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Kinetics of Water Transport in Eel Intestinal Vesicles

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Abstract. Brush border membrane vesicles, BBMV, from eel intestinal cells or kidney proximal tubule cells were prepared in a low osmolarity cellobiose buffer. The osmotic water permeability coefficient P_f for eel vesicles was not affected by pCMBS and was measured at 1.6×10^{-3} cm sec^{-1} at 23°C, a value lower than 3.6×10^{-3} cm sec^{-1} exhibited by the kidney vesicles and similar to published values for lipid bilayers. An activation energy E_a of 14.7 Kcal mol^{-1} for water transport was obtained for eel intestine, contrasting with 4.8 Kcal mol^{-1} determined for rabbit kidney proximal tubule vesicles using the same method of analysis. The high value of E_a, as well as the low P_f for the eel intestine is compatible with the absence of water channels in these membrane vesicles and is consistent with the view that water permeates by dissolution and diffusion in the membrane. Further, the initial transient observed in the osmotic response of kidney vesicles, which is presumed to reflect the inhibition of water channels by membrane stress, could not be observed in the eel intestinal vesicles. The P_f dependence on the tonicity of the osmotic shock, described for kidney vesicles and related to the dissipation of pressure and stress at low tonicity shocks, was not seen with eel vesicles. These results indicate that the membranes from two volume transporter epithelia have different mechanisms of water permeation. Presumably the functional water channels observed in kidney vesicles are not present in eel intestine vesicles. The elastic modulus of the membrane was estimated by analysis of swelling kinetics of eel vesicles following hypotonic shock. The value obtained, 0.79×10^{-3} N cm^{-1}, compares favorably with the corresponding value, 0.87×10^{-3} N cm^{-1}, estimated from measurements at osmotic equilibrium.

Key words: Eel intestinal vesicles — Osmotic permeability — Water transport

Introduction

The European eel (*Anguilla anguilla*) is a migratory, euryhaline teleost that faces widely different salinities during its life-cycle. While freshwater teleosts drink little and urinate large volumes to compensate for osmotic water uptake through the gills, the marine teleost ingests seawater and absorbs water and monovalent ions from the intestine. Together with active excretion of salt from gills and other organs, this system helps compensate for water loss from the body (Smith, 1930). Like renal processes in terrestrial vertebrates, this intestinal transport is crucial for maintenance of osmotic homeostasis in this teleost (Evans, 1979).

Salt absorption is accomplished primarily through a transcellular Na-K-2Cl cotransport system present in the mucosal barrier (Musch et al., 1982; Musch, O'Grady & Field, 1990). Although the coupling of salt and water flow has been demonstrated in the isolated sea water eel intestine in vitro, the main route for water reabsorption (transcellular or paracellular) has not been established (Oide & Utida, 1967; Utida et al., 1972; Ando, 1975; Ando, 1985). The issue has not been addressed in this tissue because of limitations of the experimental methods employed. These include (i) the use of an intestinal sac where the net water flux was measured gravimetrically (Wilson & Wiseman, 1954), (ii) monitoring the water level inside a small capillary tube connected to one side of the Ussing chamber (Wiedner, 1976; Van Os, Wied-

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ner & Wright, 1979; Eldrup et al., 1982) and (iii) measurements of net water flux in a perfusion system where the net water flux is calculated from the ratio of the concentrations of reference substances (Bunce & Spraggs, 1982; Ando, Sasaki & Huang, 1986). Measurements by these methods refer to the composite parallel flow of para- and transcellular processes. In other volume transporter epithelia like the mammalian kidney proximal tubule and small intestine, and the *Necturus* gallbladder and intestine, the contributions of the two parallel pathways, transjunctional and transcellular, to the total water flow has also been controversial (Hill, 1980; Berry, 1983; Verkman, 1989; Hill & Shachar-Hill, 1993; Loeschke & Bentzel, 1994; Hill & Shachar-Hill, 1997).

Isolated vesicles prepared from apical or basolateral membranes have been used in mammalian tissue to address other problems as well as to help separate para- and transcellular properties (Worman & Field, 1985; Van Heeswijk & Van Os, 1986; Verkman, 1989). For example, the issue of water channels was approached by measurements of osmotic water permeability coefficient (P_f) and activation energy (E_a) in both kidney and intestinal brush border membrane vesicles (BBMV). A high E_a, typical for the lipid bilayers, was found (9 to 17 Kcal mol^{-1}) in BBMV from rat intestine (Worman & Field, 1985). This value contrasts with 4 Kcal mol^{-1} for red blood cells (Farmer & Macey, 1970) or 4.6 Kcal mol^{-1} for BBMV from kidney proximal tubule (Soveral, Macey & Moura, 1997), systems where the water channel, aquaporin-1 (AQP1), was identified (Preston & Agre, 1991; Nielsen et al., 1993).

In this study we have isolated brush border membrane vesicles from eel intestine and rabbit kidney proximal tubule (both volume transporting epithelia) and examined the osmotic water flow by stopped-flow nephelometry. Using an analysis that incorporates both osmotic and hydrostatic pressure, the osmotic permeability coefficient and activation energy were calculated. The values obtained suggest that despite the physiological importance of volume flow in the eel intestine, water permeation through the transcellular path is not enhanced by water channels.

Materials and Methods

Materials and methods have been described in detail in earlier papers (Soveral et al., 1997; Alves et al., 1999). Brush border membrane vesicles were prepared from eel intestinal or kidney proximal tubule cells and relative purity of membrane preparation was assayed by measuring specific activity of enzyme markers. Vesicle size of all the membrane preparations was determined by quasi-elastic light scatter (QELS) using a particle sizer (BI-90, Brookhaven Instruments).

Stopped-flow experiments were performed on a HI-TECH Scientific PQ/SF-53 stopped-flow apparatus, which has a 2 msec dead time (mixing time less than dead time). Three runs were usually stored and analyzed in each experimental condition. In each run, 0.1 ml of vesicle suspension (0.4 mg protein/ml in 18 mosM or 113 cellobiose buffer) was mixed with an equal amount of the same buffer made hyperosmotic by the addition of cellobiose in order to make hypertonic shocks of different tonicities. The time course of 90° scattered light intensity at 400 nm was followed for different time intervals. The device was thermostated at 23°C unless otherwise specified. For the pCMBS experiments eel vesicles prepared in 113 mosM buffer were incubated for 30 min at room temperature with 10 mM pCMBS and subjected to the same hyperosmotic shocks as the control vesicles.

The calibration of the light scatter signals (I) into vesicular volumes (v) and the determination of the osmotic permeability coefficient (P_f) were done in a similar way to the one already described (Soveral et al., 1997; Alves et al., 1999, companion paper) using the linear relation between v and the reciprocal of the output $1/I$.

Results

Osmotic Permeability Coefficients

The osmotic water flow J_v out of a vesicle whose volume is V and surface area is A is proportional to the sum of the osmotic ($-\Delta\Pi$) and hydrostatic (ΔP) driving forces. Letting P_f denote the osmotic permeability coefficient and $\overline{V}_w$ the partial molar volume of water, we have

$$J_v = -\frac{dV}{A\,dt} = P_f\,\overline{V}_w\left(\frac{\Delta P - \Delta\Pi}{RT}\right) \tag{1}$$

where RT is the gas constant times the absolute temperature.

Consider the osmotic shock experiment where vesicles, prepared in a medium with osmolarity $(osm_{out})_o$ are suddenly exposed to a new medium with osmolarity $(osm_{out})_\infty$. Convenient, dimensionless variables can be obtained by utilizing the initial volume V_o and $(osm_{out})_o$ as normalizing factors, and defining:

$$\left.\begin{aligned} v &= V/V_0 \\ \Lambda &= (osm_{out})_\infty/(osm_{out})_o \\ p &= \Delta P/(RT(osm_{out})_o) \end{aligned}\right\} \tag{2}$$

Then, it follows from Eq. (1) and (2) and Soveral et al., (1997), that

$$\frac{dv}{dt} = P_f\frac{A}{V_o}\,\overline{V}_w\,(osm_{out})_o\left(\frac{(1+p_o)}{v} - \Lambda - p\right) \tag{3}$$

where p_o is the initial hydrostatic pressure difference. Defining k as the area compressibility elastic modulus, the dependence of p on vesicle radius (volume) is described in Alves et al., (1999) and can be rearranged as

$$\begin{aligned} p &= \frac{4k}{r_o(osm_{out})_o}\left(\left(\frac{1}{v_{\min}}\right)^{1/3} - \left(\frac{1}{v}\right)^{1/3}\right) && v > v_{\min} \\ p &= 0 && v \le v_{\min} \end{aligned} \tag{4}$$

where r_o is the vesicle radius when $v = 1$, and v_{min} is the volume where p vanishes.

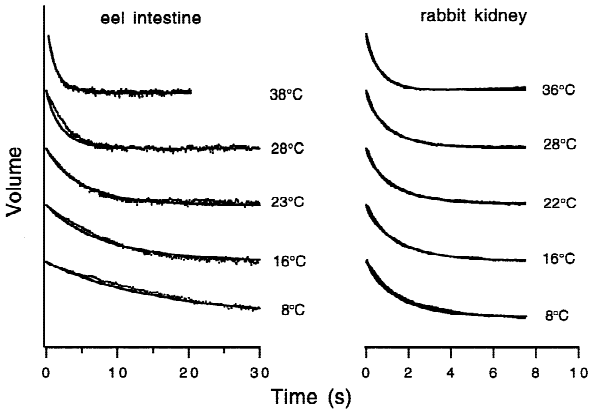

Fig. 1. Time dependence of volume changes with temperature for eel and kidney BBMV prepared in 18 mosM cellobiose buffer and subjected to the same osmotic shock ($\Lambda = 2.4$). For all traces the initial volume v is equal to 1 and they are all offset by 0.2 v from each other. The time response of the kidney vesicles is much faster (note differences in time scale) and has much less variation with temperature than the eel vesicles. This reflects a higher permeability and lower activation energy in the kidney.

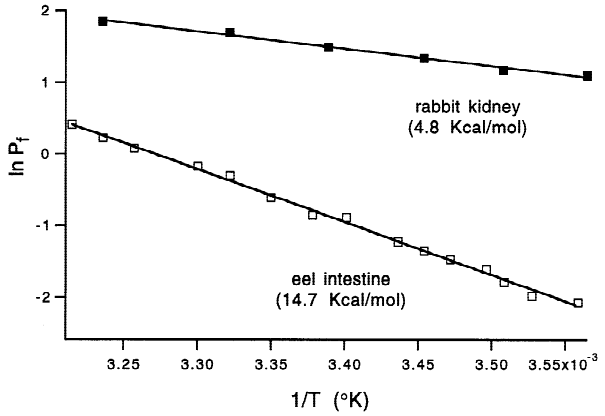

Fig. 2. Activation energy (E_a) determination of water transport. BBMV prepared from eel intestine or rabbit kidney cortex were equilibrated at different temperatures and were subjected to an osmotic shock at a $\Lambda = 2.4$. The E_a values were obtained from the slope of the lines and were 14.7 and 4.8 Kcal mol^{-1}, respectively.

With the exception of P_f, all of the constants in Eqs. (3) and (4) were determined from equilibrium data assuming a spherical vesicle (Alves et al., 1999). This allows P_f to be estimated by numerically integrating Eq. (3) and curve fitting the results to empirical data obtained by stopped flow light scattering. Both numerical integration as well as curve fitting was accomplished with the Madonna computer program (http://www.kagi.com/authors/madonna).

An example of time-dependent volume changes and P_f evaluation at different temperatures for eel and kidney vesicles for the same osmotic shock ($\Lambda = 2.4$) is shown in Fig. 1. The osmotic water permeability coefficient P_f obtained for the two vesicles populations was lower for eel than for kidney. The average P_f (± SD) for 18 mosM eel intestinal vesicles was $1.6 \times 10^{-3} \pm 0.2$ cm sec^{-1} at 23°C, while the same coefficient for kidney proximal tubule brush border membrane vesicles was $3.6 \times 10^{-3} \pm 0.3$ cm sec^{-1}, similar to the one published (Soveral et al., 1997).

The activation energy for water transport was estimated from an Arrhenius plot for both vesicles populations and is presented on Fig. 2. The slopes of the plot yields a value for the E_a of 14.7 Kcal mol^{-1} for the eel intestinal BBMV, compatible with the absence of water channels in these membrane vesicles. For rat small intestine where water permeation is thought to be through the lipid matrix, the values reported were 13.3 Kcal mol^{-1} (Van Heeswijk & Van Os, 1986) and 9.75 to 17.2 Kcal mol^{-1} (Worman & Field, 1985).

For rabbit renal BBMV, the value obtained was 4.8 Kcal mol^{-1}, a value close to the one already published (Soveral et al., 1997). This figure can also be compared with the E_a reported for red blood cells (4 to 5 Kcal mol^{-1}), where there is ample evidence for water channels (Macey, 1984), and with the E_a for diffusion and bulk flow of water in water (Wang, Robinson & Edelman, 1953; Farmer & Macey, 1970).

Experiments illustrated in Fig. 1 and 2 take place in hypertonic media where the kinetics of vesicle shrinkage is followed. In these cases the elastic stress on the membrane is short lived; internal pressures are dissipated within the first 20 to 300 msec so that the elastic properties of the vesicle play a minor role. Just the opposite is true in hypotonic media. Here the elastic stress continues throughout the experiment becoming larger as the vesicle swells. The consequences are not easily followed in kidney vesicles because kinetics of their small volume changes in hypotonic media could not be measured. On the other hand, eel vesicles have a lower elastic modulus producing a larger range of measurable volume changes. This provides us with an opportunity to assess the membrane's elastic properties solely from its kinetic response in hypotonic media. Figure 3 shows results of a hypotonic shock experiment ($\Lambda = 0.73$ for 113 mosM vesicles) which was used to extract the parameters, k and P_f by curve fitting the data to numerical integrations of Eqs. (3) and (4). The estimated values were $k = 0.79 \times 10^{-3}$ N cm^{-1} and $P_f = 2.8 \times 10^{-3}$ cm sec^{-1}. The value for k agrees very well with the value of $k = 0.87 \times 10^{-3}$ N cm^{-1} obtained from measurements of osmotic equilibria (Alves et al., 1999) and the value of P_f is similar to the one obtained by hypertonic shocks for the same vesicle preparation ($2.3 \times 10^{-3} \pm 0.2$ cm sec^{-1}).

As reported in Soveral et al. 1997 a P_f inhibition of 52% was found for kidney vesicles incubated for 30 min with 10 mM pCMBS. Under the same conditions, no inhibition was found for the eel vesicles prepared in 113 mosM buffer.

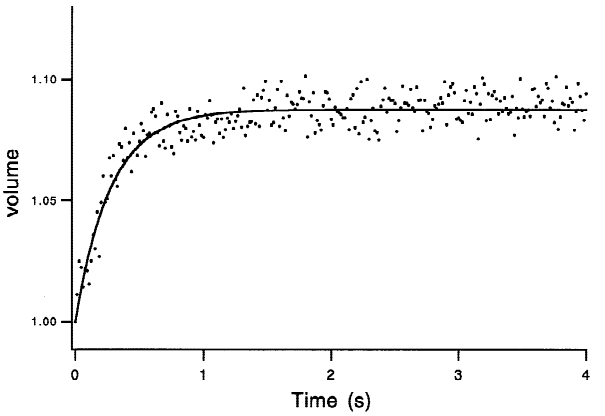

Fig. 3. Fit of a calibrated volume signal from an hypotonic shock ($\Lambda = 0.73$) of intestinal eel BBMV prepared in 113 mosM buffer. The fit was done according to Eqs. (3) and (4), using the value of $v_{min} = 0.94$ (Alves et al., 1999). The estimated values were $k = 0.79 \times 10^{-3}$ cm^{-1} N and $P_f = 2.8 \times 10^{-3}$ cm sec^{-1}.

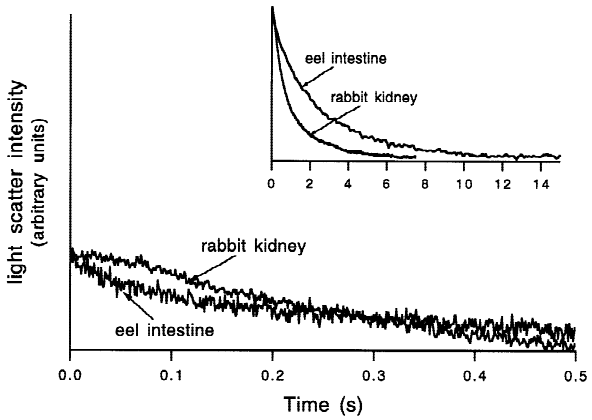

Fig. 4. Osmotic shock traces ($\Lambda = 2.4$) acquired for 0.5 sec. When the scattered light intensity signals from the two different vesicles are compared, a difference in the initial portion of the trace (about 0.3 sec) can be observed. The insert shows the light scatter behavior of the same vesicles for a longer time scale.

Discussion

Brush border membrane vesicles from eel intestine and rabbit kidney proximal tubule were prepared in low osmolarity buffer medium and were subjected to different hyperosmotic shocks. The initial volume measured by QELS was similar for both eel and kidney vesicles preparation. The average value for $P_f = 1.6 \times 10^{-3}$ cm sec^{-1} agrees with the value 1.2×10^{-3} cm sec^{-1} obtained by Worman & Field (1985) in brush border membrane vesicles taken from the rat small intestine, although it is considerably smaller than the 6×10^{-3} cm sec^{-1} reported by Van Heeswijk & Van Os (1986) for the same rat tissue. Values for water permeability of eel intestine epithelium range from 1.5 to 4.7×10^{-3} cm sec^{-1} (House & Green, 1965; Kristensen & Skadhauge, 1974; Skadhauge, 1974). However, water permeability studies on eel intestines appear to be confined to perfusion experiments. These measure the permeability of the entire tissue which consists of multiple pathways, some in parallel, some in series. Difficulties in assessing the morphological amplification factor, the size of the unstirred layer, and in separating paracellular, serosal, and mucosal pathways compromise these measurements of water permeability.

Several lines of evidence support the notion that there are no specific functional water channels in these membranes. First, the activation energies calculated (14.7 Kcal mol^{-1} for eel intestine and 4.8 Kcal mol^{-1} for rabbit kidney) strongly suggests two different mechanisms for water permeation. Second, the value of P_f is easily within the range of permeabilities found in lipid bilayers. For example a P_f near 2×10^{-3} cm sec^{-1} has been reported for lecithin cholesterol bilayers (Haydon, 1969) as well as red blood cells where the water channels have been fully inhibited by pCMBS (Macey, Karan & Farmer, 1972). Third, the pCMBS inhibition was only seen for the kidney vesicles (Soveral et al., 1997) while no inhibition was found in the eel preparations. Further, evidence comes from detailed inspection of the initial transients in the osmotic response. For the renal vesicles, an initial shrinkage delay, or lag time, corresponding to a very small change in initial volume was related to the existence of a hydrostatic pressure difference across the membrane that maintains the membrane under stress. This stress seems to be the direct cause of the inhibition of water channels (Soveral et al., 1997). In eel intestinal vesicles this lag time is not visible (Fig. 4), a result that is predicted by the absence of water channels. While water channels facilitate bulk flow in kidney proximal tubule cells (Preston & Agre, 1991), the eel intestine brush border, like its mammalian counterpart (Worman & Field, 1985; Van Heeswijk & Van Os, 1986), appears to transport water by diffusion through the lipid bilayer.

Our kidney vesicle preparations proved to contain aquaporin1 (Soveral et al., 1997). On the other hand, we were not able to test for aquaporins in our eel vesicle preparations because aquaporin antibodies are isoform specific. Immunoblotting eel vesicles with the available mammalian antibodies would not be effective.

The dependence of P_f on tonicity of the osmotic shock shown in Fig. 5 provides related evidence that channels are present in kidney vesicles, but absent (or inactive) from eel. In kidney vesicles, low tonicity shocks produce lower apparent P_fs than higher tonicities because with the smaller shrinkages taking place in the lower tonicity range, the internal pressure takes longer to dissipate. Consequently, the channel remains inhibited for a longer period during the low tonicity shocks result-

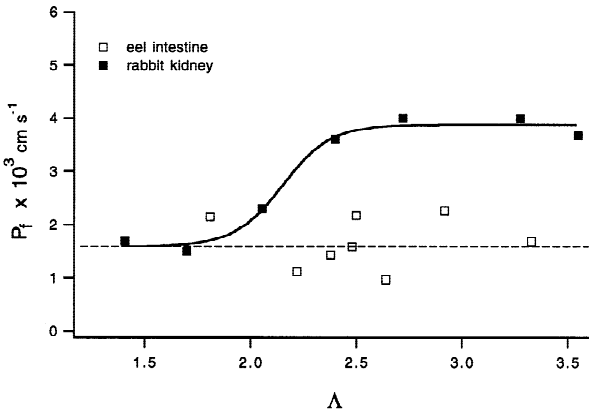

Fig. 5. Dependence of the osmotic permeability coefficient (P_f) on tonicity of the osmotic shock (Λ) for both eel and kidney vesicles prepared at 18 mosM buffer.

ing in a lower observed P_f. In eel vesicles, no such dependence of P_f on shock tonicity is seen.

In spite of the common volume transporting functions of kidney proximal tubule and intestinal epithelia, the P_f for the mucosal membranes are different and reflect different mechanisms of permeation, channels and bilayer. Inclusion of water channels within the intestinal mucosa does not appear to confer any substantial physiological advantage. Unstirred layers are notoriously large within the lumen of the intestine, and the advantages conferred by water channels would probably be severely compromised by the resistance encountered in traversing the layer. Although we are not aware of any report of its thickness in the eel, most estimates in the mammalian intestine range between 100 to 500 μm (Thomson & Dietschy, 1984). This translates into an equivalent permeability of 2.0×10^{-4} to 1.0×10^{-3} cm sec^{-1}. The permeability of the composite, P_{comp}, unstirred layer plus mucosal membrane is given by

$$P_{comp} = P_f P_u/(P_f + P_u) \quad (5)$$

where P_u is the unstirred layer permeability. Using our best estimate of $P_f = 1.6 \times 10^{-3}$ cm sec^{-1} together with $P_u = 2 \times 10^{-4}$ cm sec^{-1}, gives a $P_{comp} = 1.8 \times 10^{-4}$ cm sec^{-1}. If an infinite number of water channels were incorporated into the mucosal membrane making P_f infinite, then P_{comp} would simply increase by 10% from 1.8×10^{-4} to 2.0×10^{-4} cm sec^{-1}, and then $P_{comp} = P_u$. Taking the upper figure for $P_u = 1.0 \times 10^{-3}$ cm sec^{-1}, we find the maximal effect of incorporating water channels would be to make P_{comp} rise from 0.62×10^{-3} to 1.0×10^{-3} cm sec^{-1}. In this case there is some advantage to water channels, but it is still quite small. Further, it is likely that the lower metabolism of the cold-blooded eel is supported effectively by a more sluggish intestinal tract so that it would not be surprising to find larger unstirred layers in this species.

Water permeation through the intestine is significantly limited by transport through the unstirred layer. The same argument does not apply to kidney tubules because their luminal diameter is much smaller, about 25 μm (Gottschalk & Mylle, 1956) which would make the unstirred layer less than 13 μm corresponding to an equivalent permeability of the order of 10^{-2} cm sec^{-1}. This is an order of magnitude greater than bilayer permeability so that, in this case, increasing the mucosal permeability by insertion of channels would substantially enhance water absorption.

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