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Movement of Water in Squid Axon and Nitella Internode

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INTRODUCTION

It has been widely assumed that the cell surface structure limiting the rate of solute flux (i.e., the plasmalemma) is the same as that limiting the rate of pressure-driven solvent (water)flux. With a few exceptions (e.g., red blood cells) the surface of cells is a composite structure consisting of the plasmalemma and a variety of encapsulating materials. Until recently the contribution to pressure-coupled water flow made by the structures adjacent to the plasmalemma has not been subjected to a systematic experimental attack. A theoretical analysis of water flow in composite membranes has been undertaken by Kedem and Katchalsky (1). They pointed out that accumulation or depletion of solutes (i.e., osmotic gradients) may occur between the component elements of the composite structure. They also noted that simple additivity rules do not generally hold. My attempts at analyzing water flow through biological composite membranes are primarily experimental (2-5). I chose for this study the giant axon of Loligo vulgaris and the internode of Nitella flexilis. The surface of both these cells is a composite membrane. In the axon the surface consists of the plasmalemma, the Schwann layer and varying amounts of connective tissue, while in the internode it consists of the plasmalemma and the cell wall. The main reason for choosing these two cells is that the hydrostatic and osmotic pressure of the interior of the cell can be altered and maintained constant and water flow can be measured easily. My approach to the analysis of water flow in these cells was as follows:

When the solutes inside and outside of the cell are completely impermeable and the barrier to pressure coupled water flow is a structure in series to the plasmalemma one may expect the following: (a) that the overall (across the composite membrane) filtration coefficient determined by applying hydrostatic pressure gradients between the inside and the outside of the cell may not be affected by selective destruction of the plasmalemma; (b) that the filtration coefficient determined by applying osmotic pressure gradients between the bulk aqueous phases inside and outside the cell need not be

equivalent to that determined by applying hydrostatic even though the solutes involved are impermeable; (c) that the intra-extracellular hydrostatic pressure difference (ΔP) required to sustain zero net water flow need not be equivalent to the osmotic pressure difference ($\Delta\Pi_{th}$) applied and maintained between the bulk internal and bulk external phases. My experiments pertaining to the squid axon have been published (2-4) while those pertaining to the internode of nitella have been submitted for publication (5).

METHODS

For the determination of both the hydrostatic and osmotic filtration coefficients in both the nitella internode and the squid axon I used the intracellular perfusion technique (2,5). Periodic flushing out of the internal perfusate in conjunction with vigorous stirring of both the internal (i.e., of the perfusion channel) and the large external aqueous compartments assured that $\Delta\Pi_{th}$ could be maintained constant even after prolonged water flow across the surface of the cell. When the osmotic filtration coefficient was determined, ΔP was maintained constant and close to zero; conversely, when the hydrostatic filtration coefficient was determined $\Delta\Pi_{th}$ was maintained constant and close to zero. This situation where $\Delta\Pi_{th}$ is close to zero, although "physiological" for the squid axon, is not so for the nitella internode which normally is highly turgored (around 7 atmospheres).

Two methods were used in the axon for the determination of the ΔP required to sustain zero net water flow at various values of $\Delta\Pi_{th}$. In one method (2) the axon was perfused internally with aqueous solutions of impermeable solutes (KF or sucrose) of varying osmotic pressure. In the other method (4) the axon was bored out, perfused briefly with isosmotic KF and finally perfused with oil. From the volume of the aqueous moiety of the oil-filled axoplasmic tube juxtaposed to the plasmalemma, I estimated $\Delta\Pi_{th}$ for various values of ΔP. In this estimation I assumed that the mobile solutes (primarily KF) in the annular axoplasm were completely impermeable.

In the axon (2,4) destruction of the plasmalemma was effected by 2-4% glutaraldehyde fixation for 3 to 7 hours or by immersing the axon for days or months in isosmotic KF. The criteria of destruction of the plasmalemma were the following: (a) The osmotic filtration coefficient was reduced to immeasurably low values. (b) The rate of efflux of intracellularly injected glucose C-14, sucrose C-14, inulin C-14, and Na_{22} was increased by 2-3 orders of magnitude. The efflux rates of these tracers in the treated axon was what would have been expected had the encapsulating sheath been the only barrier. (c) The electrical properties (resistance, capacitance and potential) became those expected of the sheath alone. (d) The internal pH followed exactly the external; in normal axons the internal pH, within limits, is independent of

the external. In the nitella (5) plasmalemmal destruction was effected by flushing out the contents of the internode thereby isolating the cell wall. Some cell walls were washed with absolute ethanol and acetone to minimize contamination by intracellular materials.

RESULTS

My more relevant measurements of the filtration coefficients in both the squid axon and the nitella internode both before and after destruction of the plasmalemma are summarized in the table below. For more details cf. references 2 and 5.

Table 1

Hydrostatic (L_P) and Osmotic (L_{PD}) Filtration Coefficients*

Cell type	L_P(cm/sec/atm) x 10^5		L_{PD}(cm/sec/atm) x 10^5	
	Plasmalemma intact	Plasmalemma destroyed	Plasmalemma intact	Plasmalemma destroyed
Giant axon of Loligo vulgaris	8.1-13.9 (15)	6.1-17.2 (13)	.037-.051 (10)	0 (8)
Internode of Nitella flexilis	14.1-19.2 (4)	13.2-19.9 (8)	a. 1.55-2.0 (6)** b. 1.61-2.11 c. 1.79-2.32	0 (5)

*L_{PD} denotes water flow in cc/sec/cm^2 of surface area/atmosphere applied osmotic pressure difference between the internal perfusion channel and the outside bulk fluid phase. L_P denotes water flow in cc/sec/cm^2 surface area/atmosphere applied hydrostatic pressure difference. In L_P measurements $\Delta\Pi_{th} = 0$ and in L_{PD} measurements $\Delta P = 0$. The L_P and L_{PD} are overall filtration coefficients; they refer to water flow across the composite membrane not across the individual elements (cf. also ref. 1). The number of cells is given in parenthesis.

**In nitella L_{PD} depends upon the $\Delta\Pi$ at which it was ascertained. a = 1.35 atm, b = 2.65 atm and c = 3.95 atm.

From the table it can be seen that in both the squid axon and the internode of nitella the hydrostatic filtration coefficient (L_P) is not affected appreciably by destruction of the plasmalemma. If the L_P before destruction is compared to the L_P in the same cell after destruction (cf Table 1 ref. 5 and Table 3 ref. 2) the effect of destruction is less than may appear in the Table. The above observation that pressure-coupled water flow is not affected by destruction of the plasmalemma is consistent with my observation (2) that diffusional labeled water flow in the squid axon was not affected appreciably by destruction. In intact axons the diffusion permeability

coefficient P_d determined by measurement of the rate of efflux of intracellularly injected tritiated water in 34 axons was $1.5\text{-}2 \times 10^{-4}$ cm/sec.

It can be seen in the table that L_P is not equivalent to L_{Pd} even though the solutes involved were impermeable. The average L_P/L_{PD} in the squid axon was around 200 while in the nitella internode around 10. These experiments of non-equivalence of L_P and L_{PD} are consistent with the experiments (undertaken only in the axon) where the ΔP to sustain zero net water flow at various $\Delta\Pi_{th}$ was ascertained (2,4). ΔP was found not to be equivalent to $\Delta\Pi_{th}$. With an aqueous internal perfusate (cf, above allusion to methods) the average $\Delta\Pi_{th}/\Delta P$ in 7 axons was around 200. With oil in the perfusion channel $\Delta\Pi_{th}/\Delta P$ in 2 axons was 120 and 160. Apparently the $\Delta\Pi_{th}/\Delta P$ ratios are the same order of magnitude as the L_P/L_{PD} ratios. From the data mentioned above (especially the experiment with oil in the perfusion channel) it appears that the effective osmotic pressure due to gradients in mobile solutes is very small in the axon. I explored the possibility (3,4) that this osmotic pressure may be so small as to be of the order of the osmotic swelling pressure of the axoplasmic polymer network in which case one may expect that both these pressures should be considered in analyzing the volume relations of the whole axon. Direct measurements of the swelling pressure of the axoplasmic gel and other experiments indicate that this is the case.

Some of the experimental observations presented above are not quite new. Vargas (7), using the intracellular perfusion technique, found in the giant axon of Dosidicas gigas that L_P was two orders of magnitude larger than L_{PD}. Vargas' interpretation of the non-equivalence of L_P and L_{PD} differs from mine. He preferred to invoke the presence in the plasmalemma of one large hole or a few large holes whose reflection coefficient was close to zero. Zimmermann and Steudle (8) found, using a different technique than mine, that the L_P of near turgorless internodes of Nitella flexilis was close to the L_P of the isolated cell walls. The turgorless condition was attained somehow several hours following puncturing of the internode with a 60μ micropipette that served as an intracellular pressure sensor. Filtration coefficients in their method were determined from the elastic modulus of the cell wall and the exponential time course of ΔP following a change in ΔP or $\Delta\Pi_{th}$. Steudle and Zimmermann (6) also found that the L_P of nearly turgorless nitella internodes is markedly higher than that of highly turgored internodes. Thus in turgored internodes the L_P of the surface of the cell is far less than that of the cell wall, indicating that at high internal pressures (several atmospheres) the plasmalemma may become the rate limiting structure for pressure-coupled water flow. I have suggested (5), with little experimental supporting evidence, that at higher internal pressures the

the hydraulic conductance of the plasmalemma may be decreased by it being pressed against non-porous regions of the cell wall. The decrease in conductance of the plasmalemma should depend upon (a) the water impermeability of the non-porous regions of the cell wall, (b) the fractional area of the non-porous regions that are contiguous with the plasmalemma, (c) the proximity of the plasmalemma to the non-porous regions and (d) the macromolecular architecture of the inner face of the cell wall. Zimmermann and Steudle (9) offer a different interpretation for the pressure dependence of the hydraulic conductivity.

DISCUSSION

Obviously it is pertinent that I try to reconcile my interpretation of the water flow experiments in the axon and nitella with the generally held interpretation of water flow experiments in other cells. The widespread conviction, as mentioned at the beginning of this paper, is that, as a rule, the barrier to both solute flux and pressure-driven water flux resides in the plasmalemma, and that with impermeable solutes the overall (across the composite membrane) L_P is equivalent to the overall L_{PD} and ΔP equivalent to $\Delta\Pi_{th}$. My explanation of the aforementioned discrepancy is as follows. The measurement of L_P (with and without a plasmalemma) is <u>required</u> to show whether or not the plasmalemma is the water flow barrier. L_P measurements in conjunction with L_{PD} measurements are required in order to show whether or not L_P and L_{PD} are equivalent. A number of techniques, some simple, are available for L_{PD} measurements. L_{PD} measurements can be undertaken in most cell types; however, in order to make the crucial L_P measurement, at present at least, one <u>must</u> employ either the intracellular perfusion technique or the intracellular pressure transducer technique. Such techniques require that the cell under study be giant. Without very extensive refinement they cannot be applied to most of the cell types that have been popular for the study of water transport processes. Originally not only the type of experiments done on the giant axon and the internode, but also the interpretation of such experiments were consonant with those in other cells. It is only after the application of the intracellular perfusion and pressure sensor technique that these preparations have been regarded by some as unique or paradoxical.

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