Loops B and E form half transmembrane helices (HB and HE) and fold into the channel from opposite sides of the membrane, effectively creating a seventh broken transmembrane helix (Fig. 1a). The N-terminal ends of these half helices contain the aquaporin Asn-Pro-Ala (NPA) signature motifs that meet at the center of the pore (Fig. 1b). Aquaporins contain a few highly conserved residues in and around the two functional loops B and E that are closely positioned in the interior of the membrane. In case of bAQP1, these are residues Leu-77, His-76, Ala-75 and Gly-74 present on loop B and residues Gly-190, Cys-191, Gly-192 and Ile-193 located on loop E. Loops B and E are held together by van der Waals interactions between the prolines in the two NPA motifs. The positions of loops B and E are stabilized through ion pairs and hydrogen bonds with neighboring transmembrane helices.

Molecular dynamics simulation of aquaporins has provided useful insights into mechanism of water permeation. Simulations have revealed that water molecules move in a single file configuration through the channel. The two half helices HB and HE generate electrostatic fields directed toward the center of the channel, thereby creating an electrostatic barrier which results in a complementary alignment of the dipole moments of water molecules as they move past the NPA motifs (Tajkhorshid et al., 2002). Starting from the NPA motifs, water molecules are oriented in opposite direction in the two halves of the channel. Above and below the NPA motifs, hydrogen atoms of water molecules point towards extracellular mouth and cytoplasmic mouth of the channel respectively. This results in the bipolar orientation of water molecules inside the aquaporin channel as shown in Fig. 2. The electrostatic barrier of approximately 25–30 KJ mol^{-1} is suggested to be the predominant cause of proton exclusion (de Groot et al., 2003; de Groot and Grubmuller, 2005). Another conserved structural feature of aquaporin family is the aromatic/arginine (ar/R) constriction site located at the extracellular side of the channel (Fig. 1b). The ar/R constriction site which consists of an arginine and other three amino acids (one of which is usually aromatic) such as

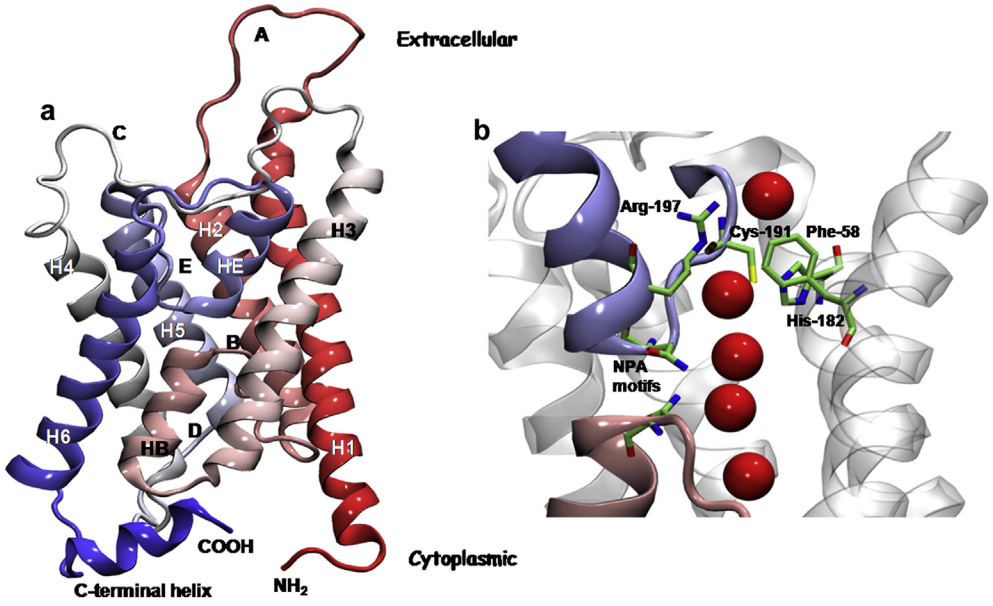

Fig. 1. Structural features of aquaporin family members. (a) The hourglass fold. Transmembrane helices are denoted as H1–H6, loops are denoted as A–E and the two pore helices formed by loops B and E are denoted as HB and HE respectively. (b) Details of NPA motifs and ar/R site. Water molecules inside the channel are shown as red spheres. The structure of bovine AQP1 (PDB ID 1J4N) is used to show the common fold.