Impedance measurement of differently treated tomato cuticle membranes with calcium solutions

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Electrical response of two differently treated tomato cuticles has been obtained by impedance measurements with CaCl₂ and Ca(NO₃)₂ aqueous solutions, and the results have been compared with those corresponding to an untreated tomato membrane for the same electrolytes. Variation of the electrical resistance with concentration was determined, and the differences are attributed to some variation in the geometry of the membrane structure, but no electrical changes in the membrane matrix were found.

1. Introduction

The study of the physico-chemical processes occurring in the cell membranes are frequently discouraged by the complex structure and multicomponent nature of these membranes and, in a great part, they are responsible for the interest to know the properties of more simple biological systems. One of these systems is the plant cuticle, which is a heterogeneous membrane mainly formed by a polyester matrix (cutin) and cuticular lipids [1]. In a series of previous papers, the transport of electrolyte solutions across isolated tomato cuticular membranes was studied and different parameters such as fixed charge, permselectivity or electrical resistance were determined [2–4].

In this work, the effect of ionic treatment on the structure of the cuticle is considered by measuring the impedance for two electrolytes (CaCl₂ and Ca(NO₃)₂) at different concentrations. A comparison of the results found for two differently treated tomato cuticles (sodium and calcium forms) with those corresponding to an untreated membrane shows some differences in the electrical response due to the treatment and the electrolyte, but no electrical changes in the cuticles structure were found.

2. Experimental

2.1. Materials

Different pieces of astomatous cuticles from tomato fruits (Lycopersicon esculentum, Mill) were used. The cuticles when isolated using the ammonium oxalate/oxalic acid method indicated in ref. [5]. These untreated or native membranes were cleaned with a jet of water and they will hereafter be called T(nat). Some of these cuticles were put in the Na⁺ or Ca²⁺ forms by equilibration with a 10⁻² M NaCl or CaCl₂ in Tris buffer solutions (pH 7), respectively. These membranes, which were washed in the way indicated in [6], will hereafter be referred to as T(Na) and T(Ca).

Measurements were carried out with aqueous CaCl₂ and Ca(NO₃)₂ solutions, at different concentrations (10⁻³ M < C < 410⁻² M) and room temperature (t ≈ 25°C).
2.2. **Impedance measurements**

Electrical impedance of three tomato cuticles, \(T(\text{nat})\), \(T(\text{Na})\) and \(T(\text{Ca})\) was measured using a frequency response analyzer FRA (Solartron 1255), for frequency ranging between 100 Hz and 10 MHz. The experimental data were corrected by software, and the influence of connecting cables and other parasite capacitances was eliminated [7].

The measuring cell is similar to that described in [8] and it basically consists of two half-cells separated by the membrane holder, which had a free area of \(1 \text{ cm}^2\). Each half-cell was filled with the different solutions, but both having the same concentration in each measurement.

3. Results and discussion

In fig. 1 a plot of the imaginary impedance \((Z'')\) versus frequency for \(T(\text{nat})\) cuticle and both electrolytes is shown \((C=0.005 \text{ M})\). From this picture, two relaxation effects can be seen: one at low frequencies \(10 < f(\text{Hz}) < 5 \times 10^3\), which corresponds to the membrane; and the other one at high frequencies \(5 \times 10^3 < f(\text{Hz}) < 10^7\) is due to the electrolytes. For this reason, only frequencies lower that \(5 \times 10^3 \text{ Hz}\) will be considered in the following discussion (membrane effect). This picture also shows the different electrical response for a given membrane and both electrolytes, at the same concentration.

In fig. 2 (a and b) the Nyquist plots for the three cuticular membranes with both electrolytes are drawn, and the influence of the exchangeable cation on the membrane impedance is evident, but also different cuticles/solutions interactions exist and the following sequence, depending on the system, is obtained:

\[
\text{CaCl}_2: T(\text{nat}) > T(\text{Ca}) > T(\text{Na}),
\]

\[
\text{Ca(NO}_3\text{)}_2: T(\text{Ca}) > T(\text{nat}) > T(\text{Na}),
\]

in both cases lower values for the sodium cuticle were found. This fact could be due to a competition between the cuticle exchange capacity and the inorganic anions to the calcium in the membrane arrangement. It seems that a higher competition exists with the nitrate ions.

![Fig. 1. Imaginary impedance versus frequency for native tomato with CaCl2 and Ca(NO3)2 solutions \((C=0.005 \text{ M})\).](image1)

![Fig. 2. Nyquist plot for three cuticular membranes at \(C=0.01 \text{ M}\): (a) CaCl2; (b) Ca(NO3)2: (+) T(nat); (O) T(Ca); (Δ) T(Na).](image2)
Fig. 3. Cuticles resistance versus concentration for the three membranes: (a) CaCl₂; (b) Ca(NO₃)₂; (✚) T(nat); (○) T(Ca); (△) T(Na).

The experimental values, for each membrane and electrolyte solution, were fitted to a circuit which consists in a parallel combination of a constant phase element \( Q_s (Z_s(\omega) = A_s (i\omega)^{-n}) \) and a resistance \( R \) using a NLLS program [9]. Variation of \( R \) values with concentration are drawn in fig. 3 for the three membranes and electrolytes. These values were fitted to the following expression:

\[
R(C) = R_0 - a C^b,
\]

where \( R_0 \) represents the polyester matrix resistance without salt contribution (\( C = 0 \)). The values of the different parameters indicated in eq. (1) are written in table 1, for the different systems studied. These results show an almost constant value for the cuticular matrix resistance, independent of the treatment, which is of the same order of magnitude to that previously found with dry samples (without any solution inside the membrane) [6].

From the circuit analysis the values of both the admittance \( Y = 1/A_s \) and the parameter \( n \) were obtained, for each membrane and electrolyte solution. Results show that they hardly depend on the concentration but some differences in the values of the treated membranes with respect to the native ones were found. Average values of both parameters for each membrane and electrolyte are shown in table 2.

From the impedance data the maximum frequency, \( f_{\text{max}} \), for each measurement was determined. An equivalent capacitance, \( C_{\text{eq}} \), which would be the membrane capacitance if it behaves as a parallel RC circuit, can be estimated by the expression:

\[
C_{\text{eq}} = \frac{1}{\pi R f_{\text{max}}},
\]

although \( R \) and \( f_{\text{max}} \) are concentration-dependent parameters, \( C_{\text{eq}} \) is almost independent of it, and their average values are also shown in table 2. For a given membrane, \( C_{\text{eq}} \) values are also independent of the electrolyte considered, but a comparison of the results found for the three membranes shows that some influence of the treatment on \( C_{\text{eq}} \) values exists. These results are attributed to some structural changes on the membrane matrix which agrees with the results previously obtained by X-ray diffraction [10].

### Table 1

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>T(nat)</th>
<th>T(nat)</th>
<th>T(Na)</th>
<th>T(Na)</th>
<th>T(Ca)</th>
<th>T(Ca)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R₀ (kΩ)</td>
<td>CaCl₂</td>
<td>Ca(NO₃)₂</td>
<td>CaCl₂</td>
<td>Ca(NO₃)₂</td>
<td>CaCl₂</td>
<td>Ca(NO₃)₂</td>
</tr>
<tr>
<td>a (kΩ/mol)</td>
<td>39760</td>
<td>39760</td>
<td>399485</td>
<td>399485</td>
<td>40336</td>
<td>40336</td>
</tr>
<tr>
<td>b</td>
<td>0.0163</td>
<td>0.0056</td>
<td>0.0043</td>
<td>0.0043</td>
<td>0.0017</td>
<td>0.0017</td>
</tr>
</tbody>
</table>

From the circuit analysis the values of both the admittance \( Y = 1/A_s \) and the parameter \( n \) were obtained, for each membrane and electrolyte solution. Results show that they hardly depend on the concentration but some differences in the values of the treated membranes with respect to the native ones were found. Average values of both parameters for each membrane and electrolyte are shown in table 2. For a given membrane, \( C_{\text{eq}} \) values are also independent of the electrolyte considered, but a comparison of the results found for the three membranes shows that some influence of the treatment on \( C_{\text{eq}} \) values exists. These results are attributed to some structural changes on the membrane matrix which agrees with the results previously obtained by X-ray diffraction [10].

### Table 1

Values determined from eq. (1) for the three tomato cuticles and CaCl₂ and Ca(NO₃)₂ electrolytes.
Table 2
Average values of the admittance, $Y$, and equivalent capacitance, $C_{eq}$, for the three tomato cuticles and electrolytes.

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>$T(\text{nat})$</th>
<th>$T(\text{nat})$</th>
<th>$T(\text{Na})$</th>
<th>$T(\text{Na})$</th>
<th>$T(\text{Ca})$</th>
<th>$T(\text{Ca})$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CaCl$_2$</td>
<td>Ca(NO$_3$)$_2$</td>
<td>CaCl$_2$</td>
<td>Ca(NO$_3$)$_2$</td>
<td>CaCl$_2$</td>
<td>Ca(NO$_3$)$_2$</td>
</tr>
<tr>
<td>n</td>
<td>0.87 ± 0.02</td>
<td>0.823 ± 0.019</td>
<td>0.77 ± 0.004</td>
<td>0.767 ± 0.004</td>
<td>0.759 ± 0.003</td>
<td>0.754 ± 0.003</td>
</tr>
<tr>
<td>C$_{eq}$ (F)</td>
<td>6.5 ± 0.09</td>
<td>0.81 ± 0.07</td>
<td>1.51 ± 0.13</td>
<td>1.27 ± 0.14</td>
<td>2.32 ± 0.18</td>
<td>1.98 ± 0.21</td>
</tr>
</tbody>
</table>

As a conclusion to this paper, we can say that ionic treatment hardly affects the electrical nature of the tomato cuticular membrane but some changes in the geometry of the matrix structure can exist.

Acknowledgement

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References