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The Membrane Potentials of Periderm and Cuticular Membranes

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The membrane potentials of periderm and cuticular membranes were measured with KCI and NaCl solution using Ag/AgCI electrodes. For the electromotive force (emf) measurements, the concentration in both compartments were brought to equilibrium with the 0.01 M concentration of KCI or NaCl solution, then the one side was kept constant and the other side changed. The estimation of the Donnan potential contribution to the membrane potential was carried out by taking into account the fixed charge concentration, C_x, value. From these measurements electrochemical characterization of the asymmetry and surface layers of the periderm and cuticular membranes can be described.

Key Words: Membrane potential, periderm membrane, cuticular membrane, fixed charge concentration, asymmetric potential

Introduction

The description of transport phenomena in biological membranes is still unclear from the viewpoint of physical chemistry. Transport phenomena in ion exchange membranes can be controlled by several factors such as ionic concentration within the membrane, fixed charge concentration, solution composition, ionic fluxes, and water content. The ionic transport as well as distinctive selectivity is provided by fixed groups in the ion exchange membrane, but that of co-ions is strongly restricted due to the Donnan effect. The fixed charge theory was first proposed by Teorell[1,2] and Meyer and Sievers[3,4], who described the Donnan equilibria at the membrane-solution interfaces. Theoretical equations for the membrane potential and salt flux based on the irreversible thermodynamics in terms of the ionic mobility and effective charge density were derived by Toyoshima et al.[5−7]. The effective charge density in ion exchange membranes using the Teorell-Meyer-Sievers (TMS) theory was determined from membrane potential measurements[8,9].

In general, periderm or cuticular membrane, which is a biological membrane, is adopted as a porous membrane model with the pore size depending mainly upon swelling in the presence of water[10]. It was pointed out that the pores in the cuticle or periderm membranes have polar regions which are weakly polar compounds due to unesterified carboxyl and hydroxyl groups and have a net negative charge above pH

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3, which affect the sorption and transport of electrolytes[11−14]. The outer and inner surfaces of periderm and cuticular membranes are different: the inner surfaces are more homogeneous with their abundance of charged groups; in contrast, the outer surface appears with a more heterogeneous ultrastructure which contains uncharged epicuticular waxes. This asymmetric behaviour was first pointed out by Yamada et al.[15]. We have a special interest in the treatment of biological membranes when investigating each face of the membranes[16]. In the present paper, the membrane potential for periderm and cuticular membranes with KCl and NaCl solutions at different concentrations was studied which in order to obtain information about the effective fixed charge concentration. The contributions of the Donnan and diffusion potentials to the measured membrane potential were also estimated.

Experimental

Electrolyte solutions of NaCI, KCI, HCl and other chemicals used were reagent grade and were obtained from Merck or BDH Chemicals Ltd. Isolation of periderm and cuticular membranes was carried out using a modification of the previous method[11,14]. Exchangeable cations were removed from periderm or cuticle samples by shaking in a 1 M HCl solution for 15 min (three changes) followed by washing with deionized water to remove sorbed HCl.

Membrane potential measurements were carried out using the borax cell. The membrane was clamped tightly between two compartments of 20 cm^3 volumes, and the exposed membrane area was 1.33 cm^2. Prior to each experiment, the membrane to be used was immersed for a minimum of 1 day in a 0.01 M solution concentration in order to achieve equilibrium. The equilibrated membrane was clamped between two half cells, which were filled with the solution at the same concentration. The membrane was positioned in the cells, and both compartments were filled with the lower concentration of the solution. The electrodes used were reversible Ag, AgCl electrodes and were connected to a galvanometer (WPA KED81 DC model). The system was stirred vigorously by magnetic stirrers at constant 500 rpm to minimize the effect of the boundary layers on the potential[17]. At this point, the membrane potential measurements were performed as one side of the cell was kept in the concentration C_1 1.0 x 10^{-2} mol L^{-1}, and on the other side C_2 was changed from 1.0 x 10^{-4} to 1.0 mol L^{-1}. The experiments were performed at room temperature. Before use, the cuticles were kept for at least 24 h in distilled water, and later they were immersed for 6 h in a solution of the appropriate concentration.

Results and Discussion

The fluxes of ions through the membrane can be given by the following equations:

$$J_+ = -\bar{D}_+\bar{C}_+\left(RT\ln\frac{d\ln\bar{C}_+}{dx} + F\frac{d\phi_d}{dx}\right) \tag{1}$$

$$J_- = -\bar{D}_-\bar{C}_-\left(RT\ln\frac{d\ln\bar{C}_-}{dx} + F\frac{d\phi_d}{dx}\right) \tag{2}$$

the electroneutrality condition is

$$J_-(x) = J_+(x) \tag{3}$$

According to the TMS theory, the electrical potential difference measured at both sides of a charged membrane when it is separating an electrolyte solution of different concentrations (C_1 and C_2) basically consists of two terms: a diffusion potential across the membrane, which is due to the different mobility of the ions in the membrane, and, the Donnan potentials at the interfaces between the membrane and the solutions[18−23]. For the described system, the diffusion potential can be expressed as;

$$\Delta\phi = \frac{RT}{Z\,\omega F}\left\{\ln\frac{C_1}{C_2}\,\frac{\sqrt{1+4(y_1)^2}+1}{\sqrt{1+4(y_2)^2}+1} + \omega\,U\,\ln\frac{\sqrt{1+4(y_2)^2}-\omega\,U}{\sqrt{1+4(y_1)^2}-\omega\,U}\right\} \tag{4}$$

where $U = (\omega_+ - \omega_-)/(\omega_+ + \omega_-)$ and $y = ZKC_s/\omega C_x$. and $K_\pm$ is the partition coefficient, C_s is the concentration of the external salt solutions and C_x is the fixed charge concentration in the membrane, ω_+ and ω_- are the mobility of cation and anion in the membrane, respectively, Z is the charge number, R is the gas constant, T is the absolute temperature and F is the Faraday constant. Taking into account the electroneutrality conditions, the following relation between the distribution of co-ions in the membrane and the solution can be derived as a function of the concentration of fixed charges[24−27]. The fitting of the membrane potential values measured at different salt concentrations allows us to determine the effective fixed charge and the parameter U. In the above equation, the concentration of fixed charges and U were evaluated from a series of potential measurements with various external concentrations (C_2) by using a curve fitting program. The procedure for the calculation of C_x values was explained in detail in a previous paper[27].

Figures 1-4 show the emf values of homogeneous surface (inner surface) constant and heterogeneous surface (outer surface) variable or vice versa for periderm and cuticular membranes versus natural logarithm of the concentration gradient ($\ln C_i/C_o$; i and o represents inner and outer surfaces, respectively) as a function of concentration gradient with KCl and NaCl solutions, respectively. As seen from these pictures, the experimental values correspond to different parabolas, with the maximum:minimum shifting to the levels, when the concentration ratio for both sides was either 10 times higher or lower. In particular, this behaviour was significantly observed for the periderm membrane. In the case of cuticular membrane, the parabolic maximum:minimum shifting was observed when the concentration ratio was 10 times higher. From this picture, the differences in $\Delta\phi$ values depending on the membrane surface in contact with the constant concentration can be observed, with this effect being more evident at higher concentrations. The negative fixed charge is due to the dissociation of nonesterified $COOH^-$ groups and -OH phenolic groups[12], depending on the electrolyte. The fixed charge concentrations of periderm and cuticular membranes were (-0.73 ± 0.15) x 10^3 M and (-0.42 ± 0.15) x 10^3 M for KCl and (-0.65 ± 0.15) x 10^3 M and (-0.37 ± 0.15) x 10^3 M for NaCl solutions, respectively. The ion exchange properties of periderm and cuticular membranes towards alkali cations have been discussed in terms of ionic mobility and hydration constants in detail[13,14].

The highest value was obtained when the concentration differences on both sides of either the inner or outer surface were large. Heredia and Benaverte[28] pointed out that membrane potentials obtained in this way give information about the electrokinetic behaviour at the other cuticular membrane surface, and on the other hand the membrane potentials at a lower ratio of concentrations are fundamentally Donnan potentials. It was also noted that the Donnan effect is more significant at the inner surface region of the periderm or cuticular membranes, because their inner surfaces contain mostly protein and lipid molecules, which have a considerable number of dissociable as well as phenolic groups from which hydrogen ions dissociate.

It is seen from Figures 1-4 that as the concentration of the salt solution increases the membrane potential $\Delta\phi$ goes through a maximum value and levels off, giving a negative slope. The results are in

agreement within reasonable ranges either experimentally or theoretically with those in the literature[28,29]. It was pointed out that the different parabolic curves are due to the Donnan potential effect[29]. In other words, co-ions are efficiently excluded from the membrane. Thus, counter-ions permeate permselectively through membranes. When the electrolyte concentration is increased, the Donnan exclusion has less effec, and thus co-ion uptake influence prevails. In this study, the aqueous solution contained only KCl or NaCl and so membrane potentials varied with concentration changes between two faces. In the higher concentrations range, the variations of diffusion membrane potential deviated from linearity. This discrepancy can be explained by consideration of the classical theories developed by TMS[30] in order to account for the potentiostatic response of ion exchange membranes. The deviations from Nerstian responses are due to the penetration of co-ions into the membrane by osmotic effects and to diffusion of the electrolyte inside the membrane. Alternatively linear relationships between $\Delta\phi$ and $\ln(C_i/C_o)$ were obtained for the calculated values, which confirms the assumption that, in this case, the membrane potential is due to the diffusion potential.

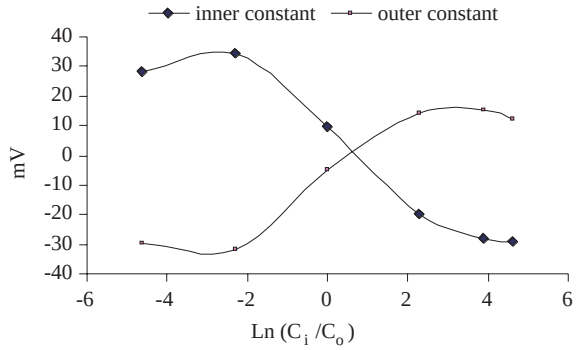

Figure 1. The changes in emf values versus $\mathrm{Ln}(C_i/C_o)$ with KCl solutions for inner surfaces and outer surfaces constant for periderm membranes.

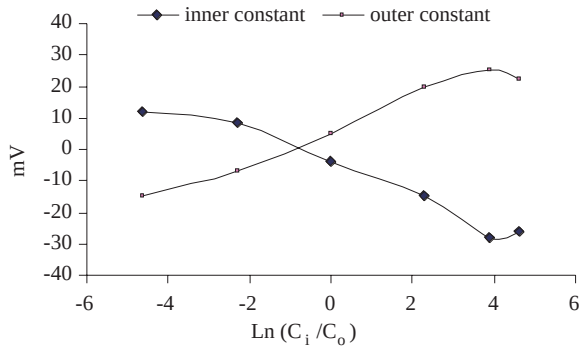

Figure 2. The changes of emf values versus $\mathrm{Ln}(C_i/C_o)$ with NaCl solutions for inner surfaces and outer surfaces constant for periderm membranes.

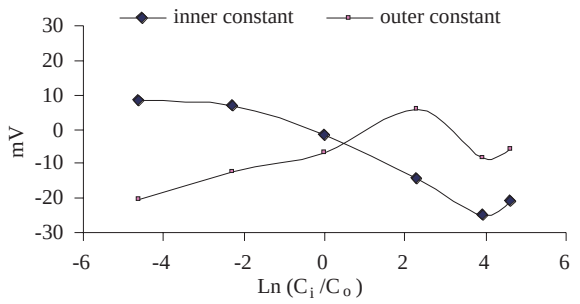

Figure 3. The changes of emf values versus $\mathrm{Ln}(C_i/C_o)$ with KCl solutions for inner surfaces and outer surfaces constant for cuticular membranes.

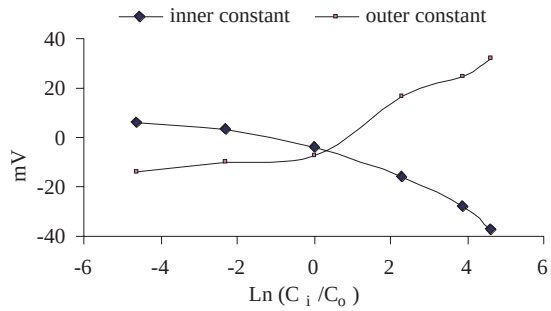

Figure 4. The changes of emf values versus $\mathrm{Ln}(C_i/C_o)$ with NaCl solutions for inner surfaces and outer surfaces constant for cuticular membranes.

The asymmetric properties of ion exchange membranes were studied electrochemically by stationary state emf measurements in well-stirred concentration cells, turning the membrane to one face or the other[30−33]. The interpretation of the emf from such experiments is difficult, because the emf is a complicated function of the salt concentration profile in a membrane with asymmetry in the fixed charge distribution[31]. The other important finding is that the extrapolation of curves do not pass through the origin. However,

an equal concentration in both sides of the membrane with $(C_1) = (C_2)$ must have been equal to zero, but the emf values for the membranes were not zero because of the heterogeneous properties of the membranes studied. In addition, emf values might be used to indicate the presence of asymmetry in biological membranes.

Conclusion

The emf measurements were specifically obtained to investigate the fixed charge concentration and asymmetric characterization of biological membranes with differences in higher or lower concentrations at both sides of the membrane. Therefore the biological membranes were first equilibrated with the same (constant) electrolyte solution rather than the lowest concentration at both surfaces of the membrane, and then the other sides of the membranes were replaced with the higher (or lower) concentration of electrolyte solution. The differences in concentration between the two solutions applied were varied and the Donnan potential contribution to the membrane potential was carried out. The fixed charge concentrations of the biological membranes were found and, due to its small value, the Donnan exclusion of co-ions appeared at lower concentrations. The membrane potential was due to the different mobilities of ions at higher concentrations and deviated from linearity.

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