

The availability of Aquaporin 1 to function as a gated cation channel is regulated by tyrosine kinase signalling

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Aquaporins (AQPs) belong to the family of major intrinsic proteins, MIPs, found across all forms of life. AQPs commonly facilitate the rapid transport of water across cell membranes with some sub-types transporting other small molecules and ions. AQP1 also has been shown to function as an ion channel when activated by cGMP, although this finding is controversial as some research groups have not detected ion permeation, or have found only a small proportion of the water channel population is available to function as gated ion channels. Molecular dynamic simulation and electrophysiological analyses suggested that cations permeate AQP1 *via* the central pore formed by the tetrameric organisation of subunits, and that a conserved intracellular loop between the 4th and 5th transmembrane domains is required for cGMP-dependent gating (Yu *et al.*, 2006).

The purpose of this study was to use cysteine residues introduced into AQP1 by mutagenesis and probed with mercuric chloride to test the central pore hypothesis, and to evaluate the role of tyrosine phosphorylation in the carboxy (C)-terminal domain for controlling availability of AQP1 ion channels to be activated by cGMP.

Human AQP1 wild type or site-directed mutant cRNAs were injected singly or in combination into *Xenopus* oocytes and incubated for at least 48h prior to experimentation. AQP1 ion channels were stimulated by the addition of extracellular CPT-cGMP (20 μ M). Conductances were measured by two-electrode voltage clamp, and compared with non-AQP-expressing control oocytes. Tyrosine kinase inhibitor (1-Naphthyl PPI, 10 μ M) and tyrosine phosphatase inhibitor (bpV phen, 100 μ M) were applied to determine the phosphorylation states necessary for AQP1 ion channel activation. A functional AQP1 mutant lacking all four native cysteines was made and used as a template for the introduction of cysteine residues into the putative central pore region and the C-terminal domain phosphorylation sites. Mercury was applied to probe the effects of the cysteine residues on the activation of the ionic conductance.

Results to date indicate that tyrosine phosphorylation is a powerful modulator of AQP1 ion channel activity, and that introduced cysteines in AQP1 mutants are effective tools for demonstrating that the central pore region is involved in the permeation of ions through the channel, and that the C-terminal region modulates ion channel availability. Continuing work is testing the hypothesis that phosphorylation serves as a master switch that controls AQP1 responsiveness to cGMP.

The presence of hierarchical levels of regulation of AQP1 could explain the differences that have been reported in ion channel activity across various experimental preparations, demonstrating that an increase in cGMP level alone is not sufficient to guarantee ion channel activation. These findings offer insight into the possible resolution of an intriguing controversy in the aquaporin field, and expand our understanding of aquaporins as complex multifunctional channels.

Yu J, Yool AJ, Schulten K & Tajkhorshid E (2006). Mechanism of gating and ion conductivity of a possible tetrameric pore in aquaporin-1. *Structure* 14, 1411-1423.