BBA 75319

ELECTRO-OSMOSIS IN CHARA AND NITELLA CELLS

P. H. BARRY* AND A. B. HOPE
School of Biological Sciences, Flinders University, Bedford Park, South Australia 5042 (Australia)
(Received April 22nd, 1969)

SUMMARY

There is evidence that current-induced volume flows which are normally considered to be electro-osmosis have two components: a true electro-osmotic component due to frictional interaction and local osmosis due to a transport number effect. Recent measurements of such components and other parameters for both cells and cell walls are used to reappraise the possibility that electro-osmosis in cells may be explained in terms of electro-osmotic coupling in cell walls alone.

INTRODUCTION

FENSOM AND DAINTY[1] observed a coupling between water flow and electric current flow in cells of *Nitella translucens*. They considered this to be evidence for the existence of water-filled, charged pores in a cell membrane. We reserve the word membrane, here, for the submicroscopic structures, assumed to be lipoprotein, bounding the cytoplasm. "Current-induced volume flows" have also been found by us[2] in cells of *Chara corallina*. In both genera the maximum flow developed was about 20 $\mu l \cdot C^{-1}$. However, such current-induced flows have been found to have two components, a truly electro-osmotic component due to frictional interaction and a contribution from local osmosis due to a transport number effect[2]. The latter is caused by local enhancement or depletion of solutes at the membrane–solution, membrane–wall or wall–solution interfaces as a result of transport number differences at these interfaces. More recently FENSOM *et al.*[3] have claimed that observed changes in the apparent electro-osmotic coupling coefficient reflected changes in the size or charge of postulated membrane pores. These claims have been queried by us because of the transport number effect.

BRIGGS[4] has questioned whether electro-osmotic coupling in the wall *per se* is enough to account for the whole electro-osmotic flow observed across the composite system (cell wall + plasmalemma). If the answer is yes, and if the volume flow predicted for this model is similar to that observed, then such observations cannot be used as evidence for water-filled pores being the conduction paths for ions in natural membranes.

In discussing the model of wall + membrane, BRIGGS[4] has used for calculations certain values which are not in agreement with several other observations. These were values for the specific (area) conductance of cell walls of Nitella and for the hydraulic

---

[*] Present address: Physiology Department, U.C.L.A. Medical Center, Los Angeles, Calif. 90024, U.S.A.

conductivity of such walls. Further calculations are made below using the model suggested by BRIGGS[4] and recently published data on whole cell and cell wall electro-osmosis[2]. Some of this data is summarized below in Table I and where possible compared with published data. The results of the experiments were briefly as follows.

Current-induced flows in both cells and cell wall preparations could be separated into two components α and β. An immediate flow, β, was established in less than the response time of the recording equipment (*i.e.* < 0.2 sec) and is interpreted as a true electro-osmosis. The flow rate than increased with time to a steady level in 60–150 sec. The increase was interpreted as due to the local osmotic component, caused by the transport number effect previously mentioned, and will be referred to as α. The magnitudes of these flows per unit current density are given in Table I together with the other parameters necessary for the calculations which follow.

It is believed that FENSOM AND DAINTY[1] assumed the sum of α and β to be the electro-osmotic permeability, *i.e.* about 20 $\mu l \cdot C^{-1}$. Their volume flows showed the characteristic increases with time which we now associate with local osmosis.

TABLE I

COEFFICIENTS CALCULATED FROM OBSERVATIONS OF CELLS AND ISOLATED WALLS OF CHARA AND NITELLA SPP.

Coefficient	*Symbol*	*Measured in*	*Value*	*Units*	*Ref. No.*
Hydraulic conductivity	$L_p = (J_v/\Delta P)_{J_e=0}$	Cells	$1.0 \cdot 10^{-5}$ $1.0 \cdot 10^{-5}$	$cm \cdot sec^{-1} \cdot atm^{-1}$ $cm \cdot sec^{-1} \cdot atm^{-1}$	7 2
Hydraulic conductivity	L_{p_w}	Walls	$3.1 \cdot 10^{-5}$–$4.0 \cdot 10^{-5}$ $1.8 \cdot 10^{-5}$–$3.5 \cdot 10^{-5}$	$cm \cdot sec^{-1} \cdot atm^{-1}$ $cm \cdot sec^{-1} \cdot atm^{-1}$	2 8
Reflection coefficient for KCl	σ_w	Walls	1.0	–	2
Conductance	$K' = (J_e/\Delta E)_{\Delta P=0}$ $K' = (J_e/\Delta E)_{\Delta P=0}$	Cells Walls	$1 \cdot 10^{-4}$ 1.0 0.25	$\Omega^{-1} \cdot cm^{-2}$ $\Omega^{-1} \cdot cm^{-2}$ $\Omega^{-1} \cdot cm^{-2}$	9 10 11
Electro-osmotic permeability	$\beta = (J_v/J_e)_{\Delta P=0}$	Cells	$7 \cdot 10^{-3}$	$cm^3 \cdot C^{-1}$	2
	$\beta_w = (J_v/J_e)_{\Delta P=0}$	Walls	$1 \cdot 10^{-2}$	$cm^3 \cdot C^{-1}$	2
Maximum volume flow due to local osmosis	α	Cells	$1 \cdot 10^{-2}$–$2 \cdot 10^{-2}$	$cm^3 \cdot C^{-1}$	2
	α_w	Walls	$2.3 \cdot 10^{-2}$	$cm^3 \cdot C^{-1}$	2
Total current-induced volume flow	$\alpha + \beta$	Cells	$1.7 \cdot 10^{-2}$ $1.9 \cdot 10^{-2}$	$cm^3 \cdot C^{-1}$ $cm^3 \cdot C^{-1}$	2 1

THEORY AND CALCULATIONS

Using the data from Table I it is proposed to calculate the contribution of the electro-osmotic coupling in the wall phase alone to the total coupling coefficient. Secondly we calculate the corrected value for the coefficient of the membrane alone from data for the isolated wall and intact cell.

It will be assumed as in the model of BRIGGS[4] that $\sigma_{(\mathrm{KCl\ or\ NaCl})}$ for both the cell wall and membrane is very close to 1.0. Calculations of σ from solute permeabilities for both systems support such an assumption. When $\sigma \neq 1$, the situation is more complex and is discussed in detail by KEDEM AND KATCHALSKY[5]. We can now use the following practical equations for electro-kinetic flows based on the thermodynamic equations, derived by KATCHALSKY AND CURRAN[6], for the steady state situations:

$$J_v = L_{p_w}(\Delta P - \Delta\pi_i)_w + \beta_w J_e = L_{p_m}(\Delta P - \Delta\pi_i)_m + \beta_m J_e \tag{1}$$

$$J_e = K'_w\beta_w(\Delta P - \Delta\pi_i)_w + K'_w\Delta E_w = K'_m\beta_m(\Delta P - \Delta\pi_i)_m + K'_m\Delta E_m \tag{2}$$

where the flows of volume (J_v) and electric current (J_e) are constant throughout the cell wall and plasmalemma in series; ΔE is the change of potential difference, ΔP is the change of hydrostatic pressure and $\Delta\pi_i$ is the change in osmotic pressure due to impermeant solutes, each across the wall (w) or plasmalemma (m). The other coefficients have been defined in Table I. When $J_e = 0$,

$$J_v = L_{p_w}\Delta P_w = L_{p_m}\Delta P_m \tag{3}$$

where, for convenience, the term $\Delta\pi_i$ has been dropped, and the term ΔP should be considered as including possible contributions from both hydrostatic pressure and osmotic pressure of impermeant solutes.

The hydraulic conductivity of the composite system (wall + plasmalemma), L_p, is defined by:

$$L_p = (J_v/\Delta P)_{J_e} \tag{4}$$

where the subscript J_e implies that the definition is for $J_e = 0$. Since the pressure is additive, the pressure, ΔP, across the composite system is given by:

$$\Delta P = \Delta P_w + \Delta P_m \tag{5}$$

and it may easily be shown from Eqns. 3–5 that

$$1/L_p = 1/L_{p_w} + 1/L_{p_m} \tag{6}$$

Since the overall electro-osmotic coefficient is defined for zero pressure across the composite system we have:

$$\Delta P = \Delta P_w + \Delta P_m = 0;\ \Delta P_w = -\Delta P_m \tag{7}$$

in agreement with BRIGGS.

From Eqns. 1 and 7:

$$J_v = L_{p_w}\Delta P_w + \beta_w J_e = -L_{p_m}\Delta P_w + \beta_m J_e \tag{8}$$

so that

$$\Delta P_w = -\frac{(\beta_w - \beta_m)J_e}{(L_{p_w} + L_{p_m})} \tag{9}$$

Now the definition of the electro-osmotic coefficient of the composite system is

$$\beta = (J_v/J_e)_{\Delta P} \tag{10}$$

Hence from Eqns. 8, 9, and 10:

$$\beta = \frac{L_{p_m}\beta_w + L_{p_w}\beta_m}{(L_{p_w} + L_{p_m})} \tag{11}$$

and combining Eqns. 6 and 11 we obtain:

$$\frac{\beta}{L_p} = \frac{\beta_w}{L_{p_w}} + \frac{\beta_m}{L_{p_m}} \tag{12}$$

If we now take the value of L_{p_w} (wall) as $3.5 \cdot 10^{-5}$ cm· sec^{-1}·atm^{-1} and L_p (wall + membrane) as $1.0 \cdot 10^{-5}$ cm·sec^{-1}·atm^{-1} (see Table I) then from Eqn. 6:

$$L_{p_m} = 1.4 \cdot 10^{-5}\ \mathrm{cm \cdot sec^{-1} \cdot atm^{-1}}$$

If we were now to propose that in fact $\beta_m = 0$ (no electro-osmotic coupling in the plasmalemma itself), then from Eqn. 12 the overall electro-osmotic coupling would be given by:

$$\beta = 2.9 \cdot 10^{-3}\ \mathrm{cm^3 \cdot C^{-1}}$$

which is less than half the value observed, even after allowance is made for the transport number effect.

A better approach, however, is to calculate the corrected value of the electro-osmotic coupling coefficient across the plasmalemma, β_m.

Using the values of L_{p_m} and L_{p_w} above and the various values of L_p, β_w and β in Table I and substituting them into Eqn. 12, which being rearranged is:

$$\beta_m = L_{p_m}\left(\frac{\beta}{L_p} - \frac{\beta_w}{L_{p_w}}\right) \tag{13}$$

we have

$$\beta_m = 1.4\left(\frac{7.0}{1.0} - \frac{10.0}{3.5}\right) \times 10^{-3}\ \mathrm{cm^3 \cdot C^{-1}}$$

or

$$\beta_m = 5.8 \cdot 10^{-3}\ \mathrm{cm^3 \cdot C^{-1}}$$

which should be compared with the observed overall coefficient, β, of $7.0 \cdot 10^{-3}$ cm^3·C^{-1}. Physically this reduction is caused by a small increase or drop in pressure at the wall–plasmalemma interface (with possible contributions from the osmotic as well as the hydrostatic pressure). For example if $J_e = 10^{-4}$A·cm^{-2}, the $\Delta P_w = -0.86 \cdot 10^{-2}$atm, in such a direction as to reduce the flow across the wall and increase it across the plasmalemma.

It is also of interest to note that from Eqns. 1 and 2, using the calculated and measured values of the electro-osmotic coefficient, the streaming potential coefficients for the plasmalemma, $(\Delta E_m/\Delta P_m)_{J_e}$ and for the composite system $(\Delta E/\Delta P)_{J_e}$ are -0.6 and -0.7 mV·atm^{-1}, respectively.

CONCLUSION

Consideration of the effect of the wall on the composite (wall + plasmalemma) system, based on recent measurements of what are considered as true electro-osmotic coefficients for both systems, indicates that though the effect is not negligible in the Characeae, it reduces the coupling coefficient for the membrane only from a former estimate of 7.0, to $5.8 \cdot 10^{-3}\,\mathrm{cm}^3 \cdot \mathrm{C}^{-1}$. This is still equivalent to about 31 moles $\cdot$ Faraday^{-1} or 31 water molecules per monovalent ion and indicates very high ion–water frictional coupling, with the resulting inference as to the existence of small, charged, water-filled channels in a cytoplasmic membrane, probably the plasmalemma.

Only further experiments will answer the doubts as to the relative contributions of (i) electro-osmosis in the cell wall, (ii) electro-osmosis in the plasmalemma and (iii) local osmosis through the plasmalemma, to the observed current-induced water flow, when the flow is altered by changing external cation species, or by adding substances such as indole-acetic acid[3].

ACKNOWLEDGEMENTS

P.H.B. was the holder of research studentships from the (Australian) Commonwealth Scientific and Industrial Research Organization (C.S.I.R.O.) and from Flinders University, South Australia, during the course of this work. We are also grateful for an extra-mural grant from C.S.I.R.O. to the Biophysics Laboratory, Flinders University.

REFERENCES

1 D. S. Fensom and J. Dainty, *Can. J. Botan.*, 41 (1963) 685.
2 P. H. Barry and A. B. Hope, *Biophys. J.*, 9 (1969) 700.
3 D. S. Fensom, D. J. Ursino and C. D. Nelson, *Can. J. Botan.*, 45 (1967) 1267.
4 G. E. Briggs, *Proc. Roy. Soc. London, Series B*, 168 (1967) 22.
5 O. Kedem and A. Katchalsky, *Trans. Faraday Soc.*, 59 (1963) 1918.
6 A. Katchalsky and P. F. Curran, *Non-Equilibrium Thermodynamics in Biophysics*, Harvard University Press, Cambridge, Mass., 1965, p. 157.
7 J. Dainty and B. Z. Ginzburg, *Biochim. Biophys. Acta*, 79 (1964) 102.
8 M. Tazawa and N. Kamiya, *Australian J. Biol. Sci.*, 19 (1966) 399.
9 A. B. Hope and N. A. Walker, *Australian J. Biol. Sci.*, 14 (1961) 26.
10 H. J. Curtis and K. S. Cole, *J. Gen. Physiol.*, 21 (1937) 189.
11 C. T. Gaffey and L. J. Mullins, *J. Physiol.*, 144 (1958) 505.