Exchange Properties of Isolated Cell Walls of *Lemna minor* L.\(^1\)

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**ABSTRACT**

From our theoretical treatment which is an extension of the classical Donnan theory, we have estimated the rational selectivity coefficients of the carboxylic groups of the walls during exchanges of divalent ions against monovalent ones (i.e., calcium and potassium or calcium and sodium ions). These coefficients express the interactions between the different ions, those between the counter-ions and the ionized groups of the wall, and the influence of water. These quantitative values are consistent with the great affinity of the carboxylic groups for the calcium ions. They vary with the experimental conditions, showing a purely physicochemical mechanism of “regulation” of the exchanges in the cells walls of *Lemna minor* L.

Among the components of the cell wall, uronic acids and proteins play a major role in ion exchanges by plants (2, 12, 14, 18). These different molecules carry carboxylic groups which can be ionized according to the pH and the ionic strength of the solution bathing the cell wall. The presence of these charges gives to the wall the properties of an ion exchanger. Mainly, the exclusion of co-ions (ions of the same sign as that of the fixed charges in the wall) and the exchange of counter-ions (ions of the opposite sign) go together with the selectivity properties.

Artificial ion exchangers have been widely studied and different models have been developed to explain the observed results (10, 13, 15, 21). The absorption of ions by plant cell walls has been analyzed using the framework of the Donnan theory which assumes a uniform electric field in the wall, or else with the help of the double layer theory which takes into account the distribution of the potential in the wall (3, 25).

In most cases, a simplified theory has been used (i.e., classical Donnan theory) where the activity coefficients and the specific interactions between the absorbed ions and the ion exchanger groups are neglected (4, 5, 17). Such an analysis leads to a distribution of ions which is controlled by the charge of the different species only; it cannot explain the selectivity resulting from the specific interactions between the charged groups in the wall and a given diffusible species.

Due to these interactions, the ionization of the carboxylic groups is modified for varying external concentrations, and therefore so is the charge density of the wall. This charge density is important when one studies the distribution of ions between the wall and the neighboring medium. It is a parameter which intervenes in ion exclusion phenomena, distribution of counter-ions, swelling of the wall, and selectivity of the sites for the different species. Most theories used to explain ion absorption in cell wall implicitly assume a constant fixed charge density, whereas our previous experiments have shown that the effect of Ca on the uptake of K and on the exclusion of chloride is characteristic of a variable charge density (6). It seems that the interaction between carboxylic groups and Ca ions is so strong that only high concentrations of K might succeed in totally ionizing the uronic acids. From these experiments it was important to study quantitatively the exchange between Ca and K (or Na).

**THEORETICAL REMINDER**

In a previous paper (6) we have shown that the distribution coefficient, as introduced from the ideal Donnan theory, cannot explain the observed results; even at low external concentrations of KCl, the chloride concentration inside the wall is far from being negligible. It has led us to introduce a coefficient of deviation (called \(\Gamma\)) to the ideal Donnan theory; this coefficient integrates all of the interactions (ion/ion, ion/charge) due to the nature of the wall (which can be considered as a concentrated polyelectrolyte solution) as well as the difference in the activity of water, inside and outside. The ionic distribution is thus written for a salt \(\text{p}_M \text{M}^{2+} \cdot \text{p}_A \text{A}^{-}\):

\[
\alpha = \frac{1}{\Gamma} \left( \frac{z_M}{c_M} \right) \left( \frac{z_A}{c_A} \right) = \left( \frac{z_M}{c_M} \right) \left( \frac{z_A}{c_A} \right) \frac{1}{\Gamma}
\]

\(\text{M}\) stands for the counter-ion, \(A\) for the co-ion, \(\Gamma\) for stoichiometric coefficients, \(z\) for valences, \(C\) for molal concentrations; the bar refers to values inside the wall. As published previously (6) this coefficient \(\alpha\) has enabled us to estimate the coefficient \(\Gamma\) to be approximately equal to 0.2 for KCl in equilibrium with Ca-saturated cell walls.

Here we shall deal with an exchange between two species of counter-ions, \(M_1\) and \(M_2\), the valences of which are, respectively, \(z_{M_1}\) and \(z_{M_2}\); and there is a single co-ion \(A\), the valence of which is \(z_A\).

The ionic distribution can be written for the two species \(M_1\) and \(M_2\):

\[
1 - \left( \frac{1}{z_{M_1}} \right) \left( \frac{c_{M_1}}{c_A} \right) = \left( \frac{1}{z_{M_2}} \right) \left( \frac{c_{M_2}}{c_A} \right)
\]

Rewriting equation 2 for the two counter-ions enables us to get the selectivity coefficient:

\[
K_1^2 = \left( \frac{z_{M_1}}{c_{M_1}} \right) \cdot \left( \frac{z_{M_2}}{c_{M_2}} \right) = \left( \frac{1}{\Gamma_1} \right) \left( \frac{1}{\Gamma_2} \right) z_{M_1} z_{M_2} = K_1^2
\]

\(K_1^2\) expresses the relative interactions of the carboxylic charges with the different species. Hence, the selectivity of the wall for species 2 as compared to species 1 is expressed by \(K_1^2\); if \(K_1^2\) is bigger than 1 it means that counter-ion 2 is fixed more tightly between the species.

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than counter-ion $1$. As it is seen in equation 3, the determination of $K_2$ needs the knowledge of the $C$, hence of the volume of swelling water (which depends on the ionic strength of the external medium); thus, the interest of introducing a rational selectivity coefficient $R_i^2$, defined from the ionic fractions $X$ (which are volume independent).

$$R_i^2 = \left( \frac{X_{M1}}{X_{M1}} \right) \cdot \left( \frac{Z_{M2}}{Z_{M2}} \right)^{Z_{M1}} \quad (4)$$

with

$$X_{M1} = \frac{Z_{M1}}{Z_{M2}} \cdot \frac{C_{M1}}{C_{M2}} \quad (5)$$

In the particular case when $Z_{M1} = Z_{M2}$, the two coefficients $K_i^2$ and $R_i^2$ are identical.

Even if $K_i^2 = 1$ (there is then no selectivity), which brings one back to the case of the ideal Donnan law, the distribution of the various counter-ions in the cell wall will not be the same as in the outside medium, when the various $Z_{M}$ have not the same value. Indeed in such a case, from equation 3, with $Z_{M1} > Z_{M2}$, one obtains:

$$\left( \frac{C_{M1}}{C_{M2}} \right)^{Z_{M1}} \cdot \left( \frac{C_{M1}}{C_{M2}} \right)^{Z_{M2}} > 1 \quad (6)$$

because, for the ideal Donnan law:

$$\frac{C_{M1}}{C_{M2}} > 1 \quad (7)$$

is always satisfied. Hence, by raising to $\frac{1}{Z_{M1}}$ power

$$\frac{C_{M1}}{C_{M2}} > \frac{C_{M1}}{C_{M2}} \quad (8)$$

This is a purely electrostatic effect (called electroselectivity) (1) which will tend to favor the accumulation of the counter-ion with the highest valence in the cell wall.

**MATERIALS AND METHODS**

**Plant Material.** The plants are *Lemna minor* L. grown in aseptic conditions (following a procedure adapted from Hillman [11]), i.e., they are kept under continuous light, given by incandescent lamps and fluorescent tubes, with 3,500 lux at the level of the plants. The temperature is maintained at $20 \pm 1 \, ^\circ C$; the nutrient medium is the same as previously (23) with a mean pH 7 over the growth period.

**Preparation of Isolated Cell Walls.** The process of cell wall extraction was adapted from Ridge and Osborne (19) Esquerre Tugaye (7), and Esquerre Tugaye and Maxau (8). It tends to minimize contamination of the cell wall material by cytoplasmic proteins. The plants are first plasmolyzed in 1 M sucrose solution for 2 hr; then they are ground in an ice-cold phosphate buffer (pH 7.2). The cell walls are extracted from the homogenate by centrifugation at 500g and 0 C, then they are washed successively in a phosphate buffer, 0.5% Triton X-100 solution, a 1 M NaCl solution, a 0.5 M NaHCO$_3$ solution, and finally in pure acetone. Several washings with distilled H$_2$O are made between each of these operations. The microscopic observations have shown no significant cytoplasmic contaminations.

**IR Characterization of Isolated Cell Wall.** In order to characterize the sites involved in the exchange of counter-ions, we have made IR measurements on cell walls neutralized with different counter-ions (Na, K, or Ca) by using their respective hydroxide in the presence of the chloride salt (0.1 M); the pH of the solution was then 7.2. Other samples were in proton form. The different samples were washed with distilled H$_2$O, filtered and dried at 40 C for 20 hr. Three mg of dry cell walls were mixed with 500 mg of KBr and pressed at 100 bars to obtain the samples to be analyzed. The IR measurements have been performed on a Perkin Elmer 177 spectrograph; the reference was pure KBr. Typical spectra are presented in Figure 1. The most interesting range is from 1,600 to 1,800 cm$^{-1}$. The wall in the protonic form shows two bands, one at 1,775 cm$^{-1}$, corresponding to COOH stretch, and another one at 1,650 which is probably due to the presence of water. The wall, in salt form, presents one band at 1,620 cm$^{-1}$ and a shoulder at 1,550 cm$^{-1}$ corresponding to COO$^-$. One must note that there are no significant differences between the spectra of the wall in the different salt forms.

The characteristics of the spectra are in good agreement with those obtained by Scharzmaier and Brehm (20) with the wall of *Sphagnum*.

**Experimental Conditions.** The samples of isolated cell walls (about 10 mg dry weight each) are placed in solutions with the same co-ion and different ratios of mono- to divalent counter-ions. Equilibrium is established by shaking the flasks for several hr; the walls are separated from the solution, and the counter-ions inside the wall are removed by a given volume of acid (same co-ion). The quantitative measurements are performed by using radioactivity for the minor ion, and flame photometry.

**EXPERIMENTAL RESULTS**

The isolated cell walls, previously put into either the Na or the Ca form, are equilibrated for several hr with different ratios of mono- and divalent ions. The counter-ions fixed in the wall are detected as indicated above. Experimental conditions and results are summarized in Table 1.

In Figure 2 we have plotted the amount of Na ions fixed in the wall when varying the external concentration of Ca and Na. From these curves it is quite clear that increasing the concentration of Ca releases the Na previously fixed in the wall. The sharp decrease of $C_{Na}$ suggests a high affinity of the carboxylic groups for the Ca ions. Such a behavior has been already demonstrated on artificial ion exchangers (9) and on alginate (22). This release is especially important when $C_{Ca}$ is below 1 mm. For higher $C_{Ca}$ values (up to 6.0) we have plotted the amount of Ca ions fixed in the wall when varying the external concentration of Na and Ca. From these curves it is quite clear that increasing the concentration of Na releases the Ca previously fixed in the wall. The sharp increase of $C_{Ca}$ suggests a high affinity of the carboxylic groups for the Al ions. Such a behavior has been already demonstrated on artificial ion exchangers (9) and on alginate (22). This release is especially important when $C_{Na}$ is below 1 mm. For higher $C_{Na}$ values (up to 6.0)

![Fig. 1. IR spectra for isolated cell walls of L. minor with different concentrations of Na and Ca.](image-url)
Table I. Estimation of the concentration of fixed charges. The value of $C_A$ is given by $C_{Na} + 2C_{Ca}$. The concentrations are given in millimoles per liter (mM). The ionic fractions X are dimensionless.

<table>
<thead>
<tr>
<th>$C_{NaCl}$</th>
<th>$C_{CaCl_2}$</th>
<th>$C_{Na}$</th>
<th>$C_{Ca}$</th>
<th>$C_{A}$</th>
<th>$X_{Ca}$</th>
<th>$X_{Ca}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>0.1</td>
<td>272</td>
<td>55</td>
<td>382</td>
<td>0.026</td>
<td>0.29</td>
</tr>
<tr>
<td>0.5</td>
<td>123</td>
<td>143</td>
<td>409</td>
<td>0.118</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>99</td>
<td>204</td>
<td>507</td>
<td>0.21</td>
<td>0.805</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>41</td>
<td>195</td>
<td>431</td>
<td>0.57</td>
<td>0.905</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>34</td>
<td>206</td>
<td>446</td>
<td>0.72</td>
<td>0.925</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>0.1</td>
<td>342</td>
<td>46</td>
<td>434</td>
<td>0.0115</td>
<td>0.21</td>
</tr>
<tr>
<td>0.5</td>
<td>134</td>
<td>96</td>
<td>326</td>
<td>0.055</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>134</td>
<td>121</td>
<td>376</td>
<td>0.104</td>
<td>0.645</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>101</td>
<td>198</td>
<td>497</td>
<td>0.369</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>119</td>
<td>303</td>
<td>525</td>
<td>0.539</td>
<td>0.84</td>
<td></td>
</tr>
</tbody>
</table>

10 mM) the remaining Na ions are only slightly displaced and thus seem to be tightly fixed in the wall; their affinity for the carboxylic groups increases. This is due to a decrease of the probability for the Ca ions to find two free neighboring sites. This effect will be discussed more thoroughly further on.

Concentration of Ionized Groups. Figure 3 gives the concentration of ionized carboxylic groups. It is determined by summing up the fixed Ca and Na ions and assuming a constant swelling (0.7 g of water/g of wet cell wall). At pH 7 the carboxylic groups seem to be totally ionized, with a mean value of 440 ± 50 mM. There is some dispersion of the experimental results; it is probably due to the fact that the washing of the samples is not too thorough (in order to avoid any hydrolysis of the carboxylates). At pH 4, only a high concentration of Ca can totally ionize the carboxylic groups, given the high affinity of the COO⁻ for protons.

Ion Exchange Isotherms. In order to analyze the ion exchanges we have plotted different isotherms in Figures 4 (exchange Ca/Na), 5 (exchange Ca/K), and 6 (exchange Na/K). Such curves are pseudoisotherms only, since the ionic strength was not kept constant. We have drawn the theoretical curves of electroselectivity assuming that the total concentration of fixed charges is equal to 440 mM as indicated above.

For the mono/divalent ion exchanges the experimental isotherms are concave to abscissa, and they lie above the corresponding theoretical electroselectivity curves. As shown in the theoretical calculations, this supports the assumption of a specific affinity of the...
wall carboxylic acids for Ca ions as compared to K or Na ions. Conversely, for the Na/K exchange, the relative affinity for the two ions depends on the concentration conditions; for $X_{Na}$ below 0.7 the Na$^+$ ions are fixed preferentially to the K$^+$ ions, whereas the reverse becomes true for $X_{Na}$ above 0.7. To quantify these phenomena the rational selectivity coefficients have been calculated and are discussed below.

**Rational Selectivity Coefficients.** Table II gives the value of the rational selectivity coefficients (see equation 4) as calculated from the smooth curves are not from the individual experimental points. One must note that due to the definition of this coefficient, it is characteristic of one experimental condition only; in the case of an exchange of a divalent against a monovalent counterion, the molal selectivity coefficient alone should be a constant. Therefore, the experimental values of the rational coefficients are discussed by correlating them with the composition and the ionic strength of the experimental medium.

**Influence of Ionic Fraction.** The rational selectivity coefficients are plotted on Figure 7 in the case of a mono/divalent ion exchange. A maximum is observed for an ionic fraction in the range of 0.1 to 0.3, showing that in these conditions, the affinity of the carboxylic groups for the Ca ions is highest. This behavior has been described by Foliard on polyacrylic membrane (9) with a maximum of selectivity for $X_{Ca}$ near 0.3. The maximum could be interpreted as a result of two antagonistic processes: when the ionic fraction is almost zero, Ca uptake is determined by the swelling rate, increasing Ca concentrations draws the pectin acid chains closer together by reducing the swelling of the wall, and it promotes the bonding of Ca to pairs of neighboring charges; when the ionic fraction of Ca in the wall ($X_{Ca}$) is high, the probability of Ca finding two free neighboring sites decreases (saturation effect). For the Na/K exchange, $R_{K}^{Ca}$ decreases over the whole range of studied concentrations.

**Influence of Ionic Strength.** At constant ionic fraction, by increasing the ionic strength of the bathing medium, the relative affinity of the wall for the Ca ions is decreased (as can be observed

![Figure 6. Pseudoisotherm of the Na-K exchange for a varying outside Na concentration and an 8.5 mM outside concentration of K (pH = 6). First bisectrix corresponds to electroselectivity. (O): experimental results.](image)

![Figure 7. Evolution of rational selectivity coefficients. (O): Ca/Na exchange $C_{Na}$ 17 mM; (A): Ca/Na exchange $C_{Na}$ 7.5 mM; (I): Ca/K exchange $C_{K}$ 8.5 mM.](image)

**Table II.** Calculation of ionic fractions and rational selectivity coefficients for exchanges between calcium and sodium, calcium and potassium, sodium and potassium for isolated cell walls.

<table>
<thead>
<tr>
<th>$C_{Na}$ : 7.5mM</th>
<th>$C_{Na}$ : 17mM</th>
<th>$C_{K}$ : 8.5mM</th>
<th>$C_{K}$ : 8.5mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_{Ca}$</td>
<td>$X_{Na}$</td>
<td>$R_{Ca}^{Na}$</td>
<td>$X_{Ca}$</td>
</tr>
<tr>
<td>0.1</td>
<td>0.67</td>
<td>49.8</td>
<td>0.65</td>
</tr>
<tr>
<td>0.2</td>
<td>0.8</td>
<td>64</td>
<td>0.72</td>
</tr>
<tr>
<td>0.3</td>
<td>0.85</td>
<td>61.7</td>
<td>0.77</td>
</tr>
<tr>
<td>0.4</td>
<td>0.87</td>
<td>46</td>
<td>0.8</td>
</tr>
<tr>
<td>0.5</td>
<td>0.89</td>
<td>36</td>
<td>0.83</td>
</tr>
<tr>
<td>0.6</td>
<td>0.91</td>
<td>30</td>
<td>0.855</td>
</tr>
<tr>
<td>0.7</td>
<td>0.93</td>
<td>24</td>
<td>0.885</td>
</tr>
<tr>
<td>0.8</td>
<td>0.95</td>
<td>19</td>
<td>0.92</td>
</tr>
<tr>
<td>0.9</td>
<td>0.975</td>
<td>16</td>
<td>0.96</td>
</tr>
</tbody>
</table>

**Table III.** Rational selectivity coefficient $R_{K}^{Ca}$ as a function of the outside concentrations of calcium, for walls in situ. At any given concentration of calcium $C_{Ca}$, the different ionic fractions of calcium $X_{Ca}$ are obtained by varying the potassium concentration.

<table>
<thead>
<tr>
<th>$C_{Ca}$</th>
<th>$X_{Ca}$</th>
<th>$X_{Ca}$</th>
<th>$X_{Ca}$</th>
<th>$X_{Ca}$</th>
<th>$X_{Ca}$</th>
<th>$X_{Ca}$</th>
<th>$X_{Ca}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.7</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>999</td>
<td>1760</td>
<td>2205</td>
<td>3436</td>
<td>4705</td>
<td>5117</td>
<td>7980</td>
<td>11100</td>
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<tr>
<td>235</td>
<td>361</td>
<td>424</td>
<td>424</td>
<td>539</td>
<td>812</td>
<td>1990</td>
<td>1773</td>
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<tr>
<td>5</td>
<td>913</td>
<td>1022</td>
<td>1022</td>
<td>1022</td>
<td>1022</td>
<td>1022</td>
<td>1022</td>
</tr>
</tbody>
</table>

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in Table II by comparing the two conditions of Na concentrations 7.5 mm and 17 mm). For instance, with $X_{Ca}$ equal to 0.2, $R_{Ca}$ decreases from 64 to 29 when $C_{Na}$ is increased from 7.5 to 17 mm. This behavior is found with synthetic resin and also with plant roots where it is called the dilution effect (16, 24). It is also observed for the wall in situ (in preparation), according to the classical procedure, when one follows against time the accumulation of a given ion in a plant system, the intercept of the first quasistationary phase of absorption with the ordinate-axis gives an estimation of the quantity of ions fixed in the cell walls in situ.

We have used this method to measure Ca and K uptake by the walls of entire $L. minor$ L. The values thus obtained for the rational selectivity coefficient $R_{KCa}$ are given in Table III. These coefficients decrease considerably when the concentration of Ca ions $C_{Ca}$ is increased from 0.05 to 5 mm. Since similar behavior and comparable quantitative values are observed with the wall in situ or the isolated cell wall, it seems that the treatment given to the $L. minor$ L. to isolate the walls does not alter their physico-chemical behavior.

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