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ELECTROMECHANICAL STRESSES AND THE EFFECT OF pH ON MEMBRANE STRUCTURE

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SUMMARY

The presence of an electric field in a membrane creates stresses which lead to a mechanical compression of the membrane material.

It is shown that considerations of this effect yield results consistent with the observed differential effect of pH on the plasma membrane substructure if the central electron-lucent layer in OsO_4-fixed membranes is identified with the ionic depletion in the double-fixed charge model of cell membranes.

INTRODUCTION

The dimensions of the central, electron-lucent layer, as well as the overall thickness of the plasmalemma of cells of *Chara corallina* fixed in OsO_4 would appear to be dependent on the pH in which the cells are cultured and fixed [1].

The presence of an electric field creates compressive stresses in a membrane. Changes in the pH, which might be reflected in changes in the intrinsic field strength in the membrane could thus also lead to changes in the dimensions of the membrane structure.

Recent work on dielectric breakdown in cell membranes suggests that such electromechanical forces may play an important role in the dimensional stability of cell membranes [2, 3].

In the present communication we are not concerned with dielectric breakdown but we wish to show that considerations of electromechanical forces are consistent with the observed effect of pH on the membrane structure if the central electron-lucent layer in OsO_4-fixed membranes is identified with the ionic depletion layer in the double fixed charge model of cell membranes [4].

Electromechanical stresses in a double fixed charge membrane

The membrane to be considered here consists of a layer of positive-fixed charges in contact with a layer of negative-fixed charges. The fixed charges are imbedded in an inert matrix which is permeated by an ionic solution. In the cell membrane the fixed charges could arise from the ionization of free amino and carboxyl groups on membrane proteins.

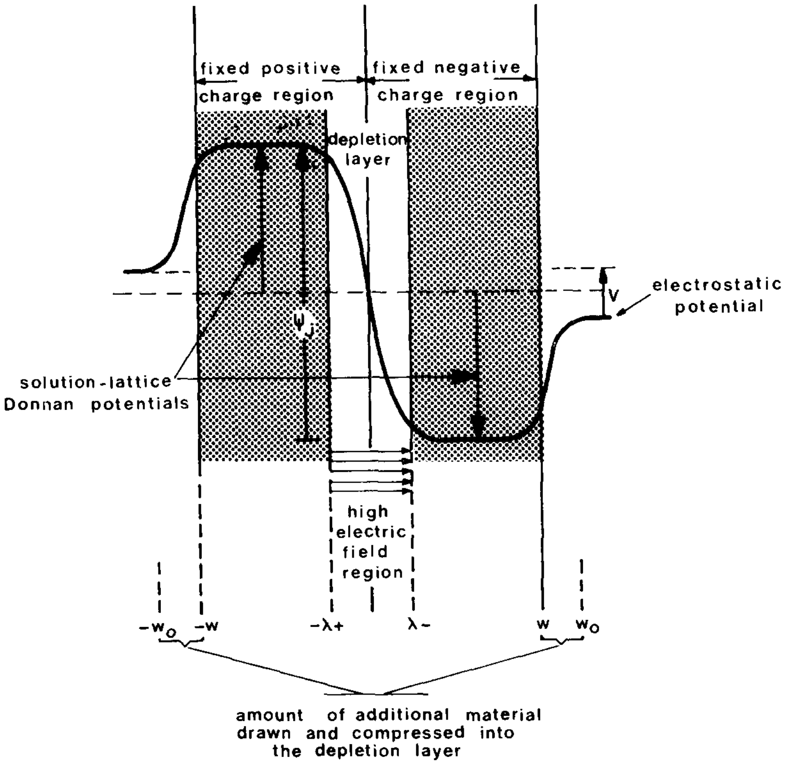

Fig. 1. Qualitative profiles for the electrostatic potential in a double fixed charge membrane in which one half of the membrane contains fixed positive charges and the other fixed negative charges. At the junction of the two fixed-charge regions, a region is generated which is largely depleted of mobile ions. The space charge density and the electric field strength here are therefore very large. The total potential across the depletion layer is $\psi_j - V$, where ψ_j is the sum of the two solution-lattice Donnan potentials and V is the (trans)membrane potential.

At the two membrane-solution interfaces, Donnan potentials are generated, one positive and one negative, see Fig. 1. In this double fixed charge membrane the counterions in one lattice are the coions in the other. At the junction of the two fixed-charge layers a region is generated (the depletion layer) which is almost completely depleted of mobile ions. In this region the fixed charges are not compensated by mobile counterions and the space charge density is therefore very high. The electric field strength in the depletion layer is hence also very high. Outside the depletion layer the field strength in the fixed-charge lattices is very weak.

The electrochemical profiles within the membrane and the electrical properties of the system have been analysed in detail elsewhere (see ref. 5 for details).

The field strength, E, in the depletion layer is readily obtained from Poisson's equation. Thus,

$$\frac{d^2\psi}{dx^2} = -\frac{\rho}{\varepsilon},$$

where ψ is the electrostatic potential, ρ the space charge density, ε the electric permittivity (dielectric constant) and x is the coordinate normal to the membrane.

In the depletion layer the space charge density is equal to the fixed-charge concentration, hence

$$\frac{\mathrm{d}^2\psi}{\mathrm{d}x^2} = \mp \frac{qN^\pm}{\varepsilon}$$

and thus the electric field, E, is given by

$$E = -\frac{\mathrm{d}\psi}{\mathrm{d}x} = \frac{qN^\pm}{\varepsilon}(x-\lambda_\pm) \qquad (1)$$

where $N^\pm$ is the positive or negative fixed-charge concentration, q is the absolute value of the charge on the fixed charges and $x = \pm\lambda_\pm$ are the edges of the depletion layer. In deriving the field strength E we have made use of the fact that at $x = \pm\lambda_\pm$ the field vanishes.

The stress, P_e, (compressive) due to the presence of an electric field is given by

$$P_e = -\frac{\mathrm{d}W_e}{\mathrm{d}x}$$

where W_e is the electric field energy.

From the rate of change of the electric field energy in the depletion layer (per unit area) with λ, the electric, compressive, stress is therefore given by

$$P_e = \frac{(qN^\pm)^2\lambda_\pm^2}{\varepsilon} \qquad (2)$$

In a region under strain the pressure in a material obeying Hooke's law is given by

$$P_m = Y\int_{l_0}^{l} \frac{\mathrm{d}x}{x} = Y \ln \frac{W}{W_0} \qquad (3)$$

where Y is the elastic modulus of the material and l and l_0 are respectively the strained and unstrained thickness of the region. For dimensional equilibrium we must have that

$$P_e + P_m = 0 \qquad (4)$$

Since the field is confined to the depletion layer, only mechanical stresses in this layer are considered.

When the transmembrane potential difference is zero and the external ionic solution concentrations are very high (so that the two solution-lattice, Donnan, potentials are also approximately zero), no depletion layer is generated and the field within the membrane vanishes. Let the membrane thickness in this unstressed condition be $2W_0$.

When the field is established in the depletion layer the electric field will lead to a compression of the membrane material in this region. The depletion layer width, during this compression, however, must remain substantially unaltered (assuming as a first approximation that the dielectric constant and fixed-charge densities remain constant). Material is therefore drawn into the depletion layer from the regions in the membrane outside it until the elastic stress balances the electric, compressive stress.

If the width of the membrane is $2W$ at electromechanical equilibrium, the compression ratio in the depletion layer is equal to $(\lambda+(W_0-\mathrm{W}))/\lambda$. Thus from Eqns 2, 3 and 4 at equilibrium,

$$\frac{(qN^{\pm})^2\lambda_{\pm}^2}{\varepsilon} - Y \ln \frac{\lambda_{\pm}+(W_0-W)}{\lambda_{\pm}} = 0 \tag{5}$$

Effect of pH on the dimensions of the membrane

The effect of pH on the electromechanical equilibrium expressed in Eqn 5 arises from its effect on the fixed-charge concentrations, $N^{\pm}$, and hence also $\lambda_{\pm}$.

An expression for the widths $\lambda_{\pm}$ of the depletion layers in each fixed-charge lattice, in terms of $N^{\pm}$ can be obtained by integration of Eqn 1. If, for simplicity, we assume for the moment that, apart from the sign of the fixed charges, the membrane is symmetrical, for zero transmembrane potential difference, $\lambda_{\pm}$ are given by (see also refs 5 and 6),

$$\lambda_{\pm} = \left[\frac{2\varepsilon\psi_{\mathrm{j}}}{qN^{\pm}}\right]^{\frac{1}{2}}$$

Here ψ_{j} is the sum of the two solution-membrane Donnan potentials.

Since the Donnan potentials and hence ψ_{j}, are weak (logarithmic) functions of the fixed-charge concentrations $N^{\pm}$, to a first approximation therefore,

$$\lambda_{\pm} \propto \left[\frac{\varepsilon}{qN^{\pm}}\right]^{\frac{1}{2}}. \tag{6}$$

Hence from Eqn 5 the contraction of the total membrane thickness,

$$2(W_0-W) \propto \lambda_{\pm}[\exp(qN^{\pm}/Y)-1]$$

that is, approximately,

$$\propto \frac{qN^{\pm}\lambda_{\pm}}{Y}. \tag{7}$$

Thus using Eqn 7, the contraction in membrane thickness varies approximately as,

$$2(W_0-W) \propto (N^{\pm})^{\frac{1}{2}} \propto \left(\frac{1}{\lambda_{\pm}}\right)^{\frac{1}{2}} \tag{8}$$

Physically the reduction in thickness, on this model, with increasing fixed-charge concentration results from the fact that material is drawn into the depletion layer where it is compressed by the intense electric field.

In the double-fixed charge membrane, changes in the fixed charge concentration cause a change in the central, high-field, region of the membrane where it is depleted of mobile ions. With considerations of the electromechanical forces, however, this also results in a change in the total thickness of the membrane. This is consistent with the experimental results previously presented [1]. As is seen from the Relation 8 a contraction of the total membrane thickness will not be directly proportional to the decrease in the depletion layer width. A differential contraction of the total membrane thickness and the central electron-lucent layer was indeed a feature of the results previously reported [1].

It is of interest to consider some numerical values of the stresses and strains involved. With plausible values [4] for the parameters such as a fixed-charge concentration of 0.1 N and a dielectric constant of $10 \cdot 8.85 \cdot 10^{-12}$, the electric field stress with $\lambda = 1.07$ nm (i.e. at pH 7) is $1.19 \cdot 10^6$ N/m^2. Given a value for the elastic modulus of the order of that for rubber ($\approx 1.7 \cdot 10^6$ N/m^2) yields a value of 2.2 nm for the contraction of the thickness of the membrane due to compression of material drawn into the depletion layer. Assuming that the fixed-charge concentration is increased by 50 % at pH 9 (in accord with the punch-through potential data [6]), the change in the width of the depletion layer observed would lead to a predicted further decrease in the membrane thickness of 1.45 nm. This is close to the value of 1.4 nm observed [1].

The punch-through data [6] further suggest that the change in fixed-charge concentration with increasing pH is greatest in the negative fixed charge layer. In this model this layer in the membrane must face the cytoplasm [6] (to accommodate the direction of rectification). Hence it would be expected from the present analysis that most of the changes in the dimensions of the structure with pH should also occur in this half of the membrane, again in accord with observation.

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