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Review

Water channel structures analysed by electron crystallography ☆

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ABSTRACT

Background: The mechanisms underlying water transport through aquaporin (AQP) have been debated for two decades. The water permeation phenomenon of AQP seems inexplicable because the Grotthuss mechanism does not allow for simultaneous fast water permeability and inhibition of proton transfer through the hydrogen bonds of water molecules.
Scope of review: The AQP1 structure determined by electron crystallography provided the first insights into the proton exclusion mechanism despite fast water permeation. Although several studies have provided clues about the mechanism based on the AQP structure, each proposed mechanism remains incomplete. The present review is focused on AQP function and structure solved by electron crystallography in an attempt to fill the gaps between the findings in the absence and presence of lipids.
Major conclusions: Many AQP structures can be superimposed regardless of the determination method. The AQP fold is preserved even under conditions lacking lipids, but the water arrangement in the channel pore differs. The differences might be explained by dipole moments formed by the two short helices in the lipid bilayer. In addition, structure analyses of double-layered two-dimensional crystals of AQP suggest an array formation and cell adhesive function.
General significance: Electron crystallography findings not only have contributed to resolve some of the water permeation mechanisms, but have also elucidated the multiple functions of AQPs in the membrane. The roles of AQPs in the brain remain obscure, but their multiple activities might be important in the regulation of brain and other biological functions. This article is part of a Special Issue entitled Aquaporins.

1. Introduction

Water channels were postulated to exist because some biological membranes are more permeable to water than can be explained by passive diffusion through a lipid bilayer. Using a *Xenopus* oocyte expression assay, Preston et al. [1] demonstrated that a 28-kDa membrane protein that is abundant in blood cells and renal proximal tubules [2] is permeable to water. The 28-kDa membrane protein was later dubbed aquaporin-1 (AQP1), and the identified homologues from many phyla, including bacteria, plant, and animal, currently number more than 300. To date, 13 water channels, AQP0 through AQP12, have been identified in humans. The AQP family is divided into two subgroups according to the permeated molecule. AQPs transport mainly water molecules, while aquaglyceroporins transport water, glycerol, urea, and small neutral molecules. The water channels have basic roles that alleviate the osmotic stress accompanied by the movement of ions though membranes in signal transduction, energy production, and other cell activities.

Despite the fast water conductance of AQPs, they are able to filter out ions as well as protons. Although the fast water movement through such a narrow space requires the water molecules to form a continuous line of hydrogen bonds (H-bonds), protons can easily permeate through such H-bonds simply by exchanging hydrogen bonding partners, as explained by the Grotthuss mechanism. How AQPs exclude proton permeation while maintaining their fast water permeability, however, has remained unclear. We proposed a H-bond isolation mechanism to explain the proton exclusion mechanism based on the first high-resolution structure analysis of AQP1 obtained by electron crystallography [3]. Our findings, however, were not consistent with models proposed based on computer simulations using structures analysed by X-ray crystallography. Therefore, many structural and mutational studies, and molecular dynamics (MD) simulations [4–8] have been performed to elucidate these issues. The latest reported structure of yeast AQP (Aqy1) in the presence of detergent at subangstrom resolution and MD simulations provide a new model for fast water transport while preventing proton conduction (see details in Section 3) [9]. It is difficult to elucidate the fast water permeation mechanism using this structure, however, because the structure represents almost the same features of the closed Aqy1 structure with very similar crystal conditions [10]. The water channels are membrane proteins whose structures should ideally be analysed in lipid membranes, similar to their inherent conditions. Electron crystallography is a very powerful method for structure analysis of

Abbreviations: 2D, two-dimensional; ar/R, aromatic/arginine; AQP, aquaporin; AQP4M1, long isoform of aquaporin-4 starting with Met1; AQP4M23, short isoform of aquaporin-4 starting with Met23; H-bond, hydrogen bond; MD, molecular dynamics; NPA, asparagine–proline–alanine

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membrane proteins in membranes, as described in the following section.

2. Electron crystallography

Henderson and Unwin [11] established the power of electron crystallography by determining the first structure of the membrane protein bacteriorhodopsin and revealing that the transmembrane region forms alpha helices. Further improvements in software and the introduction of cryo-electron microscopy allowed Henderson et al. to solve the bacteriorhodopsin structure at a resolution of 3.5 Å [12]. Application of this method to other membrane proteins provided an atomic structure of the light-harvesting complex II [13]. Although many membrane protein structures have been solved by X-ray crystallography, electron crystallography remains a very powerful tool for determining membrane protein structures for several important reasons. In particular, the advantage of electron crystallography is that the structure of a membrane protein in the lipid bilayer can be resolved under almost physiological conditions. Recent membrane protein structures determined by electron crystallography, even at medium resolution (6–10 Å), have been useful toward understanding their biological functions from a structural point of view [14–16], although higher resolution analysis is preferable.

3. Atomic structure of AQP1

As mentioned in Section 1, AQP1 was not only the first characterised water channel, but it was also the first water channel protein structure determined by electron crystallography [3]. The analysed structure revealed an unusual fold with six transmembrane helices forming a right-handed bundle, which we named the AQP fold (Fig. 1). The AQP fold comprises two tandem repeat units with a pseudo two-fold symmetry, each containing three transmembrane helices and a re-entrant loop with a half pore helix (HB or HE). The helix starts from the highly conserved asparagine–proline–alanine (NPA) motif, in which the asparagine is back bonded with the NH group of the main chain by a carbonyl group, and enters the membrane from the extracellular side or cytoplasmic side in an opposite orientation. These two repeats are connected by a long extracellular loop C on the extracellular side, and are docked to each other via an interaction between helix 2 and helix 5, as well as helical interactions by mainly proline residues of the two NPA motifs of the half-pore helices at the centre of the membrane (Fig. 1). Water channel function is strongly influenced by the stability of the interaction between helices 2 and 5, each of which has a conserved glycine residue [17]. The conserved glycine with a GxxG motif in the transmembrane helix interaction is also seen in the structures of the KcsA channel and Ca^{2+}-ATPase [18].

The AQP1 structure clearly shows a tetramer as a biological unit, and each monomer has an individual channel pore with two NPA motifs (indicated by dotted circles in Fig. 1C). Based on the structure of AQP1, we proposed that an H-bond isolation mechanism is responsible for blocking proton permeation in AQPs (Fig. 2A). In this mechanism, the water molecules in the channel are forced to orient by the electrostatic field created by the dipole moment of the two short pore helices and can smoothly form H-bonds with the amide groups of the two asparagine residues of the NPA motif by exchanging hydrogen bonding partners from adjacent water molecules, which are aligned in one line in the

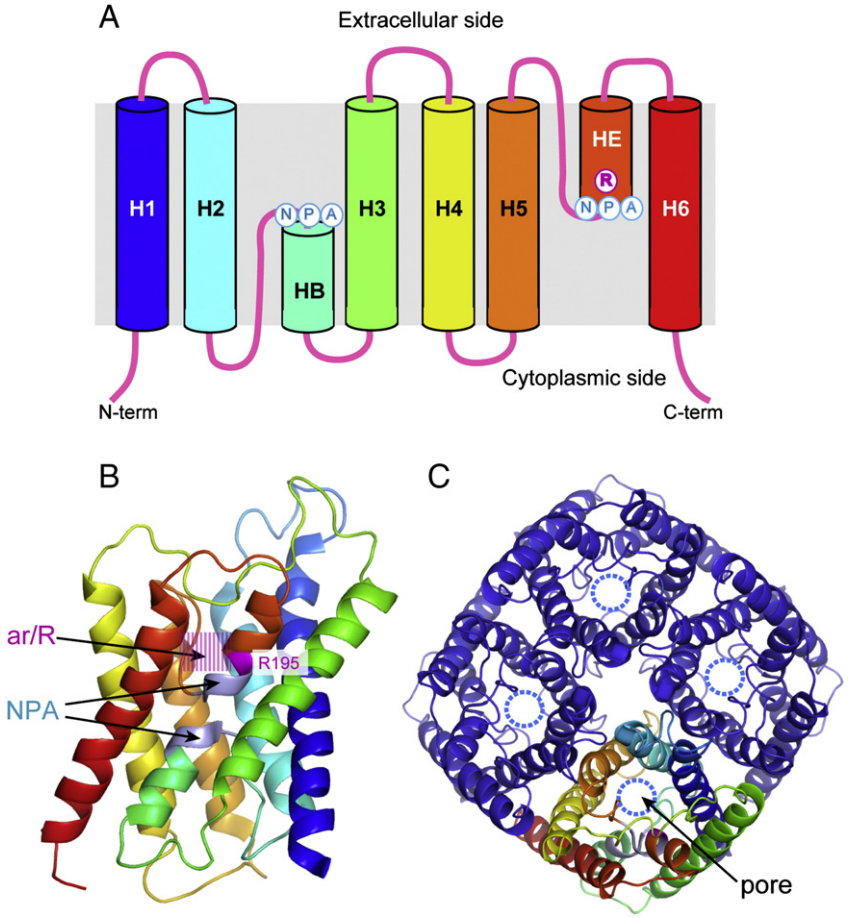

Fig. 1. Structures of AQP1 showing the typical AQP fold. (A) A secondary structure of AQP1. AQPs feature six transmembrane helices and two short pore helices, named HB and HC, which have important functions in creating the dipole moment in lipids. (B) A ribbon model of AQP1 using a rainbow colour scheme from blue (N-terminal) to red (C-terminal). The narrowest region in the AQP1 pores, previously termed ar/R [4,19], is located close to the extracellular entrance of the pore. The Arg195 and NPA motifs are shown in magenta and light blue, respectively. (C) The tetramer of AQP1 viewed from extracellular side. Each monomer has an individual pore indicated as a dotted circle.