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REVIEW

REGULATION OF WATER PERMEABILITY THROUGH AQUAPORIN-4

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Abstract—Aquaporin-4 (AQP4) is a predominant water channel protein in mammalian brains that is distributed with the highest density in the perivascular and subpial astrocyte end-feet. AQP4 is a critical component of an integrated water and potassium homeostasis. Expression and regulation of AQP4 have been studied to understand the roles of AQP4 in physiology and several pathological conditions. Indeed, AQP4 has been implicated in several neurological conditions, such as brain edema and seizure. AQP4 is dynamically regulated at different levels: channel gating, subcellular distribution, phosphorylation, protein–protein interactions and orthogonal array formation. In this review, we focus on the short-term regulation of AQP4. Phosphorylation of AQP4 is important; AQP4 is inhibited when Ser180 is phosphorylated and activated when Ser111 is phosphorylated. AQP4 is also regulated by several metal ions. These metal ions inhibit AQP4 by interacting with the Cys178 residue located in the cytoplasmic loop D, suggesting that AQP4 is regulated by intracellular signaling pathways in response to extracellular stimuli. Recently, it was demonstrated that AQP4 may be inhibited by arylsulfonamides, antiepileptic drugs and other related chemical compounds. Structural analysis of AQP4 may guide a drug design for AQP4.

Key words: water channel, proteoliposome, oocyte, metal ions, phosphorylation, arylsulfonamides.

Contents

Water balance is one of the most fundamental mechanisms for our body homeostasis. Aquaporins (AQPs) play important roles for body water homeostasis, since they function largely as water-conducting pores through cell membranes (Agre et al., 2002; Hazama et al., 2002). In mammals, there are 13 members of the AQP family (AQP0–12) (Itoh et al., 2005). Atomic structures of several AQPs have been determined (Murata et al., 2000; Ren et al., 2001; Savage et al., 2003; Gonen et al., 2005; Hiroaki et al., 2006). AQPs have six bilayer-spanning domains forming an hourglass structure, and the N- and C-termini, as well as loop B and D, are surfaced to the cytoplasm (Fig. 1). AQPs mainly exist in plasma membranes as tetramers and each monomer contains a water or solute-conducting pore. Water transport through an AQP represents facilitated diffusion driven by an osmotic gradient.

Aquaporin-4 (AQP4) is a predominant water channel protein in the mammalian brain (Hasegawa et al., 1994; Jung et al., 1994a), and is expressed in the endfeet of astrocytes surrounding the blood–brain barrier (BBB) (Amiry-Moghaddam and Ottersen, 2003). AQP4 exists in two spliced isoforms (Jung et al., 1994a): M1 and M23 (Fig. 1). A shorter spliced variant, M23, assembles into orthogonal arrays, whereas a longer spliced variant, M1, has little propensity to form the arrays. The array formation can be dynamically regulated by the modification of intracellular cysteine residues of M1 by palmitoylation (Suzuki et al., 2008). Phosphorylation of AQP4, important for trafficking of the protein (Madrid et al., 2001; Carmosino et al., 2007; Kadohira et al., 2008), can also regulate array formation (Silberstein et al., 2004; Van Hoek et al., 2009). Although the physiological relevance of the array formation is not clear, the intermolecular regulation of the array might be linked to the regulation of AQP4 water permeability.

The water permeability via AQP4 around BBB is of significance because the breakdown of BBB develops brain edema (Zador et al., 2007). AQP4 null mice are protected from several models of cytotoxic brain edema, including hyponatremia (Papadopoulos and Verkman, 2007), bacterial meningitis (Manley et al., 2000) and early focal cerebral ischemia (Papadopoulos and Verkman, 2005). On the other hand, AQP4 null mice are more sensitive for vasogenic edema due to increased permeability of capillary endothelial cells associated with brain tumors (Papadopoulos et al., 2004). In the tumor model, the AQP4 null mice developed worse neurological score and higher intracranial pressure than wild-type control. AQP4 may be involved in limiting the entry of edema fluid from the tumor bed into the brain parenchyma. In astrocyte in the vicinity of edematous brain tumors, AQP4 expression is strongly up-regulated and is not polarized to end-feet, but is seen throughout the entire cell membranes, in immunohistochemical studies of human brain tumors (Saadoun et al., 2002, 2003; Badaut et al., 2003). AQP4 null mice reveal prolonged seizure and delayed potassium reuptake from the extracellular space during cortical spreading depression (Bloch et al., 2006). These findings indicate that AQP4 plays key roles in these pathological conditions.

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Abbreviations: AQPs, aquaporins; AZA, acetazolamide; BBB, blood–brain barrier.

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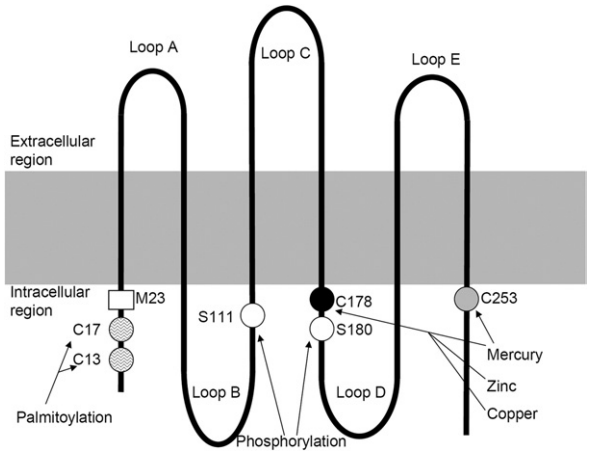

Fig. 1. Membrane topology of AQP4. Key amino acid residues of AQP4 required for functional regulation are indicated.

There are several established *in vitro* methods to measure osmotic water permeability through AQPs, in order to study regulations of AQPs (Hasegawa et al., 1993; Jung et al., 1994a,b; Shi and Verkman, 1996; Agre et al., 1999; Nicchia et al., 2000). These methods include (i) swelling assay of *Xenopus* oocytes expressing AQP, (ii) swelling assay of mammalian cells expressing AQP and (iii) stopped-flow assay of AQP-proteoliposomes (Preston et al., 1992; Zeidel et al., 1992) (see Table 1). In the oocyte swelling assay, AQP cRNA is injected into a *Xenopus* oocyte. Once AQP proteins are synthesized and expressed in the plasma membranes of the oocyte, the oocyte is transferred from isoosmotic medium to hypoosmotic medium and a volume change of the oocyte is monitored using a microscope with a CCD camera to calculate the osmotic water permeability. In the assay with mammalian cells, fluorescent materials are pre-loaded onto cells and changes of fluorescent intensity in cells are monitored with a fluorescent microscope to calculate the osmotic water permeability. This assay is performed under biological conditions since regulatory proteins or cell signaling pathways that regulate AQP activity may exist in the cells. In stopped-flow analysis using proteoliposomes, purified AQP protein is reconstituted into liposomes with or without carboxy fluorescein (CF). Any change in CF quenching or light scattering is monitored with a stopped-flow apparatus to calculate the osmotic water permeability. This method is advantageous in studying channel gating because of extremely high time resolution. In the proteoliposomes, AQP proteins are incorporated into liposome membranes with two orientations, while AQPs are located with a single orientation on the cell membranes when expressed in oocytes or mammalian cells. These methods, therefore, can be selected depending on the purpose of the study being conducted (Table 1).

In this review, we will focus on short-term regulation of AQP4 such as phosphorylation and modifications with metals or chemical compounds.

PHOSPHORYLATION

Phosphorylation regulates functions of the target proteins. For example, membrane proteins, such as transporters and channels, and AQPs are regulated by direct phosphorylation. AQP2 phosphorylation by arginine vasopressin (AVP) is required for trafficking of AQP2 to the plasma membranes in response to AVP, and phosphorylation of AQP4 has also been demonstrated in several different studies.

(1) Phosphorylation of Ser180 decreases the water permeability of AQP4

Yang et al. reported that the osmotic water permeability was not changed by forskolin or phorbol 12-myristate 13-acetate (PMA) in oocytes swelling assay (Yang and Verkman, 1997). On the other hand, Han et al. reported that the phorbol 12, 13-dibutyrate (PDBu) or PMA decreased the water permeability of AQP4 expressed in oocytes (Han et al., 1998). The discrepancy might be attributed to variation in oocyte batches undertaken for the studies or to different experimental conditions (Han et al., 1998).

In LLCPK1 cells expressing AQP4, PDBu and dopamine decreased the osmotic water permeability, which was abolished by pretreatment with a PKC inhibitor (Zelenina et al., 2002). Ser180, a consensus site for PKC, is localized in cytosolic loop D and the Ser180Ala mutant was not inhibited by a PKC activator, suggesting that Ser180 in loop D is a target residue for PKC-mediated phosphorylation of AQP4. There is no evidence of direct phosphorylation of AQP4 by PKC in primary cultured astrocyte although endogenous mouse AQP4 is constitutively phosphorylated (Nicchia et al., 2008; Kadohira, 2008). Further studies are, therefore, necessary to demonstrate physiological relevance of AQP4 phosphorylation by PKC.

(2) Phosphorylation of Ser111 increases the water permeability of AQP4

While the phosphorylation of Ser180 in loop D decreases the water permeability of AQP4, the phosphorylation of Ser111 in loop B increases the water permeability of AQP4 (Gunnarson et al., 2005; 2008). Gunnarson et al. reported that the membrane water permeability of an as-

Table 1. Methods for measurements of the water permeability

	Oocytes swelling assay	Mammalian cells swelling assay	Proteoliposomes assay
Materials	*Xenopus laevis* oocytes	Primary astrocytes or AQP4-expressing cell lines	Liposomes
AQP orientation in the membrane	Single	Single	Bidirectionally
Interaction with other proteins	○	○	×
Time resolution (s)	100	1–10	0.1
Apparatus	Stereomicroscope	Fluorescence microscope	Stopped-flow