Plant Physiol. (1997) 115: 561–567

Characterization of Water Channels in Wheat Root Membrane Vesicles[1]

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The functional significance of water channels in wheat (*Triticum aestivum* L.) root membranes was assessed using light scattering to measure vesicle shrinking in response to osmotic gradients rapidly imposed in a stopped flow apparatus. Vesicles were obtained from both a plasma membrane fraction and a plasma membrane-depleted endomembrane fraction including tonoplast vesicles. Osmotic water permeability (P_{os}) in the endomembrane fraction was high (P_{os} = 86.0 $\mu m\ s^{-1}$) with a low activation energy (E_A = 23.32 kJ mol^{-1} ± 3.88 SE), and was inhibited by mercurials (K_i = 40 μM $HgCl_2$, where K_i is the inhibition constant for half-maximal inhibition), suggesting participation of water channels. A high ratio of osmotic to diffusional permeability (P_d) (using D_2O as a tracer, P_{os}/P_d = 7 ± 0.5 SE) also supported this view. For the endomembrane fraction there was a marked decrease in P_{os} with increasing osmotic gradient that was not observed in the plasma membrane fraction. Osmotic water permeability in the plasma membrane fraction was lower (P_{os} = 12.5 $\mu m\ s^{-1}$) with a high activation energy (E_A = 48.07 kJ mol^{-1} ± 3.63 SE) and no mercury inhibition. Nevertheless, P_{os}/P_d was found to be substantially higher than one (P_{os} = 3 ± 0.2 SE), indicating that water channels mediated water flow in this fraction, too. Possible distortion of the P_{os}/P_d value by unstirred layer effects was shown to be unlikely.

Evidence has emerged that water channels that play an important role in water homeostasis in animal systems have their molecular counterpart in plants (Chrispeels and Maurel, 1994). Proteins with sequence homologies to animal aquaporins have been cloned and shown to increase water permeability when expressed in *Xenopus* oocytes (Maurel et al., 1997). However, until now there have been few data available for plants on the functional significance of water channels in the native membrane. In studies using giant algal cells parameters were found that suggested the presence of water channels. Osmotic water permeability is found to be higher than would be expected for a lipid pathway (Dainty and Ginzburg, 1964; Steudle and Zimmermann, 1974; Steudle and Tyerman, 1983) and water flow is sensitive to mercurials (Wayne and Tazawa, 1990; Henzler and Steudle, 1995; Schütz and Tyerman, 1997).

In some studies on higher plants there are indications that water channels could be functionally significant (Zhang and Tyerman, 1991; Maggio and Joly, 1995; Carvajal et al., 1996; Zhang and Jones, 1996). Zhang and Tyerman (1991) showed that a rapid decrease in L_p of single cortical cells in wheat (*Triticum aestivum* L.) roots occurs when the roots are subject to anoxia. They suggested that plasmodesmata may have been responsible, but subsequent experiments (Zhang and Jones, 1996; Cleland et al., 1994) indicated that this may not be the case. To investigate whether water channels were present in wheat root cells we studied water movement in vesicular systems in which the plasma membrane can be separated from the endomembranes of the cell, in particular the tonoplast, which represents the second cellular membrane likely to be involved in osmoregulation. This overcomes the problem with whole-cell studies such as those using the pressure probe (e.g. Zhang and Tyerman, 1991), in which it is not certain which components, i.e. plasma membrane, tonoplast, or plasmodesmata, are responsible for the large changes in overall cell L_p.

To investigate the role of water channels in their natural membrane, but in an experimentally simple system, we isolated membrane vesicles from wheat roots and measured osmotically induced changes in vesicle volume using light scattering (van Heeswijk and van Os, 1986; Solomon, 1989). This provided a measure of P_{os} that could then be tested for sensitivity to temperature and to $HgCl_2$. In addition, we could measure a P_d using a fluorochrome sensitive to D_2O (Kuwahara and Verkman, 1988; Ye and Verkman, 1989). Therefore, for the first time to our knowledge in higher plants, it was possible to test all of the indicators for the presence of water channels: a high water P_{os}, a low-E_A, and a ratio of P_{os}/P_d greater than unity. Inhibition by mercurials is another typical feature of water channels in animal systems and in some water channels from plants expressed in *Xenopus* oocytes (Maurel et al., 1993).

MATERIALS AND METHODS

Vesicle Isolation

Wheat (*Triticum aestivum* L., var 11/7; kindly provided by D. Reeves, Turretfield Research Station, Rosedale, Australia) grains were germinated on plastic grids over 1 mM $CaCl_2$ and grown under a 12-h light/dark cycle for 6 d. Eighty grams of roots was excised and macerated in a blender three times for 30 s each in about 150 mL of ice-cold isolation medium (Giannini et al., 1987) with KCl reduced to 150 mM

[1] This project was supported financially by the Australian Research Council.

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Abbreviations: ANTS, aminonaphthalenetrisulfonic acid; E_A, activation energy; K_i, constant for half-maximal inhibition; L_p, hydraulic conductivity; P_d, diffusional permeability; P_{os}, osmotic permeability; V/A, volume to surface area ratio.

and 5 μg/mL leupeptin additionally supplied. The extract was filtered through four layers of Miracloth (Calbiochem), adjusted to 200 mL, and centrifuged for 10 min at 13,000*g* to remove the bulk of the mitochondria. Subsequently, the supernatant was centrifuged for 90 min at 100,000*g* to obtain a microsomal pellet. A typical extraction of 80 g of wheat roots yielded about 70 mg of microsomal protein.

Microsomes were resuspended in 0.33 M Suc, 5 mM KCl, and 5 mM KH_2PO_4/K_2HPO_4 buffer, pH 7.8 (resuspension medium) and partitioned as described by Larsson et al. (1994) in a 6.2% Dextran T500/6.2% PEG 3350 phase system (two tubes of a 36-g phase system). After three partitioning steps the membranes in the upper (UIII) and lower (LIII) phases were each diluted to 100 mL with resuspension medium, and the membranes were pelleted for 60 min at 100,000*g*. After resuspension in a small volume of resuspension medium, they were stored for up to 6 weeks at −70°C before being used.

Light-Scattering Experiments to Determine P_{os}

Light-scattering experiments were conducted by simultaneously injecting vesicles (UIII, 0.2–0.5 mg protein/mL; LIII, 0.5–2 mg protein/mL) suspended in Iso370 (330 mM Suc, 5 mM Hepes/KOH, pH 7.0, 100 μM $CaCl_2$, at 370 mOsmol) and hyperosmotic solutions (0.2 M extra Suc in Iso370 to create an inwardly directed 100 mosmol Suc gradient) in a stop-flow fluorimeter (DX.17MV, Applied Photophysics, Leatherhead, UK). Apart from the experiments to determine the E_A, all experiments were conducted at 22°C. The time course of vesicle shrinking was followed as an increase in light scattering at 500 nm (downward deflection of the fluorimeter trace). When the isosmolar solution was injected, there was no time-dependent change in light scattering, indicating that other artifacts that can occur in stopped flow were not present (Solomon, 1989). The amplitude of the light-scattering relaxation was proportional to the osmotic gradient and was steady in time after the initial water efflux (not shown). This indicates that the vesicles were osmotically competent, and that the solutes used did not permeate rapidly across the membrane. A single exponential function was fitted to the relaxation curves for vesicle shrinking, and k was used to describe water permeability (Worman and Field, 1985; van Heeswijk and van Os, 1986; Meyer and Verkman, 1987; Jansson and Illsley, 1993). The relaxations of at least four injections (injection volume of approximately 150 μL each) were fitted, and the average of the obtained k was used in the calculations of P_{os} and P_d. The osmotic water permeability was calculated according to the following equation (van Heeswijk and van Os, 1986):

$$P_{os} = \frac{V}{A} \cdot \frac{k_{os}}{\bar{V}_w \cdot C_o} \quad (1)$$

where V/A is measured as one-third times the radius of the spherical vesicles (see below), $\bar{V}_w$ is the partial molar volume of water, and C_o is the external osmolarity.

Determination of P_{os}/P_d

For the experiments to determine P_{os}/P_d, vesicles were loaded with 10 mM ANTS by mixing equal volumes of vesicles in resuspension medium with 20 mM ANTS in resuspension medium and storing the samples overnight in the refrigerator. Vesicles were then pelleted for 20 min at 100,000*g* and resuspended in Iso370 to a protein concentration of about 400 μg/mL. Enhanced ANTS fluorescence in the presence of D_2O (Kuwahara and Verkman, 1988; Ye and Verkman, 1989) was recorded by injecting the ANTS-loaded vesicles in Iso370 simultaneously with Iso370 containing 75% D_2O. With excitation at 380 nm, fluorescence was measured at >420 nm (GG 420 cut-on filter, Schott, Applied Photophysics, Leatherhead, UK). The same batch of ANTS-loaded vesicles was used to measure light scattering at 500 nm, due to shrinking to obtain P_{os}. ANTS-loading of vesicles and the partial replacement of H_2O by D_2O had no significant effect on the observed rate constant for vesicle shrinking (not shown).

The ratio P_{os}/P_d can be obtained without knowing V/A of the vesicles (Ye and Verkman, 1989), since the rate constants for light scattering (volume changes) and changes in fluorescence due to D_2O diffusion are both directly proportional to V/A. Therefore, P_{os}/P_d can be obtained from the following equation:

$$\frac{P_{os}}{P_d} = \frac{k_{os}}{k_d \cdot C_o \cdot \bar{V}_w} \quad (2)$$

where k_d is the rate constant for the change in the fluorescence signal due to diffusion of D_2O into the vesicles.

V/A of Vesicles

Membrane aliquots in resuspension medium (UIII and LIII, 1 μg of membrane protein/mL) were fixed and stained as described in Johansson et al. (1995). Transmission electron micrographs were used to determine vesicle diameters. Freeze-fracture of comparable membrane preparations confirmed the observed size distributions.

RESULTS

Origin of Vesicles

The distribution of marker enzymes (Table I) was used to establish the composition of the fractions obtained from phase partitioning. UIII was markedly enriched in the plasma membrane marker glucan synthase II (Kauss and Jeblick, 1987). The specific activity of this enzyme in the upper phase was nine times higher than the activity in the corresponding lower phase. UIII had also been depleted of other cellular membranes; inorganic pyrophosphatase for tonoplast (Rea and Poole, 1985) and Cyt *c* oxidase for mitochondria (Widell and Sommarin, 1991). Electron micrographs of this fraction (not shown) revealed a homogeneous population of vesicles with an average diameter of 104 nm (± 3.2 nm SD, n = 348). We concluded that this fraction contained mainly plasma membrane vesicles and was minimally contaminated by other cellular membranes.

Table I. *Distribution of protein and marker enzyme activity for plasma membrane (glucan synthase II) and tonoplast (inorganic pyrophosphatase) in the membranes recovered after three partitioning steps (UIII [upper] and LIII [lower])*

Eighty grams of wheat roots yielded about 70 mg of protein, which was partitioned into two 36-g phase systems.

Component	UIII	LIII
	mg/tube phase system	
Protein	1.8 (8.4%)	19.6 (91.6%)
	μmol h^{-1} mg protein^{-1}	
Glucan synthase II	5.6	0.62
relative activity	9	1
Inorganic pyrophosphatase	2	7
relative activity	1	3.5
Cyt c oxidase	1.9	21
relative activity	1	11

Apart from the bulk of the mitochondria that got sedimented in the first 13,000*g* spin, the second fraction contained the cellular endomembranes (Larsson et al., 1994). We measured tonoplast-bound enzyme activity (inorganic pyrophosphatase; Rea and Poole, 1985) and traces of mitochondrial activity (Cyt *c* oxidase; Widell and Sommarin, 1991). Electron micrographs revealed reasonably homogeneous vesicles with a mean diameter of 132 nm (± 7.9 nm SD, n = 63) and some membrane sheets (probably ER). For the purpose of the investigation, we have assumed that osmotically responsive vesicles were most likely of tonoplast origin. Participation of other endomembranes in the light-scattering signal (fragmented mitochondria, the ER, and Golgi vesicles) cannot be discounted completely. Cross-contamination between the two fractions (UIII and LIII) was definitely low.

Light-Scattering Experiments (Vesicle Shrinking)

Figure 1, a and b, shows the time course of vesicle shrinking for UIII and LIII vesicles that were subjected to a 100 mOsmol osmotic gradient. The traces were fitted with single exponential curves to obtain k for vesicle shrinking. At 22°C k for UIII were 6.1 s^{-1} (± 0.4 s^{-1} SE, n = six separate experiments of at least four replicate injections) and for LIII were 33.1 s^{-1} (± 4.2 s^{-1} SE, n = four experiments of at least five replicate injections). Similar rate constants were obtained when Suc as extra osmoticum was replaced with isosmolar sugar alcohols (mannitol and sorbitol) or inorganic salts (KCl and NaCl). This gave P_{os} values of 12.5 μm s^{-1} for plasma membrane (UIII) and of 86.1 μm s^{-1} for tonoplast (LIII) vesicles.

To test whether our vesicle populations behaved as perfect osmometers, UIII and LIII vesicles were exposed to increasing osmotic gradients. In both cases the amplitude increased linearly with the gradient (results not shown). The rate of shrinking was differently affected by the increasing gradient size (Fig. 2). In plasma membrane vesicles P_{os} was independent of the osmotic gradient between 100 and 600 mOsmol, but LIII vesicles showed a sharp decline in P_{os} when the gradient was increased from 100 to 200 mOsmol.

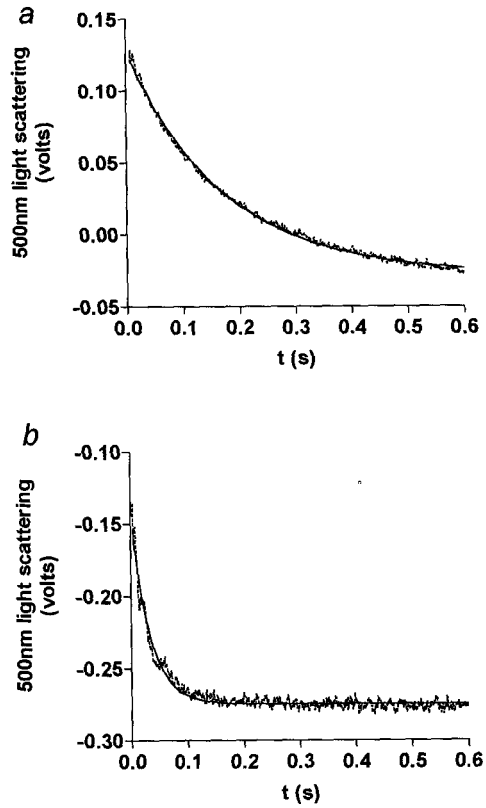

Figure 1. Time course of 500-nm light scattering for wheat root vesicles. Vesicles in Iso370 were simultaneously injected with Iso370 containing an additional 200 mM Suc to create a final 100 mOsmol osmotic gradient. To obtain a rate constant the curves were fitted with single exponentials. *a*, UIII, 200 μg protein/mL; *b*, LIII, 600 μg protein/mL.

The E_A for water flow in both the upper plasma membrane-enriched phase and the lower endomembrane phase, containing the bulk of the tonoplast, was obtained by measuring water efflux at different temperatures. From the Arrhenius plots (e.g. Fig. 3) the E_A were 48.07 kJ mol^{-1} (± 3.63 SE, n = 5) for plasma membrane vesicles, and 23.32

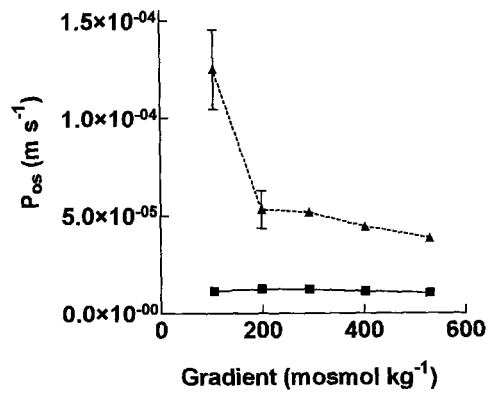

Figure 2. Effect of increasing osmotic gradient on P_{os} in UIII (■) and LIII (▲) vesicles. Vesicles in Iso370 were injected with Iso370 with extra Suc added to create the depicted osmotic gradient.

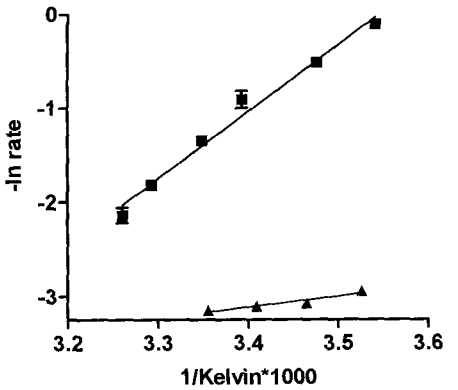

Figure 3. Temperature dependence of vesicle shrinking. Experimental conditions were as described in the legend to Figure 1. Shrinking rates were obtained for a temperature range from 10 to 34°C for UIII (■) and from 10 to 25°C for LIII (▲).

kJ mol^{-1} (± 3.88 SE, n = 5) for the tonoplast-containing lower phase.

Another feature of water channels is their sensitivity to mercury compounds (Preston et al., 1993; Zhang et al., 1993). For our experiments involving $HgCl_2$, Suc as extra osmoticum was replaced by KCl. This was necessary because vesicles mixed with hyperosmotic Suc solutions containing $HgCl_2$ displayed abnormal shrinking behavior (not shown). Compared with the control minus $HgCl_2$ the amplitude of shrinking with $HgCl_2$ increased dramatically, and the relaxation kinetics could only be well fitted to the sum of two exponentials. Whether this was due to an additional effect of $HgCl_2$ on other transport systems or was caused by some artifact changing light-scattering properties of the vesicles (e.g. vesicle agglutination) could not be resolved. When Suc as extra osmoticum was replaced by 100 mM KCl, vesicles behaved as expected and displayed shrinking amplitudes responsive to the applied gradient and unaffected by the additional presence of 400 μM $HgCl_2$ (Fig. 4a). Vesicles in Iso370 were simultaneously injected with Iso370 plus 100 mM KCl (control) or with Iso370 plus 100 mM KCl plus 800 μM $HgCl_2$ ($HgCl_2$ run). The presence of $HgCl_2$ had no effect on the k in UIII vesicles, but reduced the k in LIII vesicles (Fig. 4b). Figure 5, a and b, shows the concentration dependence of mercury inhibition of water flow in the lower, endomembrane phase. Half-maximal inhibition was reached at 40 μM $HgCl_2$ and a maximal rate reduction of 70% for water flow in LIII vesicles could be inferred from the Lineweaver-Burk plot (Fig. 5b).

ANTS Fluorescence Experiments

To measure the P_d, vesicles were loaded with the fluorescent dye ANTS, the fluorescence of which is enhanced in D_2O compared with H_2O (Kuwahara and Verkman, 1988; Ye and Verkman, 1989). The ANTS-loaded vesicles in Iso370 were then injected with Iso370 in which 75% of the water had been replaced by D_2O. The time course of D_2O diffusion into the vesicle could be followed as a change in the fluorescence signal (excitation, 380 nm; emission >420 nm) (Fig. 6, a and b).

P_d (ANTS fluorescence, Fig. 6a) and P_{os} (light scattering at 500 nm, Fig. 6b) were measured on the same batch of vesicles by testing ANTS-loaded vesicles in their response to mixing with isosmotic solution containing 75% D_2O, and then submitting the vesicles to shrinking by injecting them together with the hyperosmotic solution (100 mOsmol gradient). The observed ratio of P_{os}/P_d was 3 (± 0.2, n = 4) for plasma membrane vesicles and 7 (± 0.5, n = 3) for lower-phase vesicles containing tonoplast.

Effect of Unstirred Layers on P_{os}/P_d

An unstirred layer of solution adhering to the surface of the vesicles, when flow is stopped after mixing in the stopped-flow apparatus, will impede the diffusion of both D_2O and osmoticum to the membrane surface. This will result in an underestimation of k for the subsequent relaxations in fluorescence or light scattering. Where unstirred layer effects have been estimated they change P_d measurements more so than P_{os} (Dainty, 1963; Barry and Diamond, 1984; Solomon, 1989), resulting in an overestimated P_{os}/P_d.

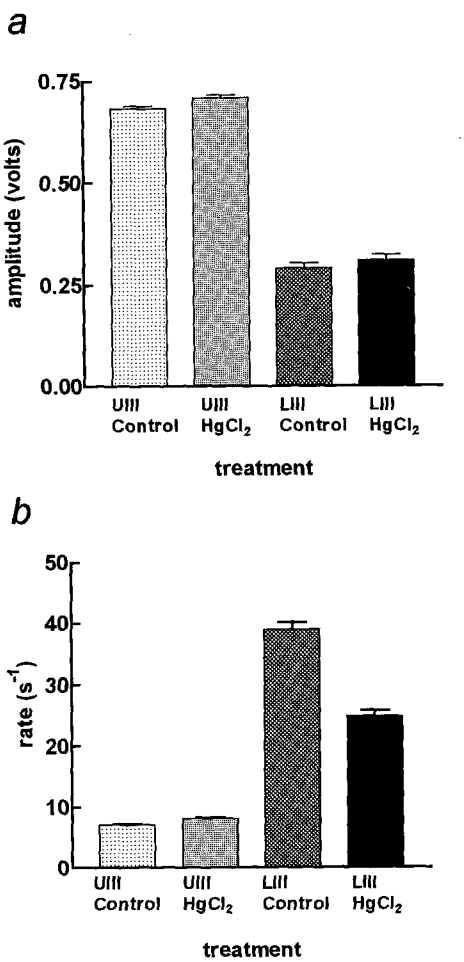

Figure 4. Effect of $HgCl_2$ on vesicle shrinking. Experimental conditions were as described in the legend to Figure 1, but Suc as extra osmoticum was replaced by 100 mM KCl and 800 μM $HgCl_2$ was added to the hyperosmotic solution. *a*, Effect of $HgCl_2$ on shrinking amplitude; *b*, effect of $HgCl_2$ on the rate constant.

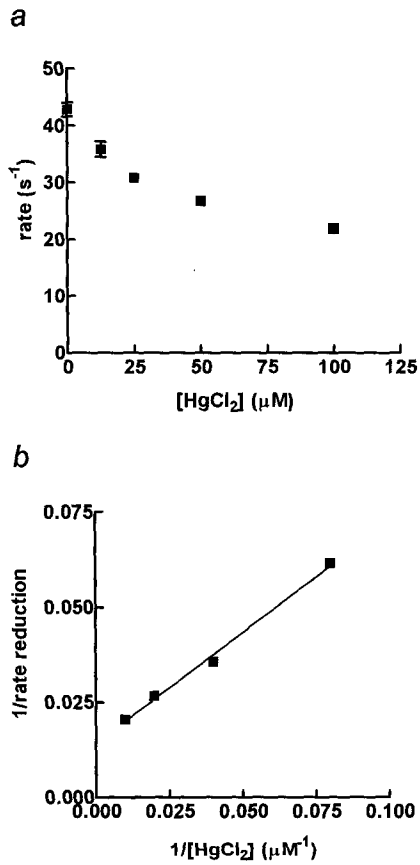

Figure 5. Concentration dependence of $HgCl_2$ inhibition of vesicle shrinking in LIII vesicles. Experimental conditions were as described in the legend to Figure 4. $HgCl_2$ was added to the hyperosmotic KCl solution. *a*, Shrinking rate versus $HgCl_2$ concentration; *b*, Lineweaver-Burk plot of data from *a* yielded K_i=41 μM $HgCl_2$ and a maximal rate reduction of 70%.

Estimates have been made of unstirred layer thicknesses for red blood cells in rapid mixing devices with a high Reynold's number (in our instrument, Re = 15,000), taking into account hydrodynamic properties (Rice, 1980; Vandegriff and Olson, 1984), and these agree with measured values (Sháafi et al., 1967; Williams and Kutchai, 1986). A theoretical estimate by Williams and Kutchai (1986) of unstirred layer thickness is equal to the radius of the cell or vesicle. For a sphere of the same surface area of a red cell this is 3.3 μm. This compares to experimentally determined values of 4.7 to 6.9 μm for red cells in a stopped-flow experiment (Williams and Kutchai, 1986). During stopped flow the situation is complicated by unstirred layer thickness changing during the experiment. The unstirred layer thickness is initially small during mixing (turbulent flow), then increases when flow is stopped (Rice, 1980). Using an upper-conservative estimate of unstirred layer thickness (δ) as 10 times the diameter of the vesicles, we calculated the underestimate in the values of P_d (using $Pd_{obs}^{-1} = Pd_{true}^{-1} + \delta/D$, where diffusion coefficient $D = 2.272 \times 10^{-5}$ cm^2 s^{-1} for D_2O at 25°C [Kohn, 1964]) at less than 1%. Even with an unstirred layer thickness of 10 μm (100 × the vesicle diameter), P_d for the endomembrane vesicles is underestimated by only 5%.

DISCUSSION

For years water flow through the plant body has intrigued ecophysiologists and plant biophysicists (Steudle, 1989). Their in-depth studies of this complex system have, however, been hampered by the fact that the contribution of individual cell compartments could not be resolved. In the present study we have used two vesicle fractions: the first enriched in plasma membrane and the second containing endomembranes, including the tonoplast, to differentially describe the water permeability of these distinctive membranes and shed light on the physiological function of the (biochemically and genetically) established presence of water channels in both of these membranes.

A comparison of the time course of vesicle shrinking revealed a much faster rate for the tonoplast-containing endomembrane fraction. For a 100 mOsmol gradient P_{os} was more than seven times higher in this fraction than the corresponding value for the plasma membrane vesicles. Plasma membrane and endomembranes also showed a different response of P_{os} to the size of the imposed osmotic

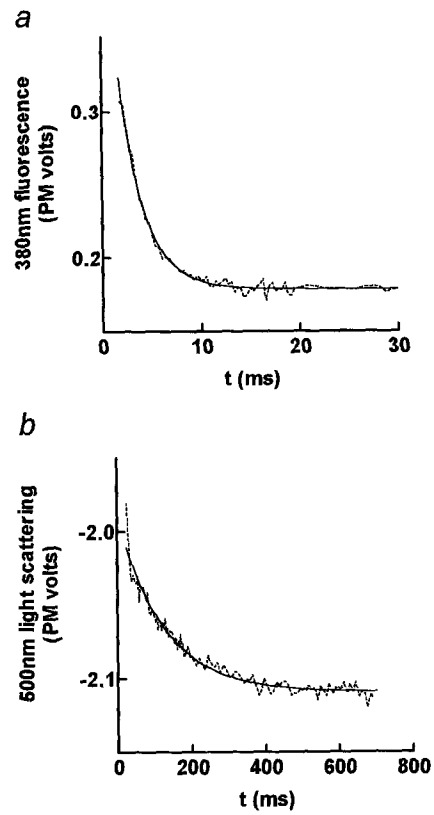

Figure 6. Determination of P_{os}/P_d. *a*, ANTS-loaded UIII vesicles were mixed with ISO370, where 75% of the water had been replaced with D_2O. D_2O diffusion into the vesicle resulted in a decrease in ANTS fluorescence. Single-exponential fits supplied the rate constant for D_2O diffusion. *b*, ANTS-loaded UIII vesicles were injected together with hyperosmotic Suc, as described in the legend to Figure 1. Identical experiments were performed with LIII vesicles (data not shown).

gradient. Whereas P_{os} for the plasma membrane was constant over the range of osmolarities tested, P_{os} for tonoplast-containing endomembranes dropped sharply for an increase in osmotic gradient from 100 to 200 mOsmol. Such a drop in P_{os} (or L_p) with increasing osmotic gradient has been observed in charophytes (Dainty and Ginzburg, 1964; Kiyosawa and Tazawa, 1972; Steudle and Tyerman, 1983) and was previously interpreted as a "dehydration effect" on membrane pores (Dainty and Ginzburg, 1964). In animal systems both dependence of P_{os} on osmotic gradient (Illsley and Verkman, 1986; Verkman et al., 1989; Jansson and Illsley, 1993) and independence thereof (Meyer and Verkman, 1986; Verkman and Masur, 1988) have been described. The decline in P_{os} that we observed in the tonoplast-enriched lower phase might well be a physiological closing-down response of water channels to a presumed water deficit.

The temperature dependence of vesicle shrinking (Fig. 3) also revealed large differences between the two fractions. The E_A for water flow in the upper, plasma membrane-enriched fraction was high (48.07 kJ mol^{-1}) and reminiscent of water flow through pure lipid vesicles with no protein mediation (Ye and Verkman, 1989). In contrast, the E_A for the tonoplast-enriched lower phase was only 23.32 kJ mol^{-1}, a value in line with energy requirements in red blood cells and other systems known for a high concentration of water channels (Ye and Verkman, 1989).

Another well-known feature from animal systems is the high sensitivity of water channels toward mercury compounds. Only vesicles from the lower, endomembrane-containing phase were inhibited by $HgCl_2$. Here mercury sensitivity was high with a K_I for mercury of about 40 μM, and a maximal rate reduction of about 70%. Such a (theoretical) reduction would in fact bring the rate down to the value of plasma membrane vesicles, again reinforcing the picture of physiologically active water channels most likely residing in the tonoplast and mainly lipid permeability-limited water flow at the plasma membrane. These data support the previous finding that tonoplast water channels are mercury sensitive (Maurel et al., 1993), whereas some plasma membrane equivalents lack the mercury-sensitive site (Daniels et al., 1994).

The experiments discussed so far have dealt with water flow under hypertonic conditions, when the vesicles were exposed to an osmotic gradient. Another set of experiments was performed under isotonic conditions. Here vesicles loaded with the fluorescent dye ANTS were injected together with an isotonic solution containing 75% D_2O. Diffusion of D_2O into the vesicles (used as a tracer for H_2O diffusion under isotonic conditions) greatly enhanced ANTS fluorescence and enabled us to use the change in ANTS fluorescence as an indicator for water diffusion rates. In combination with rates of light scattering when vesicles from the same batch of ANTS-loaded vesicles were submitted to osmotically induced shrinking, a ratio of P_{os}/P_d could be obtained. This ratio again characterizes the pathway of water flow through a membrane. If a porous pathway for water flow is present, P_{os}/P_d (bulk water flow in response to an osmotic gradient compared with water movement purely by diffusion under isotonic conditions) is likely to be larger than unity. A given ratio between P_{os} and P_d characterizes the predominant water pathway. A ratio close to unity is typical for independent diffusional flow in phospholipid vesicles, whereas systems containing water channels show higher ratios (Ye and Verkman, 1989). As it turned out, both plasma membrane vesicles and tonoplast-containing endomembrane vesicles had a ratio of P_{os}/P_d greater than 1. The value of 7 found for endomembrane vesicles was of the same magnitude as has been observed for red blood cells, which are densely studded with water channels.

The two membrane fractions studied turned out to be distinctively different in their properties. Water permeability of the endomembrane fraction is high, with the water channels likely to reside in the tonoplast, and evidently water channel-mediated. This invites speculation on the role of the vacuole as a compartment with a high and fast osmotic buffering capacity, maintaining cytoplasmic homeostasis within the cell. The situation in the plasma membrane is less clear-cut. Whereas the observed lower P_{os}, the high E_A, and the lack of mercury inhibition seem to suggest a lack of water channel participation in water flow, the high ratio of P_{os}/P_d does suggest some involvement. The value of 3 that was obtained for the plasma membrane agrees well with the results of Zhang and Jones (1996), who found a value of 2.9 for wheat root cells using NMR to measure P_d on protoplasts and using the pressure probe to measure P_{os} on intact cells. However, our value of P_{os} for plasma membrane (12.5 μm s^{-1}) is smaller than that measured in intact wheat root cells (86 μm s^{-1}) by Zhang and Tyerman (1991). The fact that the pressure-probe measurement of P_{os} in intact cells is the same as that for the tonoplast-containing endomembrane fraction measured here is probably coincidental, since, although the pressure probe is probably located in the vacuole, it is difficult to envisage how pressure relaxations can occur in a turgid cell without the involvement of the plasma membrane.

The P_{os} value we measured for plasma membrane is more similar to that measured under anaerobic stress by Zhang and Tyerman (1991), suggesting that under the conditions used for isolation and measurement of our plasma membrane vesicles the water channels may have closed. Whereas water uptake through the root cells is a necessary and desirable process, water loss by the reverse process is decidedly unfavorable. The osmotic gradients imposed on the predominantly outside-out plasma membrane vesicles in the upper phase enforces an analogous water loss. It is tempting to suggest that under such osmotic conditions water channels close down. Further experiments are warranted to reconcile the presence of water channels in the plasma membranes with their apparent lack of function.

It is also tempting to speculate on the role of water channels in water partitioning in the root. An increase in water permeability in localized regions would result in increased water supply to that particular area. Developmental processes such as cell growth in elongation zones would be selectively promoted. Conversely, closure of water channels could retard water flow in a particular area of the root, for example an area of the root with reduced ion selectivity due to an hypoxic microenvironment (Thomson et al., 1989).

Having established that water channels are present and active in our membrane preparations, further research will now be aimed at the regulation mechanisms that govern them.

ACKNOWLEDGMENTS

The authors wish to thank Geoff Findlay for helpful suggestions and Dianne Trussell for expert technical assistance.

Received April 2, 1997; accepted June 27, 1997.

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