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A Model for Predicting Ionic Equilibrium Concentrations in Cell Walls[1]

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ABSTRACT

Purified cell walls were prepared from roots of Horse bean (*Vicia faba* L., var. *minor*) and Yellow Lupine (*Lupinus luteus* L.). Two methods were used: (a) grinding and (b) lysis of the endocellular contents by Triton X-100. The Ca^{2+}, Mg^{2+}, and K^+ contents were determined after incubation in various solutions in such a manner that the measurements gave access to the undisturbed equilibrium contents. The results were used to test a model which describes the ionic atmosphere in the cell walls as a result of simultaneous electrostatic interactions between free ions (Donnan effect) and specific association equilibria, including acido-basic ones. This model correctly accounted for the whole set of experimental results and predicted the values of the unmeasurable local concentrations and pH.

Plasma membranes of root cells do not directly perceive the ionic conditions in the soil solution but are in contact with a modified medium, the composition of which is affected by the interactions between the outer solution and the pecto-cellulosic cell walls. Therefore, the analysis of the kinetic properties of the plasma membrane transport systems in response to local concentrations is a worthwhile project. Such an approach using yeast has shown that transport kinetics are considerably modified by surface ionic conditions, (18, 20). No method is available to measure directly local concentrations which must be presently estimated through theoretical models. The electrostatic field formed by fixed anionic charges of cell walls attracts cations (including protons) while, simultaneously, the cations attenuate the field strength by electrostatic screening and by binding to the charges. The attraction and screening effects have been described by the well-known Donnan (5, 17) or Gouy-Chapman models (4, 19). The analysis of the potential electrostatic energy surrounding a linear polyelectrolyte predicts that the counter-ions are condensed on the latter if its charge density exceeds a definite critical value (14). This theory has been applied to plant cell walls (8). The condensation is not a specific binding but the limiting result of the valency-dependent electrostatic attraction. The existence of a specific binding has been recognized, specially for H^+ and Ca^{2+}. It has been taken into account through the use of chelation models (23) or of activity coefficients which also describe the ion-ion and ion-water interactions (6, 7). None of these models allows a quantitative prediction of the results of the cooperation between the valency-dependent interactions and the specific bindings. The model we describe here simultaneously accounts for both effects.

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MATERIALS AND METHODS

Plant Materials. Roots were obtained from 6-day-old seedlings of Lupine (*Lupinus luteus* L.) and Horse bean (*Vicia faba* L., var. *minor*) grown in the dark. The culture medium contained 0.1 mM $Ca(NO_3)_2$, $MgSO_4$, KH_2SO_4, and oligo-elements. Two methods were used to prepare cell walls.

Cell Wall Isolation Procedure I. Roots were plasmolyzed in 0.4 M mannitol, 25 mM Tris-HCl (pH 7.4) for 30 min, and then laminated with a Pascall triple roll mill in 0.4 M sucrose, 10 mM MSH[2], 25 mM Tris-HCl. The resulting suspension was wrung in a polyacrylamide cloth, homogenized with a Virtis blender in 100 mM KCl, 10 mM MSH, 5 mM Tris-HCl, and washed twice in this medium.

Cell Wall Isolation Procedure II. Excised roots were put in 1 mM $CaCl_2$, 1% v/v Triton X-100. This solution was periodically renewed during 3 weeks, which was sufficient for the lysis of the cellular content to take place as seen by electronic microscopy (3). The surfactant was then eliminated by washing with 1 mM $CaCl_2$ for 10 days. The whole preparation was done at 5 C. The resulting cell walls retained the original shape and dimensions they had in the roots.

Experimental Treatments. Just before the experiments, the cell walls were repeatedly washed with 10 mM HCl until no further displacement of cations was observed. This treatment allowed an easier control of the ionic composition of incubation media during the experiments, especially at low concentrations. The pH and the ionic concentrations of the different incubation solutions changed following the introduction of cell walls. The solutions were renewed until their ionic characteristics remained unchanged. A 12-h incubation in the last bath insured that equilibrium was reached. The cell walls were thereafter blotted on filter paper and the fresh and dry weights of the samples were measured. The experiments were done at room temperature.

Assays. The ions were extracted from the dry samples with 100 mM HCl. The concentrations of K^+, Ca^{2+}, and Mg^{2+} in the extracts and in the incubation solutions were determined by flame spectrophotometry. Proton displacements were measured by titrations of the medium. Assays for uronic acids were made by colorimetric method (1). All contents are expressed on a dry matter weight basis.

Correcting Procedure for the Contaminating Medium. The assays of the acid extract gave the sum of the ions which were fixed by the cell walls and of the ions present in the contaminating interstitial medium. The quantity of the latter was estimated by: (fresh weight of the sample − dry weight) × (concentration in the medium) and substracted from the total. This procedure avoided rinsing, which was shown to be at variance with the equilibrium measurement requirements (see under "Discussion").

[2] Abbreviation: MSH, 2-mercaptoethanol.

THEORETICAL CONSIDERATIONS

The Association-Dissociation Model. We assume that a cation may be retained by a fixed anionic charge through two distinct mechanisms. The first one is electrostatic interaction between the two hydrated ions and the other is direct adsorption of the two ions, with formation of a common hydration shell around the pair and eventually with molecular orbital sharing (10). The first mechanism (attraction between unbound ions) is described by the Boltzmann law. Hence, selectivity is strictly valency-dependent. If the fixed charges are considered as dispersed in an homogeneous phase, the formal description of the system is that of Donnan. If the spatial heterogeneity is to be taken into account, the most classical relevant model is that of Gouy and Chapman (2). The second mechanism (binding) is described by the mass action law applied to the local concentration of unbound ions in the polyelectrolyte phase (not to their concentration in the outer phase). Selectivity then depends on molecular geometry, hence it is specific for each type of pair (10).

Symbols. Index i refers to the ion species; z_i is the valency. Cations are numbered from $i = 1$ to k and anions from $i = k + 1$ to n. H^+ and OH^- are included in this numbering. (M_i) is the concentration of ion i in the outer phase, $(\bar{M}_i^u)$ and $(\bar{M}_i^b)$ are the concentrations of unbound and bound forms, respectively, in the polyelectrolytic phase. (R_T) is the total concentration of fixed charges within the polyelectrolytic phase and (R) is the concentration of the uncomplexed form. γ_i and $\bar{\gamma}_i^u$ are the activity coefficients of the ions in the outer medium and of the free ions in the polyelectrolytic phase. $\bar{\gamma}_i^u$ relates to ion-ion interactions. Fixed charged-ion interactions are described by K_i, the intrinsic dissociation constant of the fixed charge-M_i complex. Figures without brackets denote contents. $\bar{M}_i$ is the total M_i content, *i.e.* the sum of $\bar{M}_i^b$ and $\bar{M}_i^u$.

Model.

Donnan equilibrium.

$$\frac{\bar{\gamma}_i^u \cdot (\bar{M}_i^u)}{\gamma_i \cdot (M_i)} = \left(\exp\left(-\frac{e \cdot \lambda}{kT}\right)\right)^{z_i}$$

This relation is useful to convey the concept, but, for practical use, the exponential will be written as r, the so-called Donnan ratio, so the electric potential (λ) no longer needs to be explained.

$$\frac{\bar{\gamma}_i^u \cdot (\bar{M}_i^u)}{(M_i)} = r^{z_i} \quad (1)$$

For the sake of simplicity, γ_i has been given the value 1.

Association. If z_i fixed charges bind one cation i (see under "Discussion"), the mass action law is

$$K_i = \frac{\bar{\gamma}_i^u \cdot (\bar{M}_i^u) \cdot (R)}{z_i (\bar{M}_i^b)} \quad (2)$$

(R), the concentration of unassociated fixed anions is:

$$(R) = (R_T) - \sum_{i=1}^{k} z_i \cdot (\bar{M}_i^b) \quad (3)$$

Electroneutrality in the polyelectrolytic phase.

$$(R) = \sum_{i=1}^{n} z_i \cdot (\bar{M}_i^u) \quad (4)$$

By replacing $(\bar{M}_i^b)$ and (R) in equation 3 by their expressions given in equations 1, 2, and 4, one obtains

$$(R_T) - \left(\sum_{i=1}^{n} \frac{z_i \cdot r^{z_i} \cdot (M_i)}{\bar{\gamma}_i^u}\right) \cdot \left(1 + \sum_{i=1}^{k} \frac{r^{z_i} \cdot (M_i)}{K_i}\right) = 0 \quad (5)$$

Calculations. Equation 5 can be solved for r by iteration. Then the concentrations of the free and bound ions in the polyelectrolytic phase (including the protons) are obtained from equations 1 and 2. Comparison of these theoretical values with the experimental values is achieved by converting them into contents. This can be made by using an estimate of the volume v of the polyelectrolytic phase. Hence, the parameters which should be estimated before using the model are R_T, v, the $\bar{\gamma}_i^u$ and the K_i values (see under "Results").

RESULTS

Justification of the Measurements. The validity of the correcting procedure for the contaminating interstitial medium was checked by equilibrating Horse bean cell walls (isolate II) with an aqueous 1 mM $CaCl_2$ solution and then reequilibrating them with an absolute ethanolic 1 mM $CaCl_2$ solution. A sample was blotted and assayed for Ca^{2+}. Another sample was rinsed with chloroform before the assay. Chloroform is miscible with ethanol and brings no proton capable of displacing the adsorbed Ca^{2+}. Hence, the only effect of the chloroformic rinse is the elimination of the contaminating ethanolic $CaCl_2$ solution. The measured Ca^{2+} content of the first sample was 0.641 ± 0.030 mmol·g^{-1} dry weight and the calculated correction reduced it to 0.625 ± 0.030 mmol·g^{-1} dry weight. The content of the chloroform rinsed sample was 0.627 ± 0.022 mmol·g^{-1} dry weight. The controls showed that neither ethanol nor chloroform irreversibly modify the ionic properties of the cell walls. The conclusions are that the correcting method is valid and that the correction is close to the experimental variability. To ensure that the ionic equilibria observed in the different incubation baths were truly reversible, experiments were conducted in which each sample was successively transferred into different solutions where the $CaCl_2$ concentration varied from 0 to 5 mM and then from 5 mM to 0. The Ca^{2+} exchanges were determined by measuring the variations of the concentrations of the solutions. The curves of Ca^{2+} contents *versus* Ca^{2+} concentration, respectively, obtained with increasing and decreasing concentrations were identical. Hence, the treatments induce no irreversible change. Controls showed that the same is true for the HCl pretreatment.

H^+ Exchanges. When cell walls preincubated at pH 5.0 in 1 mM KCl were transferred to 1 mM KCl plus 1 mM $CaCl_2$, there was an approximately equimolar Ca^{2+}-K^+ exchange which virtually depleted the K^+ content (Table I). The pH fell and the titrations showed that the displacement of protons was sufficient to balance the charges. These results indicate that about 50% of the fixed anionic charges in the cell walls were protonated at pH 5.0. The implication is that the pK of the fixed charges is close to the pH. The fixed charges of the cell walls are mainly those of uronic acids (5). In the cell walls of Horse bean and Lupine, the uronic acid contents correspond to the maximal cation binding capacity (about 1 mEq·g^{-1} dry weight). We measured the pK values of purified galacturonic acid and pectin and obtained

Table I. *Ca^{2+} and K^+ Contents of Lupine Cell Walls (Isolate II) and Proton Displacement (ΔH^+) due to the Transfer from KCl to KCl + $CaCl_2$*

Experimental values are given with their 95% confidence limits. Theoretical values predicted by the model are given in parentheses.

Medium	Ca^{2+}	K^+	ΔH^+
	mmol/g dry wt		
1 mM KCl	0.008 ± 0.0003	0.171 ± 0.003	
	(0)	(0.172)	
1 mM KCl + 1 mM $CaCl_2$			
Experiment 1	0.164 ± 0.004	0.005 ± 0.001	0.132
	(0.167)	(0.006)	(0.144)
Experiment 2	0.149 ± 0.006	0.009 ± 0.001	0.106
	(0.144)	(0.012)	(0.121)

values close to 3.2. This is not in contradiction with the presence of 50% protonated sites at pH 5.0, but indicates that the local pH is lowered by Donnan effect. Figure 1 shows the K^+ and Ca^{2+} contents of cell walls equilibrated with 1 mM KCl or 1 mM $CaCl_2$ at various pH values. The apparent pK values were different in the two treatments, the one in KCl being higher than that in $CaCl_2$. Such an observation is classic (9, 16). This confirms that the local pH near the fixed charges is lower than the pH of the medium due to electrostactic effects which are more effective at the lowest ionic strength (KCl) than at the highest one ($CaCl_2$).

Ca^{2+}-Mg^{2+} Exchanges. Exchanges of the two bivalent cations were measured at pH 5.5. Cell walls were equilibrated with $CaCl_2$ plus $MgCl_2$ solutions. The sum of the two concentrations was 2 mM, and the ionic fractions varied from 0 to 1. The comparison of the Ca^{2+} ionic fractions in the cell walls and in the mediums reveals a strong selectivity in favor of Ca^{2+} (Fig. 2a). Figure 2b shows that the sum of the retained cations (Ca^{2+} + Mg^{2+}) was constant (about 0.5 mmol·g^{-1} dry weight).

Mg^{2+}-K^+ Exchanges. Displacement of K^+ by Mg^{2+} was measured at pH 5.5 in 1 mM KCl plus 0 to 1.5 mM $MgCl_2$ (Fig. 3).

Ca^{2+}-K^+ Exchanges and Comparison Between the Materials. Figures 4 and 5 show the Ca^{2+}-K^+ exchanges in cell walls of Horse bean and Lupine, respectively. These measures were made with cell walls equilibrated in 1 mM KCl solutions with various $CaCl_2$ concentrations (pH 5.5). Cell walls of Horse bean retained more Ca^{2+} than those of Lupine. For both species, there is no real difference between results with isolates I and II.

Values of the Parameters Needed for Using the Theoretical Model. When the Ca^{2+} concentration in the medium approaches 0.2 mM, the Ca^{2+} content of the walls reaches a quasi-plateau (Figs. 4 and 5). The theoretical model predicts this behavior as well as the value of the quasi-plateau, which equals half the content R_T. Hence, R_T may be estimated from the experimental apparent saturation with Ca^{2+}. The values obtained (0.63 to 1.01 mmol·g^{-1} dry weight, Table II) are close to the uronic acid contents (0.63 and 0.97 mmol·g^{-1} dry weight). The dissociation constant for H^+ was given the value $10^{-3.2}$ M from the titration of galacturonic acid and purified pectin. The dissociation constants for Ca^{2+}-mono/dicarboxylic acid complexes vary from 5 to 300 mM (15, 22). That of gluconic acid is 63 mM, and we measured 30 mM for galacturonic acid with an ORION-specific electrode. We considered K_{Ca} as a semi-adjustable parameter and we retained the value which gave the best adjustment to the whole set of

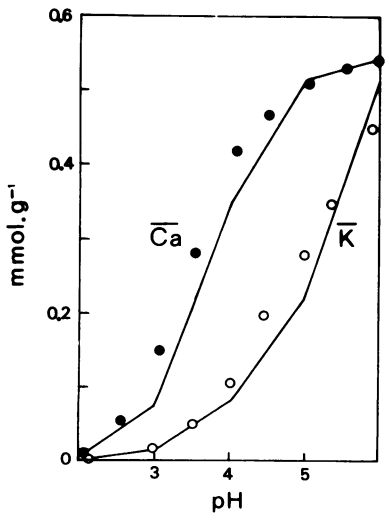

FIG. 1. Effect of the pH on the Ca^{2+} and K^+ contents of Horse bean cell walls (isolate II). The mediums contain 1 mM $CaCl_2$ or 1 mM KCl. The lines join the theoretical points. The latter are obtained from equation 5. The parameters used are listed in Table II.

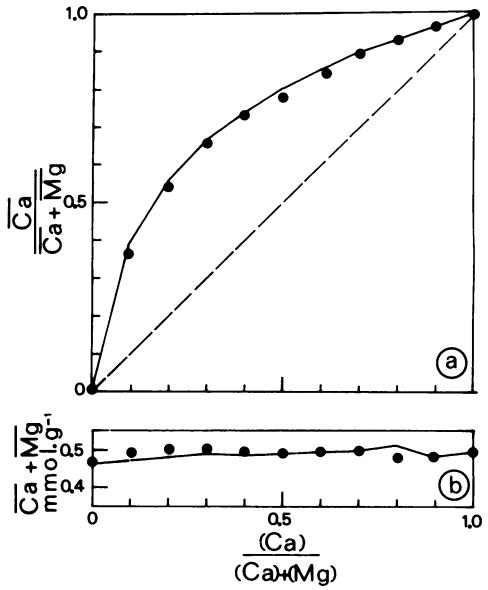

FIG. 2. Ca^{2+}-Mg^{2+} selectivity of Horse bean cell walls (isolate II). The media contained $CaCl_2$ + $MgCl_2$, total concentration 2 mM (pH 5.5). The lines join the theoretical points obtained from equation 5. Parameters: see Table II. a, Relation between the Ca^{2+} ionic fractions in the cell walls and in the mediums. b, Total (Ca^{2+} + Mg^{2+}) contents of the cell walls.

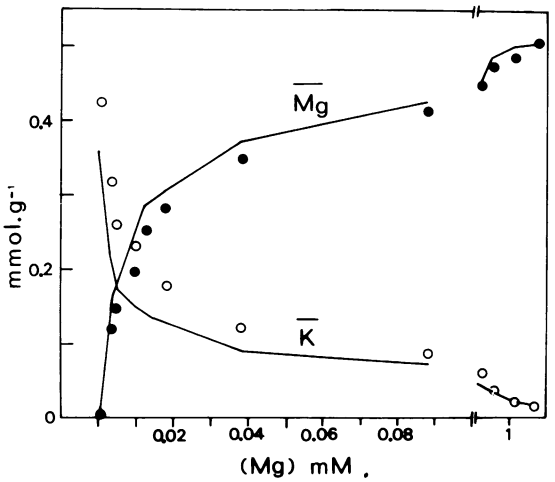

FIG. 3. K^+-Mg^{2+} exchange isotherm (Horse bean cell walls isolate II). The media contained 1 mM KCl with varying concentrations of $MgCl_2$ (pH 5.5). The lines join the theoretical points obtained from equation 5. Parameters: see Table II.

experimental data (75 mM, see Table II). There is a general consensus (15) on the absence of association between K^+ and carboxylic acids ($K_K \rightarrow \infty$). The cell walls have less affinity for Mg^{2+} than for Ca^{2+} (Fig. 2). K_{Mg} is 0.5 M for gluconic acid (15). Hence, we neglected the affinity for Mg^{2+} ($K_{Mg} \rightarrow \infty$). The cell walls isolate II retained the same volume as the roots and their dry weight was 2.5% of their fresh weight. By assuming that the volume of the walls is 3% of that of the root (12, 21), a specific volume of $\frac{3}{2.5} = 1.2$ ml·g^{-1} dry weight is obtained. This estimation is very uncertain, so we again chose to treat ν as a semi-adjustable parameter. For the $\bar{\gamma}_i^u$, the reader is referred to the discussion.

Comparison Between Experimental Results and Predictions of the Model. The theoretical results in Table I and the curves on Figures 1 to 5 were calculated with the help of equations 1, 3, and 5, and with the values of the parameters listed in Table II. Clearly,

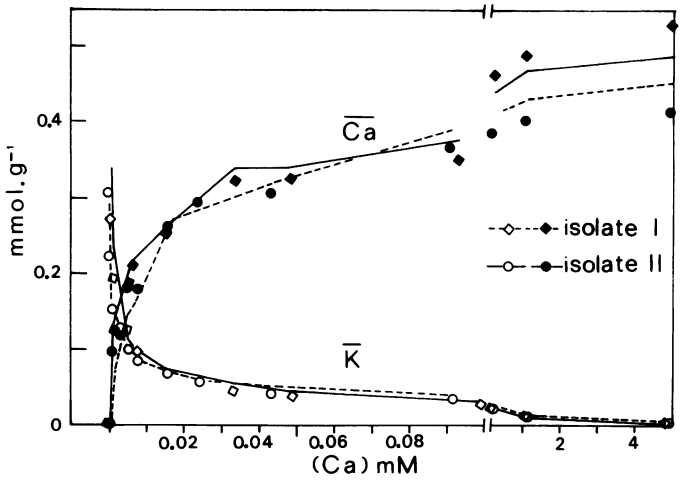

FIG. 4. K^+-Ca^{2+} exchange isotherms (Horse bean). The media contained 1 mM KCl with varying concentration of $CaCl_2$ (pH 5.5). The lines join the theoretical points obtained from equation 5. Parameters: see Table II.

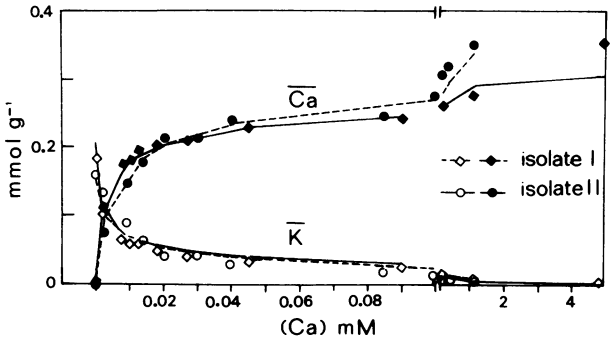

FIG. 5. K^+-Ca^{2+} exchange isotherms (Lupine). See the caption of Figure 4.

Table II. *Values of the Parameters Used for Adjustments to Experimental Results*

K_K and K_{Mg} were set so as to be sufficiently large to allow the association to be neglected in the case of K^+ and Mg^{2+}.

	pK	R_T	K_{Ca}	*vol*
		mmol g^{-1} *dry wt*	*mM*	*ml* g^{-1} *dry wt*
Horse bean				
Fig. 1, isolate II	3.2	1.080	75	0.80
Fig. 2, isolate II	3.2	1.000	75	0.85
Fig. 3, isolate II	3.2	1.050		0.85
Fig. 4, isolate II	3.2	0.925	75	0.70
Fig. 4, isolate I	3.2	1.000	75	0.70
Lupine				
Table I, isolate II	3.2	0.750	75	0.80
Fig. 5, isolate II	3.2	0.675	75	0.80
Fig. 5, isolate I	3.2	0.625	75	0.80

the model's predictions fit well with the whole set of experimental data for the two plants when a unique value for each K_i is used as well as experimentally determined R_T and values of v which are close to those estimated above (0.70 to 0.85 ml·g^{-1} dry weight). Figure 6 illustrates the predictive value of the model by depicting the local pH, the concentration of the free R groups and that of the free and bound Ca^{2+} ions. The most remarkable predicted features are the constancy of the concentration of the ionized fixed

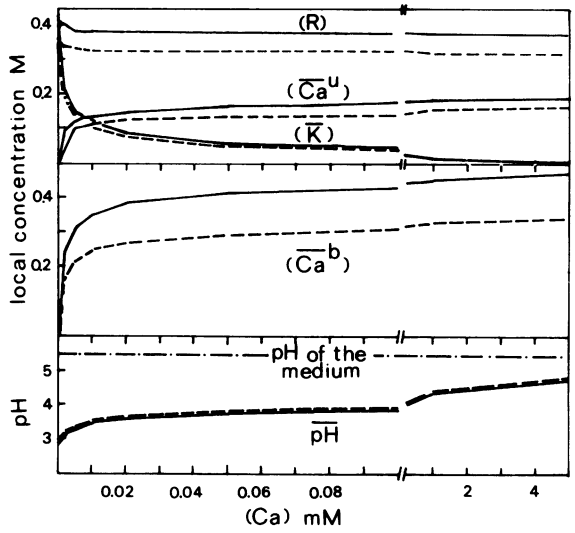

FIG. 6. Predictions of the association-dissociation model for cell walls equilibrated with 1 mM KCl plus $CaCl_2$ (pH 5.5). Concentrations of dissociated fixed acid groups (R), free cations ($\overline{Ca^u}$), ($\overline{K}$), and bound calcium ($\overline{Ca^b}$). The parameters are those used for figure 4 and Table I (see Table II). ——, Horse bean; - - -, Lupine.

groups, the low value of the local pH, and the buffering capacity of the cell walls with respect to the free Ca^{2+} concentration. The shape of ($\overline{Ca^b}$) curve is reminiscent of that of high affinity isotherms, with an apparent dissociation constant about 1 nM. The intrinsic dissociation constant is 75 mM, and the high affinity appearance is due to the local free Ca^{2+} accumulation by the electrostatic field. This effect explains the observed dependency of the pectin affinity for Ca^{2+} on the ionic strength (13). Finally, one may note that there is no major difference between the calcicole species (Horse bean) and the calcifuge one (Lupine) in spite of the former's systematically higher R_T value. This was observed for all Ca^{2+} concentrations tested in the culture medium (0.1 to 100 mM).

DISCUSSION

Are the Experimental Data Proper for an Analysis in Terms of Equilibrium? For experimental data to be treated as describing equilibria, two points need to be made clear: (a) The ionic contents must correspond to equilibrium situations. (b) The measure must give access to the ion contents at equilibrium. First, the control experiments (see justification of the measurements) indicate that none of the treatments used induces an irreversible change in the ionic properties of the cell walls. Second, the absence of rinsing after sampling preserves the previously reached equilibria. This point is especially relevant here, because the rinses, even when brief (6, 7) are likely to disturb the equilibria. We studied the composition of the rinse mediums in function of the rinsing time. Even the shortest rinse (5 s) selectively displaced the univalent cations. Since we showed that our correction procedure is valid, we may look at the points on Figures 1 to 5 as describing true equilibrium contents. Finally, it must be noted that the studies of ion exchanges at the surface of living materials do not give data which are suitable for the present purpose: the *in situ* cell walls are not at equilibrium but in a stationary state. The results we obtained with isolate I and isolate II are very close to each other (Figs. 4, 5, and Table II), which may indicate that the basic ionic properties of the walls have been preserved by the two methods of preparation.

Are the Basic Assumptions Likely? The introduction of ion-ion interactions into the model is difficult because local ionic strengths are too high for the Debye-Hückel treatment to be used to compute

activity coefficients. The empirical determination of these coefficients with solutions reconstituting the local conditions cannot be achieved due to the variety and complexity of the latters. None of the published truly predictive models actually escape this drawback (*e.g.* 14). The theoretical values on Figures 1 to 5 and Table I were calculated by using $\bar{\gamma}_i^u = 1$ for all ions. The calculations of local concentrations of free ions (equation 1) reveals that the ionic strength remains within the range 150 to 300 mM when Ca^{2+} concentrations in the mediums vary from 0 to 5 mM. The corresponding activity coefficients are about 0.75 for K^+ (11) and about 0.4 for Ca^{2+} (ORION electrode). We recalculated the K^+ and Ca^{2+} contents by using $\bar{\gamma}_i^u = 0.75$ for all the univalent ions and $\bar{\gamma}^u = 0.4$ for Ca^{2+}. The results could not be distinguished from the previous ones obtained with $\bar{\gamma}_i^u = 1$ if plotted on Figures 4 and 5. For K^+, decreasing the activity coefficient in the equation 1 and 5 leads to a similar decrease of the predicted local activity, so that there is little change in the local concentrations and contents. For Ca^{2+}, the introduction of a smaller activity coefficient leads to changes in the contents of free and bound forms which almost exactly compensate each other. Written as equation 2, the mass action law describes the Ca^{2+} receptors as formed by two near R groups (the binding reaction is first ordered with respect to the concentration $\left(\frac{R}{2}\right)$ of these receptors). A second order mechanism would imply that the R groups or the RCa monovalent complexes would be free to move and collide. The results of Table I indicate that there is no monovalent binding; since the maximal Ca^{2+} content equals the uronic acid one, we may assume that the spatial distribution of all fixed R groups is compatible with the formation of R_2 receptors. The same phenomenon has been described for purified pectin (13). From the above considerations, one might doubt whether the distribution of the R groups is compatible with the homogeneous one which is implicitly assumed in the Donnan equilibrium. In fact, there is no real contradiction because the polyelectrolytic phase is formally described as a disordered solution of R_2 groups. Nevertheless, it would be worthwhile to take the spatial organization into account, which can be done by substituting the Donnan equilibrium model by the Gouy-Chapmann treatment of the diffuse double layer. This point will be discussed in another paper. Two of the parameters were considered as semi-adjustable (K_{Ca} and ν). One may wonder whether different combinations of arbitrary values may give equally good adjustments. This risk is not serious because the analysis of the theoretical relations indicates that each given set of data (wall contents in function of the ionic parameters of the medium) is related to only one pair of values for K_{Ca} and ν.

Conclusion. The association-dissociation model uses the simplest descriptive laws for the two basic phenomena: electrostatic attraction and specific binding. It correctly describes the results of a variety of equilibrium experiments. It is likely that the cell walls *in situ* are in a stationary state which generally may not be far from the equilibrium So, they cannot modify to a great extent the electrochemical potential of the ions which enter them. Nevertheless, they affect the membrane functions because the diffusion potential, the porters and enzymes respond to local concentrations. The above model predicts the pH, the concentrations of the free ions and ionized fixed groups; it may be used without difficulty[3] for relatively complex outer ionic solutions.

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[3] The theoretical data for the illustrations may be computed with the help of a hand programmable calculator.