

5755

Equilibrium Analysis of Calcium Binding to Cell Walls Isolated from Plant Tissue

Shu-I Tu¹, Janine N. Brouillette, Gerald Nagahashi, Thomas F. Kumosinski, Deidre Patterson and Irene Hsu

Plant and Soil Biophysics Research Unit, Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118, U.S.A.

Primary cell walls were isolated and purified from potato tubers and carrots via a Parr N₂ bomb technique. Calcium binding to purified cell walls was measured with both calcium selective electrode and use of the metallochromic indicator, Arsenazo III. The cell walls used in this study were biologically active and presumably approached the physiological cell wall. Aliquots of the untreated cell walls (control) were then salt-extracted or EDTA-treated and binding properties were compared to the controls. In addition, the binding properties of freshly prepared cell walls were compared to cell walls which were stored for 1 week at 2°C. Both simple Scatchard plot analysis and an electrostatic interaction model were used to evaluate calcium binding parameters. The controls from the two tissue types had inherently different calcium binding properties and these properties were affected by treating the cell walls with salt or EDTA. Cold storage treatment drastically changed the binding properties of carrot cell walls but had negligible effect on potato tuber cell walls.

Key words: Calcium binding — Carrots — Cell wall — Ion selective electrode — potatoes.

The cell wall is negatively charged due to the presence of uronic acid residues and may act as an external compartment which affects cation nutrient adsorption and consequently nutrient absorption processes (Goldberg 1985). Calcium is an essential nutrient which can comprise up to 1–3% (w/w) of dry plant tissue and most of it is localized in the cell wall (Demarty et al. 1978). Calcium is required for many cellular functions including the stabilization of cell wall structure and regulating the activity of cell wall associated enzymes (Demarty et al. 1978).

Most studies on calcium binding properties were made on isolated cells walls (Allen et al. 1985, Baydoun and Brett 1984, Demarty et al. 1978, Morvan et al. 1979, Sentenac and Grignon 1981, Tepfer and Taylor 1981, Bush and McColl 1987) from actively growing tissues. Most of these cell wall preparations were treated with acid (Morvan et al. 1979, Sentenac and Grignon 1981), detergent (Allen et al. 1985, Demarty et al. 1978, Sentenac and Grignon 1981, Harris 1983) or salt (Demarty et al. 1978) before calcium binding was determined. These treatments will remove some cell wall components (Morvan 1979, Kato and Nevins 1984, Labrador and Nicolas 1984, Nagahashi and Seibles

1986) and possibly affect the structure and binding properties of isolated cell walls. Binding studies with purified untreated cell walls are more likely to approach physiological conditions.

Recently, we developed a rapid procedure for isolating large quantities of highly purified biologically active cell walls (Nagahashi and Seibles 1986). Using this procedure, cell walls were purified from two tissues (potato tubers and carrots) and calcium binding was examined with both a calcium selective electrode and a calcium indicator dye. Binding properties of untreated biologically active cell walls (full complement of cell wall associated proteins) were directly compared with cell walls after salt extraction or EDTA treatment. In addition, the feasibility of using stored purified cell walls for Ca²⁺ binding was determined.

Materials and Methods

Plant materials and initial tissue disruption—Russet potatoes (*Solanum tuberosum*) and carrots (*Daucus carota*) were purchased at a local market and stored in a refrigerator or used immediately. Potatoes were peeled and cut into large pieces and placed in homogenization medium (Nagahashi and Seibles 1986). Carrots were peeled, cut into

1/2 inch thick discs, and vascular tissue was removed with a cork borer. Both tissues were homogenized with homogenization medium for 30 s at 4°C with a Waring blender. The tissue homogenate was washed twice with cold water and tissue clumps were trapped on a cheesecloth sieve. The particulate sample was then suspended in fresh homogenization medium and placed in a Parr N₂ bomb.

Cell wall purification and treatments—Potato tuber tissue slices or carrot root discs were initially homogenized in a Waring blender for 60 s at 4°C with an alkaline homogenization medium (Nagahashi and Seibles 1986). Intact tissue clumps and broken cell walls were trapped on cheesecloth and washed three times with cold deionized-distilled water. The particulate matter was suspended in fresh homogenization medium and transferred to the Parr nitrogen bomb. After equilibrating at 1.032×10^7 Pa for 15 min at 4°C, the contents were extruded from the bomb, the cell walls were collected on cheesecloth and further purified by washing six times with cold deionized-distilled water (Nagahashi and Seibles 1986). Cell walls isolated with this procedure were judged to be highly purified by electron microscopy and lack of cytosolic marker enzyme activity. Purified cell walls were used directly for calcium binding studies or were treated, as described in the text, before binding studies.

All treatments of purified cell walls were performed at 0–4°C. Salt extraction was accomplished with 2 M NaCl or LiCl for 15 h with the salt solution replaced several times. For some experiments, cell walls were washed with 10 mM EDTA in 10 mM HEPES buffer at pH 7.5 for several hours. After various treatments, all cell wall preparations were washed thoroughly with ice cold water to remove treatment reagents. The treated and untreated cell wall samples were used immediately or stored in water with 1 mM β -mercaptoethanol at 2°C for 1 week. All chemicals used were of analytical grade.

Electron microscopy of cell walls—Cell wall samples were checked for morphological contaminants via microscopic techniques. Light microscopy with a microscope (BHA, Olympus, Tokyo) was used to monitor removal of starch bodies. Ultrastructural examination of purified cell walls was performed with an electron microscope (EMB 10B, Zeiss) as described by Nagahashi and Seibles (1986). Cell walls from carrots and potatoes showed no membrane, organelle, or cytoplasmic fragments as contaminants and this was verified biochemically by lack of membrane-associated and cytoplasmic-associated marker enzymes.

Calcium binding assay—A pH meter (PHM84, Radiometer Copenhagen, Copenhagen) along with a calcium ion selective electrode (Model F2002 Radiometer Copenhagen) and an accompanying reference electrode (Ag/AgCl in saturated KCl) were used to measure the concentration of free calcium present in the buffer solution of 25 ml aliquots

containing 50 mM KCl and 25 mM MOPS, pH 7.5. At this ionic strength, cell wall-associated proteins were still bound to the walls. To obtain a standard curve, increments of 1.0 M CaCl₂ were added to the buffer and millivolt readings were taken after the system re-equilibrated (1–2 min intervals) (Baydoun and Brett 1984, Hulanicki and Marek 1974). The $-\log(\text{Ca}^{2+})$ was plotted versus the millivolt readings to yield the standard curve. For the assay, a 25 ml aliquot was again used and to it was added a small amount (30–70 mg dry wt) of purified cell wall material. The system was stirred at a constant rate as increments of the standard calcium solution were added. The stirring was stopped momentarily and stable millivolt readings were taken (2–3 min after the addition of Ca-solution) and the concentration of free calcium ions present in the solution was calculated. Knowing the free calcium concentration and the amount of calcium added, the bound calcium was easily determined.

The free calcium concentration was verified by the spectral changes of a metallochromic indicator, Arsenazo III as described by Scarpa (1979). Briefly, after stable millivolt readings were recorded, a small aliquot (< 50 μ l), free from cell walls, was immediately withdrawn and diluted with an indicator solution containing 25 mM KCl, 25 μ M Arsenazo III, and 15 mM MOPS, pH 7.4. The dilution was designed to bring the calcium concentration between 1 and 10 μ M. Difference spectrum were obtained between diluted Ca solution and Ca-free indicator solution from 400 to 700 nm. The increase of absorbance at 652.5 nm was used to calculate total calcium concentration in diluted solution. A standard curve was obtained by including known amounts of Ca²⁺ in the indicator solution.

Dry wt of purified cell walls was determined as well as the amount of endogenous Ca²⁺ present. The endogenous Ca contents of potato tuber cell walls and carrot cell walls by ash analysis were 0.015% and 0.030% of the dry wt, respectively.

Simple binding analysis—The binding constant (k) and the number of binding sites (n) for calcium in treated and untreated cell walls from potatoes and carrots were obtained by both graphical and numerical methods of analysis of the experimental data. Scatchard plots, $\bar{v}/[I]$ versus \bar{v} where \bar{v} is the amount (nmols) of bound calcium per unit dry-wt of cell walls and $[I]$ is the concentration of free calcium, were drawn for all of the binding experiments. For linear Scatchard plots, straight lines were fitted by linear regression analysis and k and n were determined from the intercepts.

Complex binding analysis—Since plant cell walls may be regarded as polyelectrolytes (Sentenac and Grignon 1981) the general binding equilibrium theory which is suitable for macromolecules (Tanford 1968) may be applied to calcium binding to cell walls. We assume that cell walls contain domains with different binding affinities to

the cation. Within each domain, the cation binding is further influenced by the interactions between sites. Thus, in general, the equilibrium between cell-wall bound and free calcium cations assumes the following relationship:

$$\bar{v} = \frac{n_1 k_1 e^{-\phi_1(\bar{v}_1)} I}{1 + k_1 e^{-\phi_1(\bar{v}_1)} I} + \frac{n_2 k_2 e^{-\phi_2(\bar{v}_2)} I}{1 + k_2 e^{-\phi_2(\bar{v}_2)} I} + \dots \quad (1)$$

where \bar{v} is the total number of bound calcium ions; \bar{v}_1 is the number of calcium ions bound in domain 1, n_1 is the capacity of type 1 binding sites, k_1 is the intrinsic binding constant of type 1 sites, $\phi_1(\bar{v}_1)$ is an arbitrary function to describe the interaction among type 1 sites, and I is the concentration of free calcium cations. The second and remaining terms on the right-hand side equation (1) are used to describe the contribution of type 2 and other binding sites.

Since the binding of Ca^{2+} to the negatively charged sites of cell walls is presumably electrostatic in nature, the explicit form of $\phi_1(\bar{v}_1)$ may then be:

$$\phi_1(\bar{v}_1) = 2w_1 z_i \bar{Z}_1 \quad (2)$$

in which w_1 is a constant independent of z_i (the charge of the binding ions) and \bar{Z}_1 which is the remaining negative charges in domain 1 of cell walls with the extent of total Ca^{2+} ion binding as \bar{v}_1 . Assuming there are m total negative charges in domain 1 per unit weight of cell wall, \bar{Z}_1 assumes the form of:

$$\bar{Z}_1 = -(m - 2\bar{v}_1) \quad (3)$$

or

$$\phi_1(\bar{v}_1) = 4w_1 m - 8w_1 \bar{v}_1 \quad (4)$$

In the case of negligible site interactions ($\phi_1(\bar{v}_1) = 0$) and only one type of binding site, equation 1 reduces to:

$$\bar{v} = \frac{nkI}{1 + kI} \quad (5)$$

or the simplest form of the Scatchard equation:

$$\frac{\bar{v}}{I} = k(n - \bar{v}) \quad (6)$$

If the binding analyses (plots of \bar{v}/I vs. \bar{v}) shows a linear relationship, equation 6 is sufficient for the quantitative description of Ca^{2+} binding to cell walls. In the case of curvilinear binding plots, a more complex analysis is necessary.

In the case of a single type of binding site, characterized by a characteristic intrinsic binding constant (k) with electrostatic interaction, equation 1 would assume the form of:

$$\bar{v} = \frac{nke^{-(4wm - 8w\bar{v})} I}{1 + nke^{-(4wm - 8w\bar{v})} I} \quad (7)$$

As will be shown later, k and I (when expressed on a Molar Concentration Scale) are usually small numbers and the exponential term is less than 1, thus:

$$\bar{v} \approx nke^{-(4wm - 8w\bar{v})} I \quad (8)$$

Since n and w are constants, equation (8) reduces to:

$$\frac{\bar{v}}{I} \approx A_1 e^{A_2 \bar{v}} \quad (9)$$

in which A_1 and A_2 are nke^{-4wm} and $8w$, respectively. A non-linear regression of \bar{v}/I vs. \bar{v} will yield A_1 and A_2 .

The Scatchard plots (\bar{v}/I vs. \bar{v}) of Ca^{2+} binding to cell walls of potato and carrots under different conditions, were analyzed according to equation 6 or equation 9, or both. Curvilinear plots were fitted by equation 9 using a non-linear regression fitted program written at USDA on the Modcomp Classic minicomputer. The program was written in FORTRAN and is based on the Gauss-Newton algorithm.

Results and Discussion

Calcium ion concentration determination—In the absence of cell walls, the ion selective electrode method applied in the current work (Fig. 1A), exhibited a good linear relationship between mV readings and the logarithm of Ca concentration over a wide-range. This linear response was used to estimate free calcium concentration in equilibrium with bound calcium of cell wall samples as described in Material and Methods. The possible interference by direct contact of cell walls with the electrode was discounted because the equilibrium potential readings were taken without stirring. To further ascertain this estimation, free calcium concentration in equilibrium with cell walls was also determined by the metallochromic indicator method.

The spectral properties of Arsenazo III, a metallochromic indicator, exhibit a pronounced red-shift upon binding with Ca^{2+} ions. The binding may be represented as $[\text{Ca}][\text{Ars}] \rightleftharpoons [\text{CaArs}]$. Thus:

$$K_b = \frac{[\text{CaArs}]}{[\text{Ca}][\text{CaArs}]} \quad (10)$$

in which Ars , CaArs , and k_b represent free indicator, Ca-bound indicator, and the binding constant, respectively. The concentration terms $[\text{Ca}]$ and $[\text{Ars}]$ refer to free Ca and free Arsenazo III in the solution with the total concentrations of Ca and Ars as m and n , the equilibrium k_b is then:

$$k_b = x/(h-x)(i-x) \quad (11)$$

Since the binding of Ca induces maximum absorbance in-

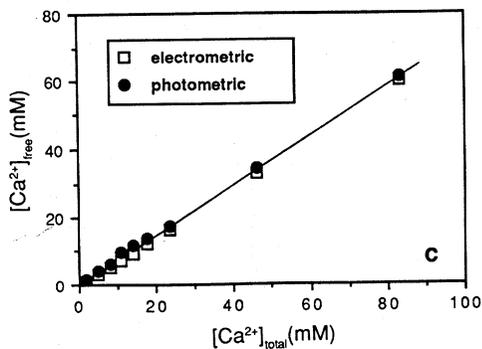
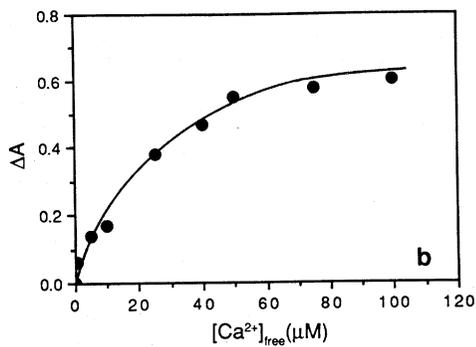
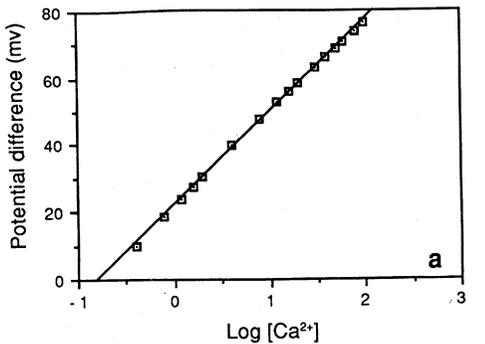


Fig. 1 (A) Standard curve of free Ca^{2+} concentration (in mM) determined by ion-selective electrode method. The relationship between Ca^{2+} ion concentrations and observed voltage readings was established by the procedure described in the text. The replacement of MOPS by Tris did not cause any noticeable change of the standard curve. (B) Absorbance changes of a metallochromic indicator, Arsenazo III, induced by the presence of Ca^{2+} ion. The exact procedure of measuring Ca^{2+} ion induced absorbance increases at 652.5 nm was described in text. The binding constant of Ca-Arsenazo III was determined from the data shown and equations (11) and (12). (C) Comparison of free Ca^{2+} ion concentrations in equilibrium with cell walls determined by ion-selective electrode and metallochromic indicator. The exact procedure was described in text. The free Ca^{2+} concentrations estimated from the mV readings (\square) and the absorbance increases (\bullet) were plotted against the total Ca^{2+} added.

crease at 652.5 nm, the term x may be related to absorbance as:

$$x = \frac{\Delta A}{\Delta A_{max}} \cdot i \quad (12)$$

where ΔA and ΔA_{max} represent the absorbance increase and the maximum absorbance (saturation level) due to the formation of CaArs under the condition of a fixed i but variable h . The application of equations (11) and (12) allowed us to determine k_b (Fig. 1B). In agreement with (Scarpa 1979), k_b was found as $0.1191 \mu\text{M}^{-1}$ under present experimental conditions. The term A_{max} is estimated as the ab-

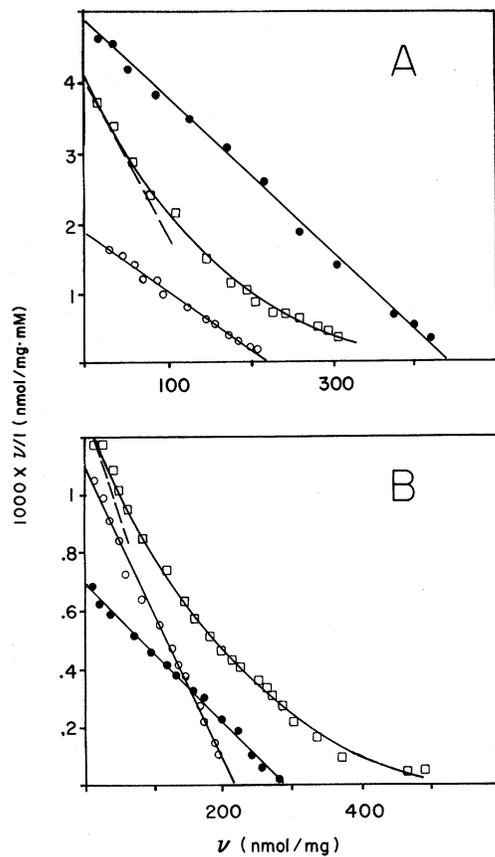


Fig. 2 (A) Calcium binding to freshly isolated carrot cell walls. Carrot cell walls were isolated and further treated with salt or EDTA as described in Materials and Methods. \bullet — \bullet Represents untreated carrot cell wall. \square — \square Represents EDTA treated carrot cell wall and \circ — \circ represents salt-extracted cell wall. Solid lines represent the best-fit curves obtained by either equation (6) or equation (9). Dotted lines represent lines determined from the best linear fit of first few points (no less than six, not all shown) for the estimation of the thermodynamic quantities k and n . (B) Calcium binding to 1 week old carrot cell walls. Carrot cell wall samples previously isolated and treated were stored at 2°C for 1 week. Symbols are the same as in (A).

sorbance increase generated in a solution containing Ca one hundred times in excess of Arsenazo. Thus, the unknown calcium concentration of a solution can be determined from the absorbance increase ΔA caused by the presence of a fixed concentration Arsenazo III from equations (11) and (12).

As described in Material and Methods, once the equilibrium binding to cell walls was reached and the stable potential reading was taken, a small aliquot of the clear supernatant was withdrawn and immediately diluted with buffered Arsenazo III solution. Usually, no more than 50 μ l of the aliquot was diluted to 2 ml with the buffered solution. The exact volume of the aliquot used was calculated, by assuming zero Ca binding to cell walls, to assure the total Ca in diluted solution was less than Arsenazo III in the buffer. The concentration, of free Ca in equilibrium with cell wall solutions were determined by ion selective electrode and compared to the dye binding assay. In Fig. 1C, the estimated free calcium concentrations in equilibrium with a fixed amount of EDTA-treated potato cell walls, under various concentrations of total Ca, are compared. As shown, the indicator method appears to give a slightly higher estimation of Ca concentration. Nevertheless, the difference obtained by two different methods appears to be negligible. Thus, in the following experiments, only ion selective electrode measurements were used to determine Ca concentrations. Although atomic absorption method can also be applied to determine Ca concentration, the indicator method offers a greater sensitivity and requires only minimal sample volume. The latter advantage

Table 1 Effects of different treatments on Ca^{2+} binding parameters^{a, b} of purified cell walls

Sample	n (nmol mg^{-1})	k (mM^{-1})
Carrot		
Fresh unextracted	439	11.11
Fresh salt extracted	222	8.33
Fresh EDTA treated	200	20.10
1 week unextracted	286	2.44
1 week salt extracted	219	5.00
1 week EDTA treated	210	6.67
Potato		
Fresh unextracted	102	19.50
Fresh salt extracted	82	15.38
1 week unextracted	97	19.07
1 week salt extracted	92	12.50

^a The values for potato cell walls and EDTA treated carrot samples were estimated from the initial linear portions. In all cases, the binding parameters were determined by linear regression method.

^b The reported values have no more than 10% error.

allowed us to accurately and simultaneously determine Ca concentration in the same sample used for Ca ion electrode method without significant interference.

Calcium binding to freshly isolated carrot cell walls—

Table 2 Negative cooperativity measured by electrostatic interaction model^{a, b}

Sample	A_1	w	Estimated nk	
			(Linear model)	(Electrostatic model)
Carrot				
Fresh unextracted	—	—	8,210	—
Fresh salt extracted	—	—	1,849	—
Fresh EDTA treated	7,860	-0.00088	4,020	3,888
1 week unextracted	—	—	698	—
1 week salt extracted	—	—	1,095	—
1 week EDTA treated	1,862	-0.00072	1,401	1,017
Potato				
Fresh unextracted	1,991	-0.00119	1,989	1,225
Fresh salt extracted	1,640	-0.00115	1,261	1,125
1 week unextracted	1,905	-0.00138	1,850	1,115
1 week salt extracted	1,544	-0.00122	1,150	985

^a The values of w (the electrostatic interaction factor) and A_1 were numerically determined by fitting equation (9) with a non-linear regression program based on the Gauss-Newton algorithm.

^b The values of the product nk were estimated by either from the data listed in Table 1 (line) or from determined A_1 and w and estimated n of Table 1.

The binding data of the carrot cell wall control (Fig. 2A) was simply described by Scatchard analysis (equation 6). The binding parameters were graphically estimated and numerically calculated by linear regression (Table 1). Salt extracted carrot cell walls also showed a linear Scatchard plot. Surprisingly, salt extraction decreased the number of binding sites (n) and slightly lowered the binding constant (k). Salt extraction can remove ionically bound cell wall proteins (13) or possibly remove fragments of cell wall polysaccharides which bind calcium. If cell wall proteins or polysaccharide fragments bind Ca^{2+} , then salt extraction would be expected to lower the Ca^{2+} binding capacity (nk).

EDTA treatment resulted in a dramatic change compared to the untreated control (Fig. 2A and Table 1). The plot was curvilinear; however, the initial linear portion of the curve (Table 1) could be used to estimate k and n . EDTA treatment decreased the binding capacity by 50%, but k was doubled (Table 1). EDTA may chelate endogenous tightly bound Ca^{2+} so that sites previously occupied became available during the binding assay.

For the curvilinear binding plots, we found that the application of equation 9 was sufficient to quantitatively describe the whole profile. The obtained A_2 was used directly to calculate w which is really a measure of binding cooperativity. On the other hand, as a complex expression involving k , n , and w , the exact physical meaning of A_1 is difficult to decide. Nevertheless, it offers a way to determine the validity of k and n estimated from the initial linear portion of the curve. This can be achieved by comparing the values of nk calculated from linear method to that calculated from A_1 and w . The binding parameters obtained by the electrostatic model are shown in Table 2. It appears that the values of nk product are reasonably close to each other suggesting the estimation obtained by the initial linear portion was acceptable. The binding of Ca^{2+} to EDTA treated cell walls appears to be strongly negatively cooperative and most likely fits nicely with the electrostatic interaction model.

Calcium binding to freshly isolated potato tuber cell walls—In contrast to carrot cell walls, calcium binding to potato tuber cell walls was more complex. For both the control (untreated cell walls) and salt extracted cell walls, the electrostatic interaction model was needed to fit the curvilinear binding curves (Fig. 3A). As shown in Table 1, the binding capacity (n) and binding constant (k) of untreated cell walls, estimated from the initial linear portion of the curve, decreased only slightly after salt extraction. The extent of negative cooperativity in calcium ion binding, as measured by w , is not affected (Table 2) and this indicates that the electrostatic interaction between binding sites in potato cell walls remains relatively unchanged by salt treatment. Again, the estimated n and k values from the initial linear responses were acceptable judging from the agreement with the electrostatic interaction parameters.

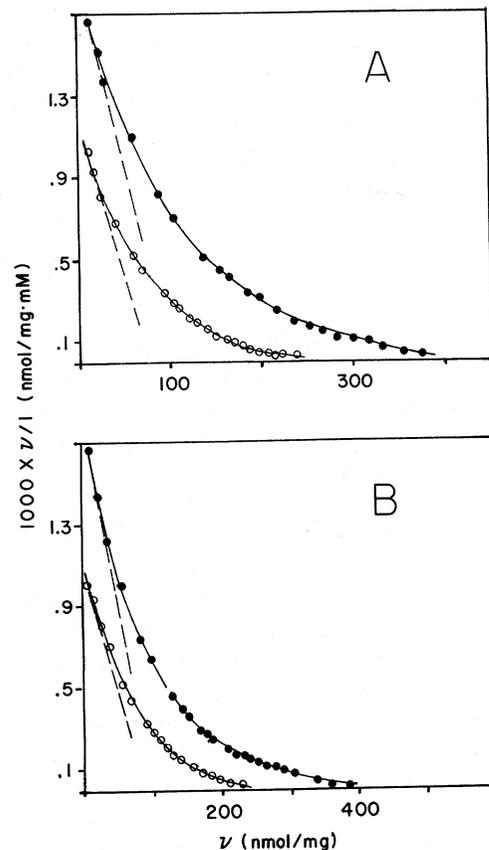


Fig. 3 (A) Calcium binding to freshly isolated potato tuber cell walls. Potato tuber cell walls were isolated and treated as described in the Materials and Methods. ● Represents untreated potato cell wall. ○ Represents salt-extracted potato cell wall. Solid lines represent the best-fit curves obtained by equation (9). Dotted lines represent the lines drawn for the graphical estimation of the thermodynamic quantities k and n . (B) Calcium binding to stored potato tuber cell walls. Potato tuber cell walls isolated, salt-extracted and stored for 1 week at 2°C . Symbols are the same as in (A).

Effects of cold storage on the Ca^{2+} binding properties of purified cell walls—Aliquots of purified cell walls were initially frozen but after thawing, the cell wall suspension had many clumps which were hard to disperse. Instead of freezing, cell walls were stored in a refrigerator for 1 week to determine if the Ca^{2+} binding properties were affected by storage. Storage of untreated carrot cell walls lowered both the binding capacity and binding constant when compared to the fresh preparation (Fig. 2 and Table 1). The storage of salt extracted cell walls did not drastically change the binding constant or binding capacity (Table 1). These results both suggest that the cell wall autolytic process (self-digestion) reported for some plant cell walls (Labrador and Nicolas 1984) occurs in carrots and was like-

ly to be enzymatic. The reduction of k and n during storage and the stabilization of these parameters after salt extraction is consistent with the ability of carrot cell walls to autohydrolyze. Salt extraction has been shown to prevent autolysis by removing cell wall-associated hydrolytic enzymes (Labrador and Nicolas 1984). Storage did not affect the binding capacity of EDTA treated cell walls but after storage, Ca^{2+} did not bind as tightly as before (Table 1). This observation was also consistent with the degradation or loss of the native structure of the cell wall during storage.

In contrast, storage treatment had minimal effects on Ca^{2+} -binding (Fig. 3 and Tables 1 and 2) properties of the control or salt-extracted potato tuber cell walls. These results were consistent with the fact that very little enzymatic autolysis of tuber cell walls occurred after 96 h of cold storage (unpublished results).

In conclusion, we demonstrated that both Ca-ion selective electrode and Arsenazo III dye binding methods are suitable to measure calcium binding to purified cell walls under proper conditions. The Ca^{2+} binding profile (Scatchard plot) to cell walls is affected by different treatments, e.g., mild salt extraction or cold storage. These treatments induce significant changes in the intrinsic binding constant (k) of Ca^{2+} to cell walls. Dramatic decreases in total binding capacity (n) were observed in carrot cell walls but not in potato cell walls. Yet, it is interesting to note that cell wall structure appears to contain only one type of intrinsic Ca^{2+} binding arrangement as supported by the need of only one intrinsic binding constant (k) to describe the overall process. The negative cooperative Ca^{2+} binding of potato cell walls and EDTA-treated carrot cell walls appears to reflect an electrostatic interaction, suggesting a close spatial arrangement of the binding sites in the cell wall. However, whether this relatively simple Ca^{2+} binding model can be applied to cell walls prepared by other methods remains to be determined.

References

- Allen, M.S., McBurney, M.I. and Van Soest, P.J. (1985) Cation-exchange capacity of plant cell walls at neutral pH. *J. Sci. Food Agric.* 36: 1065–1072.
- Baydoun, E.A.H. and Brett, C.T. (1984) The effect of pH on the binding of calcium to pea epicotyl cell walls and its implications for the control of cell extension. *J. Exp. Bot.* 35: 1820–1831.
- Bush, D.S. and McColl, J.G. (1987) Mass-action expressions of ion exchange applied to Ca^{2+} , H^+ , K^+ , and Mg^+ sorption on isolated cell walls of leaves from *Bassica Oleracea*. *Plant Physiol.* 85: 247–260.
- Demarty, M., Morvan, C. and Thellier, M. (1978) Exchange properties of isolated cell walls of *Lemna minor* L. *Plant Physiol.* 62: 477–481.
- Goldberg, R. (1985) Cell-wall isolation, general growth aspects. In *Modern Methods of Plant Analysis Vol. I. Cell Components*. Edited by Linskens, H.F. and Jackson, J.F. pp. 1–30. Springer-Verlag, Berlin.
- Harris, P.J. (1983) Cell walls. In *Isolation of Membranes and Organelles from Plant Cells*. Edited by Hall, J.L. and Moore, A.L. pp. 25–53. Academic Press, London.
- Hulanicki, A. and Marek, T. (1974) Direct potentiometric determination of calcium in water with a constant complexation buffer. *Anal. Chim. Acta* 68: 155–160.
- Kato, Y. and Nevins, D.J. (1984) Enzymic dissociation of *Zea* shoot cell wall polysaccharides. *Plant Physiol.* 75: 740–744.
- Labrador, E. and Nicolas, G. (1984) Autolysis of cell walls in pea epicotyls during growth. Enzymatic activities involved. *Physiol. Plant.* 64: 541–546.
- Morvan, C., Demarty, M. and Thellier, M. (1979) Titration of isolated cell walls of *Lemna minor* L. *Plant Physiol.* 63: 1117–1122.
- Nagahashi, G. and Seibles, T.S. (1986) Purification of plant cell walls: isoelectric focusing of calcium chloride extracted enzymes. *Protoplasma* 134: 102–110.
- Scarpa, A. (1979) Measurements of cation transport with metallochromic indicators. *Methods Enzymol.*: 301–338.
- Sentenac, H. and Grignon, C. (1981) A model for predicting ionic equilibrium concentrations in cell walls. *Plant Physiol.* 68: 415–419.
- Tanford, C. (1968) Multiple equilibria. In *Physical Chemistry of Macromolecules*. pp. 526–545. John Wiley and Sons, New York.
- Teper, M. and Taylor, I.E.P. (1981) The interaction of divalent cations with pectic substances and their influence on acid-induced cell wall loosening. *Can. J. Bot.* 59: 1522–1525.