Ionic Permeabilities of Different Isolated Fruit Cuticles Calculated by Conductivity and Membrane Potential Measurements

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Membrane potential and electrical resistance for two isolated fruit cuticular membranes (tomato and pepper cuticles) were measured for different NaCl solutions (10^{-3} M < C < 5×10^{-2} M). From these experimental results some membrane characteristic parameters were obtained such as cation transport number, t_+, which represents the fraction of the electric current transported by the cation, I_+, with respect to the total current, I_T ($t_+ = I_+/I_T$), and ionic permeabilities, P_+ and P_-, for both membranes. Concentration dependence of these parameters was also considered. A comparative study between both types of fruit cuticles is also made. It was found that cation transport number, ionic permeabilities and electrical resistances are quite similar for both membranes.

Introduction

The plant cuticular membrane can be considered as the interface between the plant and the environment. The cuticle is a heterogeneous membrane consisting of a polyester matrix (cutin) and cuticular lipids, but carbohydrate, phenolic acids and other compounds may also be present (Holloway, 1982; Martin and Juniper, 1970). Some evidences suggest that fruit cuticles are weak ion/exchange membranes (Schönherr and Bukovac, 1973; Heredia and Benavente, 1991). This fixed charge is an important characteristic which could affect both the sorption and transport of water and ions through the cuticle, and it also can play an important role in some interfacial processes related to agricultural or ecotoxicological problems. For this reason, measurements of some electrokinetic parameters could give us information about the electrical behaviour of fruit cuticles.

In this work, membrane potential and conductivity for isolated pepper and tomato fruit cuticles were measured with NaCl solutions, for concentration ranging between 10^{-3} M and 5×10^{-2} M. From these experimental values some membrane characteristic parameters such as transport numbers, ionic diffusion coefficient ratio and ionic permeabilities in the membranes can be determined. Concentration of these parameters was varied as well as a comparison was made between both kinds of cuticles.

Experimental

Materials

Astomatous cuticles from tomato (*Lycopersicon esculentum* Mill.) and pepper (*Capsicum anuum* L.) fruits were used. The cuticles were enzymatically isolated following the procedure described by Shafer and Bukovac (1987).

Wet membranes thickness, δ, was measured with a precision micrometer MX-Matrix-II, and the following average value, <δ>, which corresponds to six measurements covering the whole membrane area, for each sample, were obtained as follows: tomato: <δ> = (23 ± 2) μm; pepper: <δ> = (40 ± 2) μm.

Experiments were carried out using NaCl (Merck$^{\text{TM}}$, 99.5% purity) aqueous solutions at different concentrations. Before use, the cuticles were kept for at least 48 h in distilled water, and

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later they were immersed for 12 h in a solution of the appropriate concentration.

The cation exchange capacity of both cuticles was determined equilibrating several membrane pieces of known weight in 10 ml of a 0.1 M NaCl solution for 24 h at room temperature (around 25 °C). Thereafter, the cuticle pieces were washed ten times in 10 ml of deionized water (5 min each) to removed sorbed cations. The exchangeable ion was eluted from cuticular membranes by washing three times with 3 ml of 1 M HCl solution for 15 min each. The cation concentration was measured by flame photometry (Corning 410 C model). A standard curve for sodium ion over an appropriate concentration range was used. A 1 M HCl solution was used as blank. The following values for the cation exchange capacity values for each fruit cuticle were determined: tomato: (2.1 ± 0.8) 10^{-4} mol of Na^+/g cuticle; pepper: (0.74 ± 0.03) 10^{-4} mol of Na^+/g cuticle.

These results are in good agreement with those indicated in the literature (Schönherr and Huber, 1977).

In order to obtain some structural parameters an X-ray analysis was made for the two cuticular membranes. X-ray diffraction patterns of both isolated cuticles are very similar and they show a common basal spacing around 4.5 Å (Heredia *et al.*, 1991).

Membrane potential measurements

The membrane potential measuring cell is similar to that described by Heredia and Benavente (1991). The cell itself was formed by two half-cells separated by two silicone rings (membrane holder), where the cuticle was placed. Membrane potential measurements were carried out by keeping constant and equal to 2, the concentration ratio of the solutions on both sides of the membrane, $\gamma = C_o/C_i = 2$, for concentrations ranging between 10^{-3} M and 5×10^{-2} M, being C_o the salt concentration in contact with the outer cuticle surface (high concentration) and C_i the concentration in contact with the inner membrane surface (low concentration).

In all cases the membrane potential, $\Delta\phi$, was measured at a constant temperature (t = 25 °C) with Calomel electrodes introduced into the NaCl solutions *via* KCl-agar saline bridges. The electrode placed into the solution of low concentration, C_i, was grounded; that means: $\Delta\phi = \phi(C_o) - \phi(C_i)$.

Electrical resistance

The experimental device for measuring the electrical resistance was described by Cabeza *et al.* (1991). An alternating current bridge (Wayne Kerr, Automatic Precision Bridge, model B 905) with four different frequencies (f = 100 Hz, 400 Hz, 1 kHz and 10 kHz) was used. Because of the variation of cuticular resistance with frequency (1% for the interval 100 Hz–1 kHz, and 10% for 1 kHz–10 kHz), results at the frequency of 1 kHz are only presented (Nakanishi *et al.*, 1991).

The electrical resistance was determined for different NaCl solutions, for concentrations ranging between 2×10^{-3} M and 5×10^{-2} M, with the solution at both sides of the membranes having the same concentration. All resistance measurements were made with the cuticular membranes placed in the membrane holder (R_{md}) and without them (R_d). Difference between both values was taken as the membrane resistance (R_m). The contribution of surface contact resistances are the same in both R_{md} and R_d, and therefore they cancel out (Lakshminarayanaiah, 1966).

Results and Discussion

Membrane potential, $\Delta\phi$, as a function of the NaCl concentration in contact with the internal face of the membrane, C_i, is shown in Fig. 1 for both cuticles. From this picture, increasing $\Delta\phi$ values when the concentration increases can be observed for both membranes, which is more significant at low concentrations (for concentrations ranging between 2×10^{-3} M and 10^{-2} M).

For these systems, the membrane potential is mainly due to the different mobility (or transport number, t_i) of the ions across the membrane, and for external salt concentration higher than the concentration of fixed charge in the membrane (diffusion potential), it can be expressed by (Jonsson and Benavente, 1992):

$$\Delta\phi = (RT/F)[(t_-/z_-) - (t_+/z_+)]\ln(C_o/C_i), \qquad (1)$$

where t_- and t_+ are the anion and cation transport numbers respectively, and they represent the fraction of the total intensity carried by each ion (t_i =

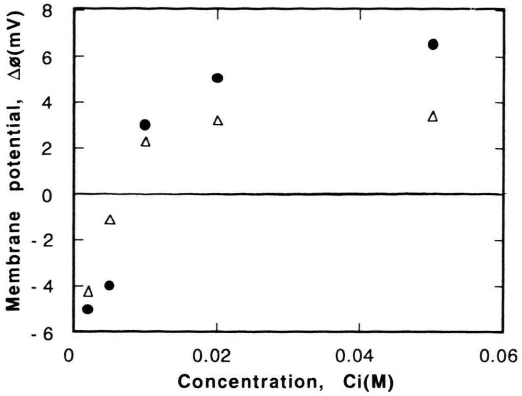

Fig. 1. Membrane potential, Δø, *versus* concentration at the inner surface of the cuticular membranes, C_i: (△) tomato; (●) pepper.

I_i/I_T); z_- and z_+ are the anion and cation valencies; *R* and *F* are the gas and Faraday constant, *T* is the temperature of the system and the other symbols have already been explained. Taking into account that: $t_+ + t_- = 1$, the cation or anion transport number in the cuticles can be calculated from the experimental values by Eqn. (1).

Variation of the cation transport number with the average concentration C_{avg}, ($C_{avg} = (C_o + C_i)/2$), for both cuticles are drawn in Fig. 2. A decrease of t_+ values when the concentration increases can be observed for both membranes, but at high concentrations t_+ remains almost constant. This kind of behaviour is similar to that indicated in the literature for artificial membranes (Lakshminarayanaiah, 1966).

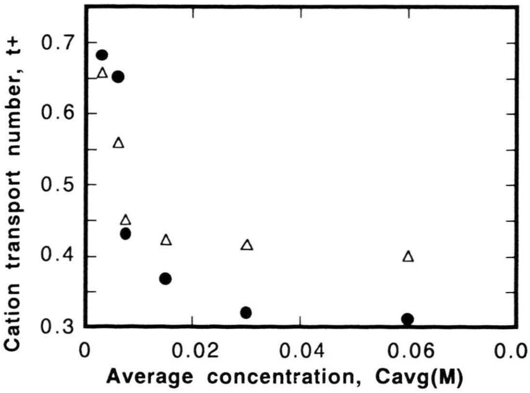

Fig. 2. Variation of the cation transport number, t_+, with the average concentration, C_{avg}: (△) tomato; (●) pepper.

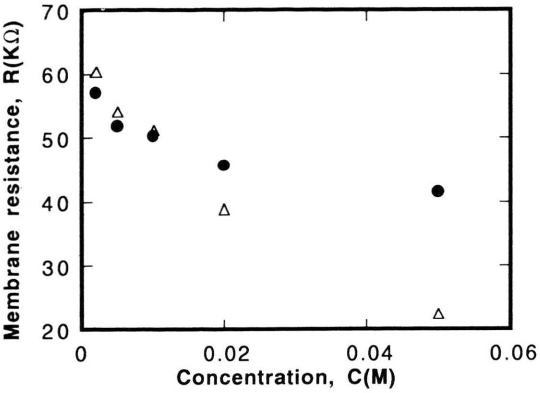

Fig. 3. Membrane resistance, *R*, *versus* concentration *C*, (C_i=C_o=C), (△) tomato; (●) pepper.

Fig. 3 shows the variation of the electrical resistance as a function of the external salt concentration (C_i=C_o=C). The decrease of R_m values when the concentration increases is attributed to the salt invasion into the cuticular matrix (Benavente and Jonsson, 1993).

From membrane potential and resistance values (or membrane conductance, $\lambda = 1/R$), the ionic permeabilities, P_i, through the cuticular membranes can be determined by the following expression (Tyree *et al.*, 1991):

$$P_+/P_- = \exp[(F/RT)\Delta\varnothing] - \gamma/(1 - \gamma \exp[(F/RT)\Delta\varnothing] \qquad (2)$$

$$P_+ + P_- = \lambda RT/F^2Sz^2C \qquad (3)$$

P_+ and P_- are the cation and anion permeability, respectively (P_i = ionic diffusion coefficient/membrane thickness); *S*, the membrane area; $\gamma = C_i/C_o = 2$, and the other parameters have already been indicated. Conductance values at the average concentration were obtained by extrapolation of the results in Fig. 3.

Variation of the ionic permeabilities with concentration is shown in Fig. 4, for tomato and pepper cuticles. A decrease of the P_i values when the concentration increases is also found, being this fact more significant for the pepper cuticle; in all cases the values for the ionic permeabilities are around 10^{-9} m/s, which is in good agreement with values in the literature for different fruit cuticles (Tyree *et al.*, 1991).

Results obtained for the different electrokinetic parameters determined for the two fruit cuticles studied (isolated tomato and pepper cuticular membranes) indicate a weak ion exchange character for these membranes, because their electrical properties are more evident at low external concentration, while the effect of the fixed charge is almost masked at high values of the external salt concentrations. Although the chemical composition of both types of cuticles is different (Martin and Juniper, 1970), the electrical behaviour with respect to the characteristic parameters studied in this paper, such as cation transport number, ionic permeability and electrical resistance is quite similar for both membranes.

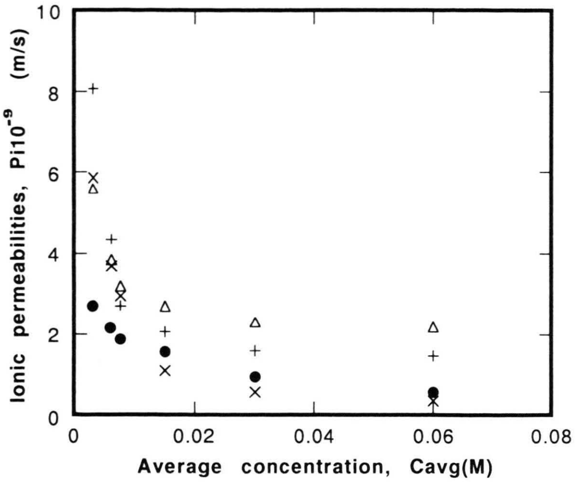

Fig. 4. Ionic permeabilities, P_+ and P_-, as functions of the average concentration, C_{avg}, tomato: (+) P_+, (△) P_-; pepper: (×) P_+, (●) P_-.

Acknowledgements

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