REVIEW

Aquaporins in plants

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Abstract

Although very often exposed to a rapid changing environment, plants are in general unable to evade from unfavourable conditions. Therefore, a fine tuned adaptation of physiology including the water balance appears to be of crucial importance. As a consequence a relatively large number of aquaporin genes are present in plant genomes. So far aquaporins in plants were shown to be involved in root water uptake, reproduction or photosynthesis. Accordingly, plant aquaporin classification as simple water pores has changed corresponding to their molecular function into channels permeable for water, small solutes and/or gases. An adjustment of the respective physiological process could be achieved by regulation mechanisms, which range from post-translational modification, molecular trafficking to heteromerization of aquaporin isoforms. Here the function of the four plant aquaporin family subclasses with regard to substrate specificity, regulation and physiological relevance is described.

Keywords  gas transport, plant-aquaporin, water channel.

Water is the most important molecule in any kind of living cells. An efficient regulation of water supply is essential for many biological processes. Aquaporins (AQPs) are a family of small pore forming integral membrane proteins which facilitate the transport of small molecules such as water and glycerol, or volatile substances like CO₂ or NH₃ (Nakhoul et al. 1998, 2001, Biela et al. 1999, Uehlein et al. 2003). They were described in diverse archea (Kozono et al. 2003), eubacterial and eukaryotic species (Park & Saier 1996).

In biological membranes aquaporins occur as tetramers and each monomer operates as a separate water channel. The monomer is composed of a characteristic conserved arrangement of six membrane-spanning helices linked by three extra- and two intra-cellular loops. Both the N and C termini facing the cytosol (Borgnia et al. 1999a,b). In the pore region a highly conserved amino acid-motive (asparagine-proline-alanine, NPA) appearing twice forms a selective threshold. Hydrophobic regions near the NPA motives are rate-restrictive water barriers and reduce interactions between water molecules. Together with an aromatic/arginine-region, an effective proton filter, and the NPA motives the protein possess a two-stage filter.

The first aquaporin described was the mammalian AQP1. It was found in erythrocytes and renal tubuli facilitating the osmotical driven permeation of water across membranes (Denker et al. 1988, Preston & Agre 1991). It was classified into a large superfamily of intrinsic membrane proteins named major intrinsic proteins (MIP) according to the prototype from bovine lens. Because of the discovery of various aquaporin homologues in different organisms and according to their respective function, the MIP superfamily was subdivided into two major protein family clusters: the water selective channels (aquaporins, AQP) and glycerol-transporting homologues (glycerol-uptake facilitator like proteins, GLP) with varying water permeabilities (Heymann & Engel 1999).

A distinctive diversification of the protein family was obtained in vertebrates and higher plants (Fig. 1). By phylogenetic analysis, animal AQPs were classified into nine groups (AQP0-2, 4–6, 8, 11, 12). GLPs were divided in four main groups (AQP3, 7, 9, 10) (Zardoya
2005). The GLP orthologous are not abundant in plants. However, 35 different AQP genes were identified in Arabidopsis (Johanson et al. 2001) and in maize 33 MIP like isoforms were identified by transcriptome analysis (Chaumont et al. 2001). Higher plant aquaporins are subdivided into four subfamilies, the tonoplast intrinsic proteins (TIPs), nodulin 26-like intrinsic proteins (NIPs), small basic intrinsic proteins (SIPs) (Johanson & Gustavsson 2002) and plasma membrane intrinsic proteins (PIPs). In this review we focus on the properties of the four aquaporin subfamilies of higher plants (Table 1). In particular we will discuss their physiological role in planta and during different regulation mechanisms. Beside the water permeability, the gas conductivity of plant aquaporins is of rising importance and will be discussed on CO₂ and NH₃ permeability. CO₂ permeability as demonstrated for distinct aquaporins implies a function in one of the most important metabolic process in plants, the photosynthesis.

**Higher plant aquaporins**

**Tonoplast intrinsic proteins**

The plant vacuole is a cellular storage compartment with functions in turgor regulation, cell signalling and degradation. High flux rates of water and small solutes across the vacuolar membrane (tonoplast) suggested a contribution of aquaporins. In 1999 Gerbeau et al. analysed water transport rates in purified tobacco tonoplast vesicles and showed an increased permeability of urea or glycerol compared with plasma membrane vesicles. A cDNA encoding a TIP homologue (NtTIPa) was isolated and the protein was found to be localized in the tonoplast membrane. Functional expression of NtTIPa in Xenopus oocytes indicated that this protein was permeable to water, urea and glycerol. Likewise different TIP isoforms were isolated from Arabidopsis thaliana and were characterized in the oocyte system as urea permeases (Liu et al. 2003). Thus, in addition to their role as water channels, TIPs were assigned to...
be important for equilibrating urea concentrations between different cellular compartments.

**Nodulin 26-like intrinsic proteins**

The soybean nodulin 26 was the first described member of the NIP subfamily. It was found to be expressed during the formation of symbiotic nitrogen fixing root nodules (Fortin et al. 1987). NIPs are multifunctional transporters and their suggested function is to mediate the bidirectional flux of water, glycerol, NH₃, and other small solutes between plant cytoplasm and symbiotic bacteroids. Homology modelling indicated that the pore selectivity region of the nine NIP genes from *A. thaliana* are organized into two subgroups (Wallace & Roberts 2004). The NIP group I (NIP1;1, NIP1;2, NIP2;1, NIP3;1, NIP4;1, NIP4;2) is similar to nodulin 26 and the proteins function as aquaglyceroporins. Many NIP I exhibit a CDPK phosphorylation site at the carboxyl terminus (Weaver & Roberts 1992). Phosphorylation of soybean nodulin 26 for example is stimulated in response to water deficit, resulting in enhanced transport activity (Guenther et al. 2003). Interacting proteins that recognize the carboxyl-terminal region of nodulin 26 were identified, suggesting that NIP I proteins may possess a protein binding epitope that might modulate NIP function (Biswas 2004).

The NIP group II (NIP5;1, NIP6;1, NIP7;1), the aromatic Arginine selectivity filter close to the NPA pore region differs to that of NIP I and the proteins do not exhibit the conserved CDPK phosphorylation site. Functional analysis of NIP6;1 confirmed its glyceroporin function and revealed an unusually low water permeability. The amino and carboxyl terminal regions of NIP5;1, 6;1 and 7;1 share consensus phosphorylation sequences for MAP kinases. Taken together, it is evident that the NIP subfamily can be divided into two structurally and functionally distinct groups with different transport selectivity and potentially distinct regulatory properties.

**Small intrinsic proteins**

The SIP subfamily comprises not only the smallest molecules but is also the smallest family cluster in plants. The SIP family was identified by database mining and phylogenetic analysis (Johanson & Gustavsson 2002). Proteins of this subfamily are highly basic proteins. The main reason for their small size is a very short cytosolic N-terminal region compared with other plant MIPs. The SIP N-terminal region resembles that of AqpZ from *Escherichia coli*. Current publications report about localization studies with fusions of *Arabidopsis* SIPs to green fluorescent protein (GFP) expressed in suspension cultured cells (Ishikawa et al. 2005). Strong fluorescence signals from GFP–SIP fusion were detected in the ER and not in the plasma membrane or tonoplast. By heterologous expression in yeast and vesicle permeability studies, SIP1;1 and SIP1;2 from *Arabidopsis* were characterized as aquaporins, while SIP2;1 showed minor water conductance. Until now, this was the first approach in analysing the SIP physiological function and substrate specificity.

**Plasma membrane intrinsic proteins**

The plasma membrane intrinsic proteins (PIP) represent the subfamily with the largest number of members. It consists of 13 members in *Arabidopsis* and maize. As the subfamily name indicates, the majority of PIPs is localized in the plasma membrane. The PIP subfamily can be divided into two phylogenetic groups, named PIP1 and PIP2. They differ in length of N-
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C-termini and in water permeability characteristics as analysed in different heterologous expression systems. PIP2 aquaporins usually induced comparably higher water permeability than PIP1 in the same assay.

**PIP1 subfamily.** As indicated by sequence comparison, the amino acid residues at the selectivity filter were similar in PIP1 and PIP2 aquaporin isoforms. However, their permeability and cellular function seems different. Although, in different functional characterization assays PIP1 aquaporins displayed very low water permeability, a participation in plant water relations was indicated by the results of a number of experiments. Decreasing NtAQP1 transcripts in tobacco plants by means of RNA antisense expression resulted in reduced root hydraulic conductivity and lower water stress resistance (Siefritz et al. 2002). In studies analysing PIP1 antisense *Arabidopsis* plants, also a decreased hydraulic conductivity in roots and root protoplasts was observed. Moreover, in these plants the hydraulic conductance and transpiration rate recovered slower after drying and rewetting cycles. For protoplasts from tobacco or *Arabidopsis* PIP1 antisense plant root cells a lower water permeability than for protoplasts from controls was obtained, indicating for a significance in root water transport (Martre et al. 2002).

In contrast to results in planta, functional studies of heterologous expressed PIP1 isoforms in *Xenopus* oocytes or other expression systems revealed no or very low aquaporin activity (Biel et al. 1999, Dean et al. 1999, Chaumont et al. 2000). None the less, permeability for other small solutes like glycerol, urea or gases like CO\textsubscript{2} or NH\textsubscript{3} was observed. Accordingly, PIP1 aquaporins could be transporters for small solutes and/or gases, or they need to be activated in the plant in order to function as water channels. A regulation mechanism such as phosphorylation was demonstrated for different plant aquaporin isoforms and could be one of the molecular mechanisms modifying plant aquaporin activity (Johansson et al. 1998, Tornroth-Horsefield et al. 2005).

The PIP1 isoforms in tobacco and maize were localized in nearly all parts of the plant. Roots and leaves differ in morphology and physiological function, e.g. with regard to water or CO\textsubscript{2} conductance. Thus, in planta, cells more permeable for water or gas could be distributed differently and it can be speculated that the PIP1 aquaporin function is modified according to the requirements of the respective tissue or cells. In leaf cells PIP1s might act as transporters for small solutes or gases. In root plasma membranes the same protein type could display water channel activity mediated by modifications or interaction with other aquaporins (see below). Western blots with an NtAQP1 antibody led to signals of different size depending on the origin of proteins, i.e. from roots or leaves. In the former an additional band was observed besides the aquaporin-specific 28 kDa. This slightly larger signal was not abundant in leaf protein preparations and could be interpreted as a result of a post-translational modification in roots.

**PIP2 subfamily.** In many studies aquaporins of the PIP2 subfamily exhibited more efficient water channel activity than members of the PIP1 cluster, which could be due to the different molecular structure of PIP2s in comparison with PIP1 isoforms. The former have a shorter amino-terminal extension, a longer carboxy-terminal end and in an additional stretch of eight amino acids located in the first extracytosolic loop. In different heterologous expression systems PIP2 aquaporins established five to 20-fold increased water permeability compared with control values (Daniels et al. 1994, Weig et al. 1997, Johansson et al. 1998).

To date members of the PIP2 subfamily were functionally characterized in different species and a role in different physiological processes was assumed. The proteins could be involved in cellular water transport in roots, leaves (Martre et al. 2002, Lopez et al. 2003), reproductive organs (Bots et al. 2003a,b) and seed germination (Schuurmans et al. 2003). A member of the PIP2 family with an extraordinary high water channel activity when expressed in *Xenopus* oocytes was found in the leguminous Mimosa tree *Samanea saman* (Moshelion et al. 2002). Functional characterization of SsAQP2 induced up to 20-fold increased $P_t$ values compared with oocytes expressing SsAQP1 affiliated to the PIP1 family. The water permeability could be inhibited by HgCl\textsubscript{2} and additionally by millimolar concentrations of phloretin, another transport blocker (Dordas et al. 2000). In plants, SsAQP2 was found in the pulvini, which are motor organs responsible for the movement of leaves and leaflets.

Sequence comparison of maize EST clones led to the identification of a member of the PIP2 family named ZmPIP2;1 (ZmPIP2b; Chaumont et al. 2000). Transient expression in *Xenopus* oocytes increased the $P_t$ of the membranes eightfold above water-injected control cells. HgCl\textsubscript{2} reversibly inhibited its water channel activity.

**CO\textsubscript{2} permeability of aquaporins**

In general, gases such as NH\textsubscript{3} or CO\textsubscript{2} simply cross membranes by diffusion through the membrane lipids. Because some cell membranes exhibit different gas permeability, it was suggested that in the case of an increased gas transport rate, it was mediated by aquaporins (Prasad et al. 1998, Terashima & Ono 2002).
CO₂ transport capability was initially demonstrated for the human AQP1 (Nakhoul et al. 1998) and could be associated to a gas channel function. Effects of the membrane lipid composition or expression pattern of intrinsic genes that could modify oocyte CO₂ permeability were excluded (Cooper & Boron 1998). However, physiological consequences of AQP1 facilitated CO₂ transport are still a matter of debate (Cooper et al. 2002). As AQP1 knock-out mice were similar with regard to CO₂ exchange rates (Sun et al. 2001, Fang et al. 2002) a significant role of aquaporins in animal CO₂ transport was challenged. On the other hand, results obtained on human erythrocytes at low chemical CO₂ gradients, demonstrated that nearly the entire CO₂ transport across the membrane was mediated by AQP1 and the HCO₃⁻Cl⁻ transporter (Blank & Ehmk 2003).

In conclusion it was hypothesized that some aquaporins may function as high affinity CO₂ transporters in the erythrocyte membrane. Taken together, the situation in animals appears rather controversy because diverse tissues, cells and membranes were analysed and different experimental setups were applied.

Physiological studies in plants provided evidences for relevance of an aquaporin mediated CO₂ transport. Tobacco plants with an increased or decreased intrinsic aquaporin expression were changed in attributes towards water transport as well as CO₂ dependent processes like photosynthesis (Siefritz et al. 2002). When Vicia faba or Phaseolous vulgaris leaf discs were treated with minimum concentrations of HgCl₂, the hydraulic permeability of the plasma membrane was decreased by 70–80%. In a similar matter, photosynthetic CO₂ fixation and conductance of CO₂ from the intercellular spaces to the chloroplast stroma were restricted by mercury treatment. Although, the application of heavy metals should be considered with the same carefullness as in experiments investigating water conductivity, it was assumed that the photosynthetic CO₂ uptake across the plasma membrane of the mesophyll cells was facilitated by HgCl₂ sensitive aquaporins (Terashima & Ono 2002). Under favourable growth conditions Arabidopsis AtPIP1;2 (AtPIP1b) overexpressing tobacco plants revealed significant increased transpiration rate, higher stomatal density and a greater photosynthetic efficiency (Aharon et al. 2003). Nevertheless, the authors did not relate the effects to an increase in CO₂ transport rate, but to facilitated water transport.

Functional characterization of tobacco NtAQP1 in Xenopus oocytes indicated for a high conductance to CO₂ (Uehlein et al. 2003). The oocytes were injected with NtAQP1 cRNA and carbonic anhydrase, which accelerates the conversion of CO₂ to HCO₃⁻. In this experimental set up a decrease of intra-cellular pH indicates the transport of CO₂ into the oocyte. CO₂ membrane transport is rate limiting for HCO₃⁻ accumulation rather than the conversion reaction to HCO₃⁻. For NtAQP1 it was found that CO₂ uptake was 45% higher compared with control oocytes injected with water.

CO₂ transport was suggested for PIP2 aquaporins, too. The barley aquaporin HvPIP2;1 was overexpressed in rice in order to examine if members of the PIP2 subfamily contribute to facilitated CO₂ transport (Hanba et al. 2004). The internal conductance for CO₂ diffusion (gᵢ) and CO₂ assimilation rate was determined on intact leaves by concurrent measurements of gas exchange and carbon isotope ratio. It was found that gᵢ strongly related to rate of HvPIP2;1 expression and the results were interpreted in a way that HvPIP2;1 has a role in CO₂ diffusion in rice leaves. However, it remained to be determined whether the correlation between aquaporin expression and CO₂ permeability increase was just a side-effect or causative to HvPIP2;1 expression.

NH₃ permeability of aquaporins

Besides the debate about CO₂ conductivity there is also a lively discussion on ammonia (NH₃) and ammonium (NH₄⁺) permeability of aquaporins and recent studies led to novel insights to this issue. Plant ammonium uptake at low extracellular concentration is catalysed by members of the ammonium transporter/methylammonium permease (AMT/Mep) family (Ninnemann et al. 1994), but there is also evidence from inhibitor studies in plants in favour of an NH₃ permeability by aquaporins (Niemetz & Tyerman 2000). Using functional complementation of yeast ammonium transport mutant (Dmep1–3), three wheat (Triticum aestivum) TIP2 aquaporins were characterized, which complement the effect of the deletion mutations on growth medium with reduced ammonium supply (2 mM). When expressed in oocytes an additional conductivity for the NH₄⁺ analogues methylammonium and formamide was registered. Homology modelling of the TIP2 combined with data from site directed mutagenesis and electrically measurements suggested that NH₃ enters the pore, is protonated and released as NH₄⁺ (Jahn et al. 2004). The specific TIP2 seems to fulfil the requirements for the predicted low affinity NH₄⁺ transporter. In a recent study the mammalian aquaporins AQP8, AQP9, AQP3, AQP1 and the plant aquaporin TaTIP2;1 (see above) were expressed in Xenopus oocytes to analyse the transport of NH₃ and NH₄⁺ in a solution-exchange chamber or voltage clamp conditions (Holm et al. 2005). In order to investigate NH₃ conductivity, the aquaporin expressing oocytes were placed in a well stirred bathing medium with low buffer capacity. NH₃ transport into the oocytes was accompanied by an
acidiﬁcation of the bathing solution. By employment of this technique AQPs, AQP9, AQP3 and TaTIP2;1 could be identiﬁed as ammonia transporters. NH$_4^+$ transport was measured via voltage clamp techniques and conﬁrmed NH$_4^+$ conductance for AQPs, AQP9, AQP3 and TaTIP2;1. Physiological relevance was suggested for the mammalian aquaporins. AQP8 can be found in mitochondria of hepatocytes (Ferri et al. 2003), suggesting involvement in NH$_4^+$ uptake to supply the urea cycle. In hepatocyte plasma membrane AQP9 may play a role in NH$_4^+$ transport from the blood into the perportal hepatocyte. AQP3 is proposed to be involved in acid secretion by NH$_4^+$ across the collecting duct epithelium in the kidney. The suggested functions were supported by phenotypes of knock out mice, but still have to be further conﬁrmed to assess a function of aquaporin mediated NH$_4^+$ transport in animal physiology. A physiological function of the plant aquaporin TaTIP2;1 has not been circumstantiated to date.

**Regulation of aquaporins**

On view to the high diversity of plant aquaporins several regulation mechanisms could be conceivable. Post-transcriptional modiﬁcation like phosphorylation was shown for AtTIP3;1 (ζ-TIP) from *Arabidopsis* (Maurel et al. 1995) and the soybean nodulin 26 as mentioned above. In recent studies the crystal structure of the tetrameric protein of *Spinacia oleracea* PIP2;1 [PM28A, (Johansson et al. 1996)] could be obtained (Kukulski et al. 2005). The 3D structure of the tetramer suggests a novel mechanism for the regulation of distinct aquaporins. In oocyte expression assays PIP2;1 was shown to be activated by phosphorylation at two serine residues. Moreover, phosphorylation of one of these residues by a plasma membrane-associated Ca$^{2+}$-dependent protein kinase was demonstrated in response to a high water potential gradient (Johansson et al. 1998). Besides the suggested phosphorylation at several serine residues a highly conserved cystein (Cys) at the C terminus of loop A is proposed to be involved in regulation of the channel. Together with three Cys residues at the N-terminal part of helix 2, the conserved Cys may stabilize the SoPIP2;1 monomer by hydrogen bonds or complexing a metal ion initiating an opening or closure of the protein.

The plasma membrane permeability appears also to be inﬂuenced by divalent cations and the pH (Gerbeau et al. 2002). Cell hydraulic conductivity ($L_p$) was measured on *Arabidopsis* suspension cells using a cell pressure probe and varying bathing solutions. Ca$^{2+}$ added to the pipette and bathing solution reduced $L_p$ fourfold. The results were conﬁrmed on puriﬁed plasma membrane vesicles and stopped ﬂow spectrophotometer measurements. Furthermore H$^+$ was shown to reversible decrease water channel activity. This led to the assumption that divalent cations and the pH inﬂuences directly membrane permeability and may allow coupling of water transport to cell signalling and metabolism.

Intra-cellular acidiﬁcation through anoxic stress led to a decrease in water permeability of root cell membranes in *Arabidopsis* (Tournaire-Roux et al. 2003). In experiments expressing *Arabidopsis* aquaporins in Xenopus oocytes a drop of intra-cellular pH resulted in a diminishment of water conductance implicating a closure of aquaporins by protons. A histidin (His) residue at position 197 in Loop D of AtPIP2;2 was identiﬁed to be the major pH-sensing site under physiological conditions (Tournaire-Roux et al. 2003, Chaumont et al. 2005). In a structural model of AtPIP2;2 with protonated His$^{197}$, Loop D is folded over the pore and caused the closure of the protein.

Molecular trafﬁcking is also an optional regulation mechanism of aquaporins. The regulation and redistribution of a TIP (McTIP1;2) from *Mesembryanthemum crystallinum* (ice plant) was investigated by sucrose density gradient separation and immunofluorescence microscopy (Vera-Estrella et al. 2004). Osmotic stress induces a delocalization of the protein from the tonoplast to other membrane structures and could be blocked by inhibitors of vesicle trafﬁcking-related processes. The early effects of osmotic stress on aquaporin expression and regulation were further analysed by exposure of *Arabidopsis* roots to salt (Boursiac et al. 2005). A decrease of AQP mRNA could be recognized after 2–4 h after application of salt. The protein abundance decrease was different within the aquaporin subfamilies. The amount of PIP1 protein decreased within 30 min, whereas members of PIP2 and TIP subfamily showed reduction after 6 h of treatment. Results from fusion with GFP indicated for relocation of TIP and PIP into intra-cellular spherical structures after 45 min and 2 h, respectively. The traﬃcking of the mammalian AQP2 is well ascertainment in apical membranes of kidney collecting duct epithelia. AQP2 containing vesicles were routed to the membrane in response to a hormonal stimulus (Brown 2003).

Above and beyond the regulation by mechanisms mentioned above, a multimerization of membrane proteins can regulate their activity and function (Veenhoff et al. 2002). Coexpression of maize ZmPIP1;2 (PIP1) and different PIP2 isoforms (ZmPIP2;1/2;3/2;4/2;5) in Xenopus oocytes resulted in an increase in $P_t$ compared with individual expression of the PIP2 isoform (Fetter et al. 2004). The authors assume, that the increased $P_t$ indicates that heteromerization of PIP1 and PIP2 isoforms is required for both to act as functional water channels. Heteromerization was also demonstrated for *Mimosa pudica* aquaporins PIP1;1 and PIP2;1 (Temmei et al. 2005).
References


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