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A study of membrane potential across isolated fruit cuticles for NaCl and $CaCl_2$ solutions

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Fixed charge density and ionic diffusion ratio in an isolated tomato fruit cuticular membrane have been estimated from membrane potential measurements for NaCl and $CaCl_2$ electrolyte solutions. The values of the parameters studied show marked differences in the electrical behaviour of the electrolytes, and evidence the asymmetric character of the cuticular membrane. While the membrane potential values for NaCl solutions can be explained in terms of a Donnan potential plus diffusion potentials, for $CaCl_2$ solutions the membrane potential values agree very well with the diffusion potential. These facts could be explained by taking into account some structural features of the cuticular membrane.

Introduction

All aerial parts of higher plants are bounded by the plant cuticle that forms the interface between the plant and its environment. The plant cuticle is a heterogeneous membrane, consisting of a polyester matrix, cutin, and cuticular lipids [1]. Additionally, carbohydrates, phenolic acids and other compounds may also be present [2].

There are some studies focusing on the interactive relationship between plant cuticles and permeation of nonelectrolytes [3], but current information about the mechanism of ion permeation through cuticles is very poor although the ion exchange capacity of plant cuticles has been known for many years [2]. In this sense, some evidence suggests that cuticles are weak ion exchange membranes: the polymer matrix is a polyelectrolyte with an isoelectric point around three [4] and, above pH 3, the cuticle carries a net negative charge due to acid groups from several kind of components of the cuticular membrane [5]. This fixed charge is an important characteristic affecting the sorption and transport of water or electrolytes, and some physiological and environmental aspects such as cuticular transpiration [6], agricultural spray applications [7] and ecotoxicological problems [8], are influenced by it. For these reasons, measurements of the membrane potential could give us some information about the electrical behaviour of the cuticular membrane.

When two solutions of the same electrolyte but different concentrations are separated by a membrane, various transport phenomena may be observed. If there are no pressure and temperature gradients across the membrane, the electrical potential difference produced is due to the concentration difference, and it is called 'membrane potential' ($\Delta\phi$). The Teorell-Meyer-Sievers (TMS) theory [9] has been used by several authors to study the membrane potential through artificial membranes [10–12]. This theory assumes, in essence, that the membrane potential is due to the sum of two Donnan potentials at each membrane-solution interface, plus a diffusion potential within the membrane. For symmetrical electrolytes ($z_+ = z_- = z$), $\Delta\phi$ can be expressed as:

$$\Delta\phi = \frac{RT}{wzF}\left|\ln\frac{C_2}{C_1}\frac{(1+4y_1^2)^{1/2}+1}{(1+4y_2^2)^{1/2}+1} + wU\ln\frac{(1+4y_1^2)^{1/2}-wU}{(1+4y_2^2)^{1/2}-wU}\right| \quad (1)$$

w is $+1$ or -1, for anionic or cationic membranes respectively; $y_j = z_j k_s C_j / wX$, k_s being the salt partition coefficient, X the fixed charge concentration in the membrane and z_j the valence of the ions. C_j represents the concentration of the solutions separated by the membrane (C_i = inner solution, C_o = outer solution). R and F are the gas and Faraday constant, T the temperature of the system, and the parameter U is related to

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the diffusion coefficient of the ions in the membrane ($U = [D_+ - D_-]/[z_+D_+ - z_-D_-]$), where z_+, z_-, D_+ and D_- are the valencies and the diffusion coefficients of the cation and anion, respectively. Taking into account the TMS theory, the membrane potential values measured at different concentrations allow us to calculate the membrane fixed charge density as well as the variation of the mobility ratio, or the diffusion coefficients ratio, of the ions in the membrane as a function of the external salt concentration.

In this work we have used NaCl and $CaCl_2$ solutions, which also permitted the study of the membrane potential dependence on the concentration for two different kinds of electrolytes. Significant differences for $\Delta\phi$ values have been found depending on the electrolyte considered, which could be related to the different exchange capacity and affinity of the cuticular membrane for the two electrolytes.

Material and Methods

Cuticular membrane

Tomato fruit cuticular membranes (*Lycopersicum esculentum* Miii, cv. Pik Red) were provided by Dr. M.J. Bukovac (Michigan State University, East Lansing, U.S.A.); the cuticular membranes were enzymatically isolated using the procedure described by Shafer and Bukovac [13].

Small pieces of tomato fruit cuticular membranes were observed in a JEOL JSM-840 scanning electron microscope (SEM) using accelerating potentials of 15 keV for the lower magnifications and 30 keV for the higher resolution images.

Cation exchange capacity

The cation exchange capacity was determined by equilibrating several membrane pieces of known weight in 10 ml of a 0.1 equiv./l $CaCl_2$ solution for 24 h at room temperature (around 25° C). Results are presented as mequiv./g cuticle of $CaCl_2$, assuming the Ca^{2+} ions have displaced all cations previously bound to the cuticle in an exchangeable form. This assumption is based on the length of incubation time and the high affinity of Ca^{2+} for binding sites [14]. A similar procedure was used with NaCl solution.

Thereafter, the cuticular membrane pieces were washed ten times in 10 ml of deionized water (5 min each) to remove sorbed cations. The exchangeable ion was eluted from cuticular membranes using three washes with 3 ml of 1 M HCl solution for 15 min each.

The cation concentration was measured by flame photometry (Corning 410C model). Standard curves for both cations over an appropriate concentration range were used. In all cases, 1 M HCl solutions were used as blank. All solutions were prepared from analytical grade reagents.

Membrane potential: experimental set up and procedure

The experimental device used to measure membrane potential is basically the same as that described in Ref. 15, and it is schematically shown in Fig. 1. The membrane was placed between two half-cells, supported by rubber rings, and the exposed area was 0.5 cm^2. Two circulatory systems (C.S.), each one with a centrifugal pump (P) whose output was approx. 600 cm^3/min, provided a turbulent flow within the membrane cell to minimize the effect of the boundary layers on the membrane potential. The C.S. connected each half-cell to each one of the solution tanks (D), which had thermostatic jackets to keep the solution at a constant temperature. All the experiments were carried out at 25° C.

To avoid a difference in the hydrostatic pressure between the two half-cells, which could cause a streaming potential across the membrane, each half-cell was

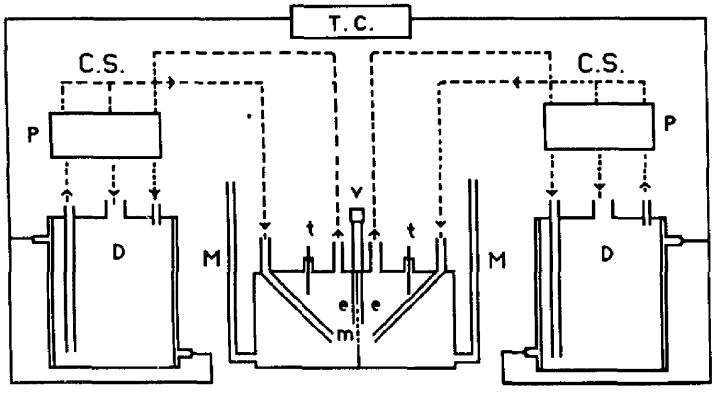

Fig. 1. Experimental apparatus for membrane potential measurements. m, membrane; e, electrode; t, thermometer; V, voltmeter; M, manometer; D, solution tank; P, pumps; C.S., curculatory system; T.C., temperature control.

provided with a vertical capillary tube acting as a reference manometer. The pressure of the two pumps was carefully adjusted so that the meniscus levels in the two tubes were equal.

Aqueous solutions of NaCl and $CaCl_2$ were used. Before measuring, the membranes were immersed for at least 18 h in a 10^{-3} M solution of the corresponding electrolyte. The membrane potential measurements were carried out keeping constant the concentration of the solution on one side of the membrane, while the concentration of the solution on the other side was changed gradually. The constant concentration value was 10^{-2} M, and the other ranged between 10^{-3} M and 10^{-1} M.

The electrical potential difference between both sides of the membrane were obtained using calomel electrodes connected to a digital voltmeter of 1000 MΩ input impedance and joined to the solution via KCl-agar bridges. The electrode connected with the inner solutions was grounded, so $\Delta\phi = \phi(C_o) - \phi(C_i)$. Membrane potential values were corrected to compensate for electrode asymmetry but not for the liquid junction potentials at the tips of the saline bridges.

Results and Discussion

The outer and inner surfaces of isolated tomato fruit cuticle viewed by scanning electron microscopy are shown in Fig. 2. The two surfaces appear to have different morphology. The inner surface is more homogeneous and the micrograph illustrates cuticular pegs

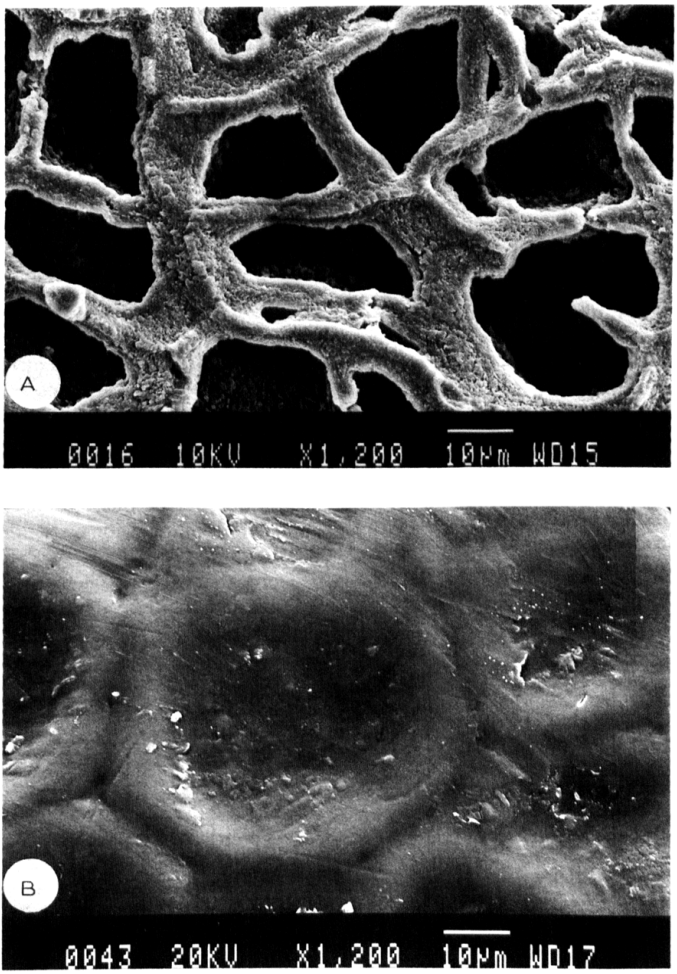

Fig. 2. Scanning electron micrographs of isolated tomato cuticular membrane. (A) outer surface; (B) inner surface.

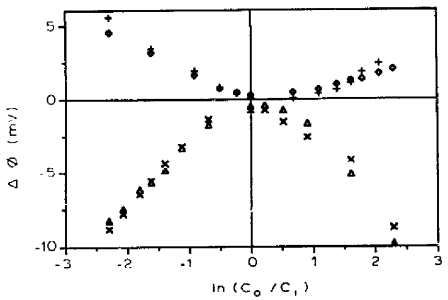

Fig. 3. Membrane potential versus $\ln(C_o/C_i)$ for NaCl solutions. $C_i = 10^{-2}$ M: (+) and (◇); $C_o = 10^{-2}$ M: (×) and (△).

that project between the anticlinal walls of epidermal cells. According to Holloway [1], the inner surface blends into the cell wall with its abundance of charged groups as proteins and carbohydrates. On the other hand, the outer surface appears with a more heterogeneous ultrastructure showing the epicuticular waxes. For this reason, this surface is predominantly uncharged [1].

The cation exchange capacity of the cuticular membrane for Ca^{2+} and Na^+ ions was determined. These membranes have a considerable cation exchange capacity showing that they have negative fixed charged. We obtained an exchange capacity of 0.36 ± 0.04 mequiv. of Ca^{2+} and 0.21 ± 0.08 mequiv. of Na^+ per gram of cuticular membrane. These results are in good agreement with data previously obtained by Schönherr and Bukovac [5] also for tomato fruit cuticle. The exchange capacity data obtained show that these membranes have a pronounced selectivity for Ca^{2+} and Na^+.

Membrane potential values versus $\ln(C_o/C_i)$ for $C_o = 0.01$ M NaCl and variable C_i, and vice versa are shown in Fig. 3. For $CaCl_2$ solutions activities instead of concentrations were used, and the experimental results are shown in Fig. 4. In each one of these graphs $\Delta\phi$ values, keeping constant the solution close to the outer (C_o) or inner (C_i) membrane surfaces, were drawn for two series of measurements in each case, and no significant variations were obtained. The differences found in the membrane potential values, depending on which was the constant concentration solution, give an idea of the asymmetry of the cuticular membrane concerning to the electrical parameters. Similar results were also reported by Tyree et al. [16] for different isolated leaf cuticles and KCl solutions. On the other hand, a comparison between Figs. 3 and 4 makes evident the different behaviour of the membrane potential, for the same experimental procedure, depending on the kind of electrolyte considered.

For NaCl solutions, the curves obtained are similar to those found in the literature for slightly charged cellulosic membrane [10,12]. Taking into account the mathematical condition for the minimum (or maximum) of a curve $(d[\Delta\phi]/dC) = 0)$, Demish and Push [12] derived the following expression for the concentration of fixed charge in the membrane:

$$X = 2C_{ext}U/(1-U^2)^{1/2} \qquad (2)$$

where C_{ext} represents the concentration at the minimum (or maximum) of the experimental curve, and the parameter U was defined above. By fitting the experimental points to a parabolic curve, the following average values for X were obtained, depending on which was the membrane surface in contact with the concentration keeping constant during the experiments:

$$C_o = \text{cte} \quad \langle X \rangle = -(3.3 \pm 0.5) \cdot 10^{-3}\ \text{M}$$
$$C_i = \text{cte} \quad \langle X \rangle = -(1.9 \pm 0.6) \cdot 10^{-3}\ \text{M}$$

For $CaCl_2$ solutions, membrane potential values versus $\ln(C_o/C_i)$ yield almost straight lines. This different behaviour with respect to that found for NaCl solutions could be due to some special affinity of the cuticular membrane for Ca^{2+} ions, as was reported in the literature [5].

Comparisons between experimental and calculated membrane potential values obtained by means of Eqn.

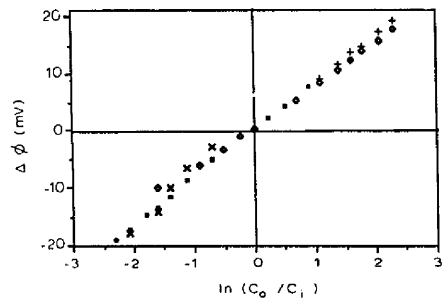

Fig. 4. Membrane potential versus $\ln(C_o/C_i)$ for $CaCl_2$ solutions. $C_i = 10^{-2}$ M: (+) and (◇); $C_o = 10^{-2}$ M: (×) and (■).

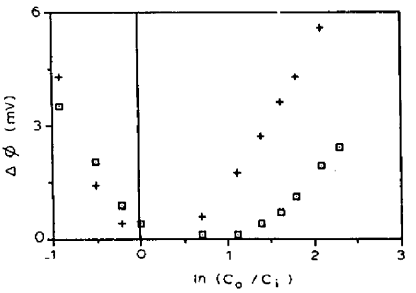

Fig. 5. A comparison between theoretical (+) and experimental values (□) for NaCl solutions ($C_i = 10^{-2}$ M).

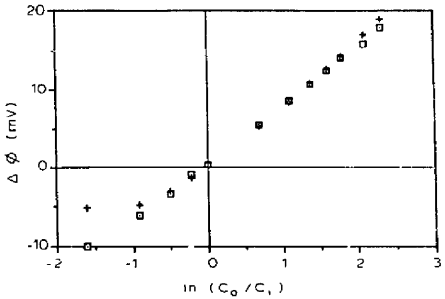

Fig. 6. A comparison between theoretical (+) and experimental values (□) for $CaCl_2$ solutions ($C_i = 10^{-2}$ M).

1, are shown in Figs. 5 and 6. For determining the calculated values we used the following parameters:

$X = -5 \cdot 10^{-3}$ M, $U = -0.208$ (NaCl solutions)
$X = -2 \cdot 10^{-3}$ M, $U = -0.355$ ($CaCl_2$ solutions)

in both cases, the U values correspond to free solution [17]. For both pictures, a similar tendency for both values, experimental and theoretical, can be observed. For NaCl solutions, the similarity between both kinds of value is greater at low concentrations ($\Delta\phi$ values are mainly due to the Donnan potential) than at high concentrations ($\Delta\phi$ mainly due to the diffusion potential), which implies some kind of interaction between the ions and the cuticular membrane. For $CaCl_2$ solutions a quite good agreement is obtained when $C \gg X$, which indicates the diffusion of the ions across the cuticle hardly differs from that in free solution.

At high concentrations ($C \gg X$) the diffusion ratio of the ions in the membrane, D_+/D_-, was calculated by means of an approximation of Eqn. 1, which assumes the membrane potential is only due to a diffusion potential:

$$\Delta\phi = (RT/F)[(D_+ - D_-)/(z_+ D_+ - z_- D_-)] \ln(C_o/C_i) \qquad (3)$$

The results for both electrolytes are shown in Fig. 7, where the variation of D_+/D_- with the average concentration ($C_{avg} = [C_o + C_i]/2$) is drawn.

The different results obtained might be explained taking into account some structural information about the cuticular membrane. The membrane has polar region composed of cuticular waxes with polar substituents, the hydroxylic polymer matrix (cutin) and the polyuronic acids associated with polysaccharides in the secondary cuticle [5]. The arrangement of both polar and non polar substances in the cuticular membrane could give a molecular structure with polar pathways, that could explain the membrane potential measured when electrolyte solutions are made to diffuse across isolated cuticular membranes [16]. Additionally, the arrangement above mentioned gives the cuticular membrane some asymmetric electrical properties: the inner surface is more charged than the outer surface, which is predominantly uncharged. This asymmetric behaviour was firstly documented by Yamada et al. [18] in some studies on the undirectional cuticular membrane penetration and the binding on cuticular surfaces of several cations and anions.

From our data the asymmetric behaviour appears very clear in the experiments preformed with NaCl solutions (Fig. 3). Depending on where the constant solution is located we found different membrane potential values. The highest ones were obtained when the constant concentration solution was placed at the outer cuticular surface. Membrane potentials obtained in this way give information about the electrokinetic behaviour at the other cuticular membrane surface. On the other hand, the membrane potentials at lower C_o/C_i ratio are fundamently Donnan potentials (Fig. 5), and our data suggest that there is a more significant Donnan effect just at the inner surface region of the cuticular membrane. Pectin and the other macromolecules near the inner membrane surface have a large number of immobile carboxyl groups from which hydrogen ions dissociate. This gives the cuticular membrane a net negative charge, which can be calculated from the minimum, or maximum, of the experimental curves (Fig. 3). In relation to these comments, Yamada et al. [18] obtained more ion binding on the inner than on the outer cuticu-

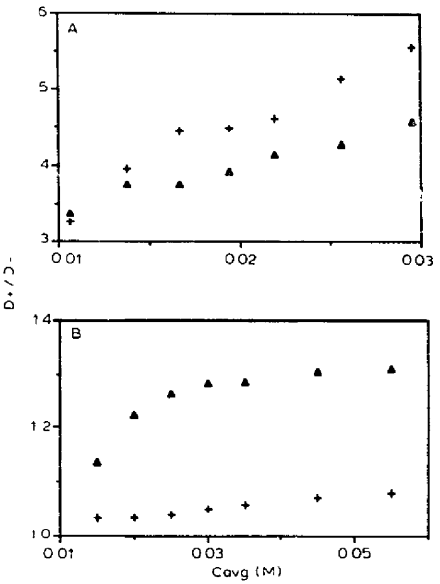

Fig. 7. Variation of $D+/D-$ versus the average concentration, C_{avg}: (A): (+) $C_i = 10^{-2}$ M and (Δ) $C_o = 10^{-2}$ M, $CaCl_2$ solutions. (B): (+) $C_i = 10^{-2}$ M and (Δ) $C_o = 10^{-2}$ M, NaCl solutions.

lar surfaces. Finally, above a given C_o/C_i electrolyte ratio the membrane potentials measured are fundamently diffusion potentials where some specific interactions between the Na^+ and the cuticular membrane could occur (Fig. 5).

The analysis of the data obtained for $CaCl_2$ solutions reveals very different behaviour. The measured potential values for a large number of C_o/C_i ratio appeared always like diffusion potentials (Fig. 4). In this case there is not an appreciable Donnan potential contribution to the total membrane potential value. On the other hand, Fig. 6 shows that the diffusion of calcium ions across the cuticular membrane is very similar to that in free solution. Additionally, it has been documented that the cuticular membrane has a pronounced selectivity for Ca^{2+} over Na^+ [5]. This fact could be due to some special affinity of the cuticular membrane for calcium ions. Attempts to explain the above data must have present some special arrangement in the polymer matrix of the cuticular membrane. Thus the polymer matrix would prefer a counter-ion which minimizes both the electrostatic and stretching free energy, and which maximizes the configurational entropy of the polymer chains. Additionally, it is known that there is a high affinity of Ca^{2+} for some polysaccharides, mainly polyuronic acids, present as component of the cuticular matrix as was reported by Rees and Welsh [19]. This fact, in addition to the formation of highly hydrated environments produced by the calcium solvation potential, could explain the different behaviour observed for $CaCl_2$ solutions.

A more detailed discussion on the calcium interaction with the different components of cuticular membrane will be necessary for a better understanding of these systems. Work is currently underway.

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