Molecules in focus
The aquaporins. A family of water channel proteins
D.L. Connolly, C.M. Shanahan, P.L. Weissberg *
Department of Medicine, University of Cambridge, Cambridge, UK
Received 3 June 1997; accepted 23 September 1997

Abstract

The recent discovery of aquaporins, a family of highly conserved water channel proteins, which are expressed in both eukaryotes and prokaryotes, has provoked a re-evaluation of the physiology of water transport in all organisms. So far, seven distinct aquaporins have been characterised in mammals, but highly homologous family members have also been found in amphibians, insects, plants and bacteria. These transmembrane proteins serve to facilitate water transport down osmotic gradients with low activation energy. Alterations in channel expression, cellular targeting and perhaps channel permeability regulate membrane water transport. Naturally occurring and experimentally produced mutations in aquaporins cause a variety of perturbations of water homeostasis. Manipulation of aquaporin expression may have a therapeutic role in several disease processes including cardiac failure and the ascites associated with liver disease. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: Aquaporin; Water-channel; CHIP 28; AQP1

1. Introduction

Until recently, all water transport across biological membranes was thought to be via simple diffusion. However, experimentally measured rates of water transport across the membranes of red blood cells and kidney tubular cells were faster than simple diffusion would allow and predicted the existence of specific water channels (for a review, see Ref. [1]). The discovery of the first water channel, initially called CHIP 28 and now renamed aquaporin-1, occurred when Agre and colleagues in Baltimore fortuitously detected a highly abundant 28 kDa protein in red cell membranes that co-purified with, but was immunologically distinct from, the Rhesus antigen [2]. High expression of the same protein in the renal proximal tubule suggested a role in water transport. Confirmation of this function was provided by experiments in xenopus oocytes, which must survive in fresh water and are therefore highly impermeable to water, in which expression of aquaporin-1 rendered them permeable to water. Furthermore, mercuric chloride, which blocks red cell water permeability, also blocked the aquaporin-1-induced water permeability in xenopus oocytes, indicating that aquaporin-1 was the archetypal water channel [2]. Since the discovery of aquaporin-1, seven mammalian aquaporins have been characterised. In addition, over 30 other family members have been described in amphibians, insects, plants and bacteria, most of
which act as water channels, although some also act as urea and glycerol transporters (reviewed in Ref. [3]).

2. Structure

The amino acid sequence of aquaporin-1 revealed homology with an expanding group of homotetrameric proteins named the ‘MIP family’ after the first described, Major Intrinsic Protein of the lens. In this family, each subunit has six transmembrane domains in which the first three domains are homologous to the second three, suggesting an evolutionary internal duplication. Recent crystallographic studies have confirmed that these two sections are oriented at 180° to each other allowing a highly preserved asparagine–proline–alanine [NPA] motif, present in two intra-domain loops, to fold inwards and create a channel (see Fig. 1) [4, 5]. Degenerate PCR amplification across this highly conserved motif has led to the discovery of all the other identified aquaporins. All known aquaporins have a similar genomic organisation with a large first exon encoding the amino terminal half of the molecule and smaller exons 2 through 4 encoding the remainder of the protein.

3. Biological function and pathophysiology

Oocytes expressing aquaporin-1 are ten times more permeable to water than control cells. Osmotically induced water transport through these aquaporin-1 channels requires very little energy (Arrhenius activation energy of <3 kcal/mol). In experiments using aquaporin-1 deficient red cells, aquaporin-1 also contributes to diffusional water transport. Merccuric chloride inhibits aquaporin-1 water channel permeability by binding to a cysteine residue close to the NPA motif. Other aquaporins with similar cysteine groups are also mercury sensitive, but those without (e.g. aquaporin-4) are mercury insensitive. As yet, no other inhibitors of aquaporin-mediated water transport have been described. Aquaporin-1 is thought to be highly selective for water, in that

![Diagram](image_url)
voltage clamp experiments in oocytes showed no increase in conductance on exposure to an osmotic gradient. A controversial report suggesting that cAMP stimulation of aquaporin-1 resulted in ion flux \[6\] has not been reproducible by many other groups (see Ref. \[7\]).

The abundance of aquaporin-1 in red cells and kidney proximal tubules led to the prediction that aquaporin-1 mutations would result in a severe or lethal phenotype. However, a handful of kindreds world-wide have been identified who have no functional aquaporin-1 and yet have no overt phenotype \[8\], although minor abnormalities in water permeability have been identified in their osmotically stressed red cells. These findings argue strongly for redundancy of aquaporins and the extremely low incidence of aquaporin-1 deficiency in the population may have implications for fetal survival. Aquaporin-1 has been found also to be highly expressed in vascular endothelial and smooth muscle cells, suggesting a specific role in vascular function \[9\].

Aquaporin-2 is expressed exclusively in the apical membrane of the renal collecting duct \[10\] where it is stored in subapical vesicles. Stimulation of renal vasopressin V2 receptors results in cAMP-dependent translocation of aquaporin-2 to the apical membrane where it allows water to enter the cell. This water then leaves the cell via basolateral aquaporins 3 and 4 and enters the circulation (Fig. 2). This results in urinary concentration. Water restriction, or treatment with vasopressin, leads to a long term upregulation of aquaporin-2, probably via stimulation of a cAMP regulatory element in the 5′ flanking region of the aquaporin-2 gene. Mutations in the aquaporin-2 gene result in autosomal recessive nephrogenic diabetes insipidus (NDI) \[11\], whilst the commoner acquired form of NDI is caused by lithium treatment (for manic depressive disorder), hypokalaemia, low protein diets, bilateral ureteric obstruction and puromycin aminonucleoside nephrosis, all of which are associated with downregulation of aquaporin-2 expression \[12\]. Conversely, aquaporin-2 may be upregulated in cardiac failure and cirrhosis, both of which are associated with water retention.

Aquaporin-3 can also be induced by vasopressin and is found in the basolateral membrane of renal collecting duct cells where, along with aquaporin-4 which is expressed in the same cells, it may have a role in water exit from the collecting duct. Recently, a further renal aquaporin has been described, aquaporin 2L, but as yet it’s role in physiology is undetermined \[13\].

Aquaporin-4 is not upregulated by vasopressin and, unlike the other mammalian aquaporins, it has multiple isoforms which originate from a single gene \[14\]. It is expressed throughout the brain with highest expression in the supraoptic and paraventricular nuclei, consistent with a postulated role in osmoregulation \[15\]. It is also expressed in the lung and stomach were it may have a role in water secretion, and is very highly expressed in skeletal muscle indicating a potential role in contraction.

Aquaporin-5 is highly expressed in salivary and lachrymal glands, corneal epithelium and lung with postulated roles in saliva and tear production and in transpiration \[16\]. As yet, no disease associated with altered Aquaporin-5 expression has been identified, but it has been suggested that induction of Aquaporin-5 expression by ‘gene therapy’ may enhance saliva

Fig. 2. The mechanism of urinary concentration in the renal collecting duct. Vasopressin stimulation of V2 receptors leads to insertion of aquaporin-2 into the apical membrane allowing resorption of water down the osmotic gradient.
and tear production in Sjogren’s syndrome following therapeutic head and neck irradiation.

Major Intrinsic Protein has recently been shown also to be a water channel and has therefore been renamed aquaporin-0 [17]. It is highly expressed in lens fibres where it is thought to be responsible for lens translucency since deficiency or degradation of aquaporin-0 in mice results in congenital cataracts [18].

In summary, the aquaporins are a family of highly conserved transport proteins that are required to transport water rapidly across cell membranes and are expressed in a wide variety of mammalian tissues. Their evolutionary conservation implies that many will have important physiological and pathophysiological roles and that agents which modify their function may have significant therapeutic potential.

Acknowledgements

PLW holds a British Heart Foundation Chair. CMS is a BHF lecturer and DLC is the recipient of a BHF Ph.D. Fellowship.

References