demonstrated in vitro is probably enhanced in plums under natural conditions. Indeed, polyphenoloxidases and *o*-diphenols come into contact very rapidly at the beginning of drying through a cellular decompartmentation phenomenon (Raynal et al., 1985). In addition, polyphenoloxidase is very active at pH close to that of plums and displays great affinity for *o*-diphenols (Raynal et al., 1986). These compounds are oxidized rapidly and the products of oxidation are able to oxidize anthocyanins rapidly. This degradation is enhanced by their sensitivity to heat.

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Inhibition of Cucumber Tissue Softening in Acid Brines by Multivalent Cations: Inadequacy of the Pectin "Egg Box" Model To Explain Textural Effects[1]

Roger F. McFeeters* and Henry P. Fleming

Low concentrations of added calcium ions were shown to effectively reduce the first-order softening rate of cucumber (*Cucumis sativus*) mesocarp tissue stored in acid brines with 1.5 M NaCl. Softening rates of cucumber tissues without added calcium were hyperbolically correlated with the natural level of calcium in the cucumber (1.8–8.2 mM). The relationship between inhibition of softening rates and the concentration of added calcium was also hyperbolic. Inhibition of softening by added multivalent ions was in the relative order Ca ~ Sr > Ba ≫ Cu > Al. Cd, Co, Zn, and Mg were not effective softening inhibitors. All 12 lanthanide ions tested inhibited tissue softening. On a molar basis, La, Ce, Pr, Nd, Sm, and Eu had a greater inhibitory effect than calcium. Calculated low frequencies of calcium/pectin cross-linkages, the pattern of softening inhibition by other metal ions, and the inability of Cd ions to affect softening inhibition by calcium all suggested that the egg box model does not provide an adequate explanation for the observed textural effects of multivalent metal ions on cucumber tissue.

Recently, it was observed that there is a salt-softening effect when NaCl or other alkali-metal salts are added to

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acidified cucumber tissue (McFeeters et al., 1989). This effect is similar to that observed in a number of low-acid vegetables (Van Buren, 1986), but the softening mechanism involved may be different due to the low pH (Doesburg, 1965). Since calcium has been found to inhibit softening of cucumber tissue in a variety of conditions (Buescher et al., 1979, 1981; Fleming et al., 1978; Thompson et al., 1979; Tang and McFeeters, 1983; McFeeters et al., 1985), it was of interest to determine the effect of calcium ion under conditions in which salt softening occurred.

Calcium appears to help maintain cellular adhesion in fresh potato tissue (Linehan and Hughes, 1969) and to hold

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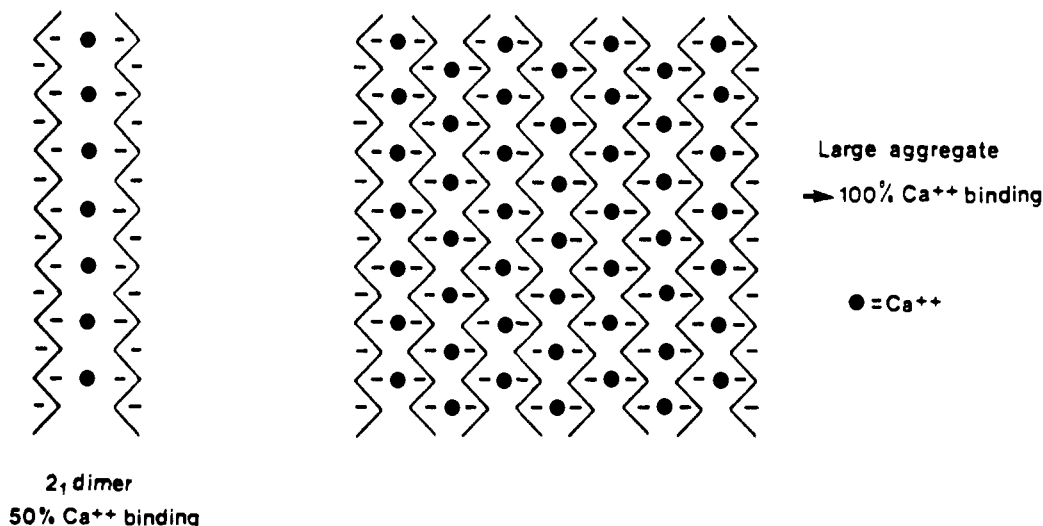
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Figure 1. Egg box model for calcium ion binding by polyuronate chains. Reprinted with permission from Rees (1982). Copyright 1982 Elsevier.

pectic substances in cell walls prepared from fresh tissues (Jarvis, 1982). In processed fruits and vegetables, calcium can cause firming of the tissue when it is added (Van Buren, 1984; Sterling, 1968; Archer, 1962), as well as inhibit softening of the tissue during heat processing or storage (Hudson and Buescher, 1985; Hughes et al., 1975; Personius and Sharp, 1939; McFeeters et al., 1985). Grant et al. (1973) first proposed an "egg box" mechanism to explain aggregation and gelation of alginate and polypectate molecules in dilute solutions of neutral pH when calcium or other divalent cations are added. It was suggested that divalent cations of an appropriate size could fit into ionic pockets formed by adjacent, negatively charged polysaccharide chains (Figure 1). The ionic cross-linkages could be formed cooperatively within negatively charged blocks in the polymers to give strong gels at low polymer concentrations. This mechanism of ion binding has received considerable support in subsequent studies (Thom et al., 1982; Rinado et al., 1980; Kohn, 1975; Powell et al., 1982). To explain calcium effects on the structure and texture of fruits and vegetables, it has often been assumed that an egg box type cross-linking of pectin molecules also occurs in plant cell walls (Selvendran, 1985; Jarvis, 1984; Demarty et al., 1984; Bartley and Knee, 1982).

However, the egg box model poses certain difficulties when it is used to explain textural effects in plant tissues, as opposed to gelation in pectate solutions. The most serious mechanistic problem is that, in many cases, the pectin in fruit and vegetable cell walls has a high proportion of methyl-esterified carboxyl groups, which usually appear to be randomly distributed in the polymer chains (Anger and Dongowski, 1985). It has been shown that the binding affinity of calcium declines as methylation increases (Kohn and Furda, 1967). Powell et al. (1982) reported that approximately 25 uninterrupted uronic acid residues are required to form stable junction zones in a pectate gel. Such blocks of nonesterified carboxyl groups should rarely occur with the degree of esterification commonly found in fruits and vegetables if the methyl ester groups are randomly distributed. Thibault and Rinado (1986) showed that pectin preparations with randomly distributed methyl ester groups would not gel in the presence of calcium when the degree of esterification was above about 40%. Thus, there is a question whether calcium or other divalent cations could cause substantial gel formation in the cell walls of fruits and vegetables. The purpose of this paper is to describe the textural effects of

multivalent cations on softening of blanched cucumber tissue and to consider the implications of these effects for the mechanism of calcium action.

MATERIALS AND METHODS

Cucumbers were either Calypso cultivar grown under irrigated conditions using standard cultural practices or fruit of unknown cultivar obtained from local processors. Fruit size was 44–51-mm diameter (commercial size 3B).

Metal ions were used as their reagent-grade chloride salts with the exception of aluminum. NaCl was obtained from Fisher Scientific Co. (Raleigh, NC). Other metal chlorides were purchased from Aldrich Chemical Co. (Milwaukee, WI). Aluminum was used in the form of food-grade alum, $AlK(SO_4)_2 \cdot 12H_2O$ (Holland Co., Adams, MA).

The procedure of McFeeters et al. (1989) was used to measure tissue softening rates. Briefly, a food slicer was used to prepare 7-mm-thick slices from whole cucumbers after removal of about 15-mm tissue from both the blossom and stem ends of the fruit. Mesocarp pieces were prepared by peeling the fruit prior to slicing (7-mm thickness) and then cutting the mesocarp tissue sections from each of the three fruit carpel sections. The seed area of the peeled slices was discarded. Cucumber slices or mesocarp pieces were then blanched for 3 min in boiling, distilled water, cooled, and packed into 360-mL (12-oz) or 60-mL (2-oz) jars, respectively. The samples were covered with a volume of brine equal to the volume of cucumber tissue, which contained 3.0 M NaCl, 0.2 M acetic acid, 400 ppm SO_2 added as sodium metabisulfite, and the appropriate multivalent ion. The sample jars were held at either 17 °C (data in Figures 2 and 5) or 4 °C for 3 days to allow equilibration. They were then placed in a 44 °C incubator to accelerate softening reactions. The firmness of the cucumber mesocarp tissue was measured on 15 slices or tissue pieces per jar from duplicate jars at the time samples were placed in the 44 °C incubator and at four subsequent sampling times. The only exception to this practice was the calcium/cadmium competition experiment in which single jars were analyzed at each sampling time. Firmness measurements were made with a 0.315-cm-diameter, flat-tipped, stainless steel punch mounted on an Instron Universal Testing Machine (Instron Corp., Canton, MA) (Thompson et al., 1982).

A calcium/cadmium competition experiment was carried out by preparing 36 brines at a 2× concentration to give all combinations of 0, 2, 5, 10, 20, and 80 mM calcium and cadmium after equilibration. All samples contained an equilibrated concentration of 1.5 M NaCl. Five jars with 30 g of blanched cucumber mesocarp pieces were covered with each brine. One jar from each treatment was analyzed at each sampling time.

The inhibitory effect of lanthanide ions on the rate of cucumber softening relative to calcium ion was determined. Ten jars of cucumber mesocarp pieces were equilibrated with 1.5 M NaCl and 0, 2, 5, 10, 20, and 80 mM added calcium. Softening rate
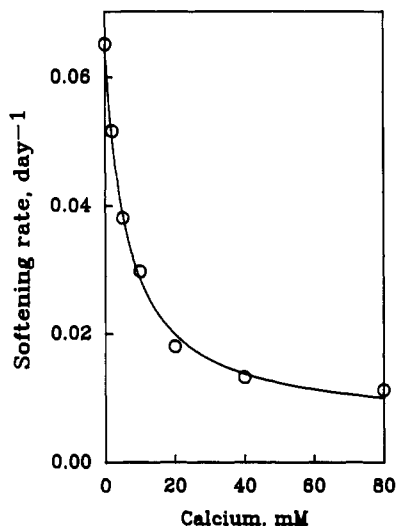
Inhibition of Cucumber Tissue Softening

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Figure 2. Effect of calcium concentration on the inhibition of mesocarp tissue softening in blanched cucumber slices. The line is the least-squares fit of the observed rates to a hyperbola. Parameters of the hyperbola: $r_m = 0.0655$ day^{-1}; $r_s = 0.0058$ day^{-1}; $c_{0.5} = 6.17$ mM; $R^2 = 0.9992$.
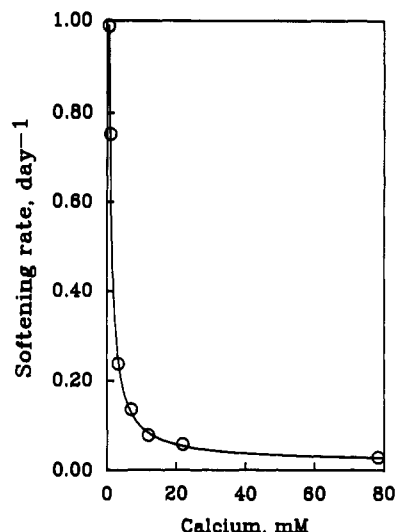

Figure 3. Effect of calcium concentration on the inhibition of softening of cucumber mesocarp tissue pieces. The line is the least-squares fit of the observed rates to a hyperbola. Parameters of the hyperbola: $r_m = 0.993$ day^{-1}; $r_s = 0.018$ day^{-1}; $c_{0.5} = 1.51$ mM; $R^2 = 0.9998$.

constants were determined and fit to a hyperbolic curve. This curve was used as the standard curve for comparison of the inhibition by the lanthanide ions. Each of 12 lanthanide ions was added at a 10 mM equilibrated concentration to 10 jars of cucumber tissue. The first-order rate constants for softening were compared to the calcium standard curve. The amount of calcium ion required to give a softening inhibition equivalent to each lanthanide ion was calculated.

Pectin Methylation Analysis. Uronic acid and pectin methylation in the cell wall samples were analyzed as described previously (McFeeters and Armstrong, 1984), except that the Saeman hydrolysates (Blakeney et al., 1983) after ammonium hydroxide addition were used for the colorimetric analysis of the uronic acids. Use of these hydrolysates rather than the hydrolysis procedure described by Scott (1979) reduced the variability in the analysis (McFeeters and Lovdal, 1987).

Calcium Analysis. Calcium was analyzed by the colorimetric procedure of Gindler and King (1972).

pH Determinations. Measurement of the pH of brines after equilibration with cucumber tissue was done on an Orion Model 901 pH meter with a combination electrode.

Data Analysis. First-order softening rates (Huang and Bourne, 1983) were calculated by linear regression analysis using the mean tissue firmness calculated for each sample jar. Usually, the r^2 value for the linear fit was 0.95 or greater. Data on tissue softening rates as a function of calcium ion concentration were fitted to a hyperbolic curve by the nonlinear regression analysis available in SAS (1982) or Asystant+ (MacMillan Software Co., New York, NY). Both software packages gave the same result. The parameters of the hyperbola were as follows: $r_m =$ softening rate at the minimum calcium concentration; $r_s =$ softening rate at saturating calcium levels; $c_{0.5} =$ calcium concentration giving a softening rate halfway between r_m and r_s; and $R^2 =$ goodness of fit of experimental data to the hyperbolic model. Analysis of the reciprocal plots of tissue softening rates as a function of cadmium concentration to determine whether curves at different calcium concentrations were parallel or intersecting was done by weighted linear regression analysis.

RESULTS AND DISCUSSION

Cucumbers with 1.5 M added NaCl softened rapidly at 44 °C. The quantitative effect of added calcium ion in inhibiting this softening was first determined on cucumber slices (Figure 2). The inhibition followed a hyperbolic curve in which 50% of the maximum calcium ion inhibition occurred with 6.2 mM added CaCl₂. In addition to the added calcium, the cucumber slices contributed 3 mM calcium ion to the equilibrated calcium concentration such

that half-maximal softening inhibition occurred at 9.2 mM total calcium. Figure 3 shows a second calcium inhibition experiment in which pieces of mesocarp tissue, rather than whole slices, were used. It can be seen that again there was a hyperbolic inhibition of softening. In both experiments, the hyperbolic model accounted for greater than 99.9% of the experimental variation in softening rates. Two differences in this experiment were that softening rates were much higher than the slice experiment and that half-maximal inhibition of softening occurred at only 1.5 mM total calcium. An important point to make is that the degree of pectin methylation in these experiments was expected to be quite high since the blanch treatment used was designed to inactivate pectinesterase activity present in cucumber tissue (McFeeters et al., 1985). In the experiment shown in Figure 3, the degree of pectin methylation was found to be 62%.

The pattern of metal ion effects was found to be very reproducible among different lots of cucumbers in these experiments and in other experiments on the salt-softening effect (McFeeters et al., 1989). However, it was found that the absolute softening rates could be very different in different lots of fruit (compare Figures 2 and 3). Such differences in softening rates could be caused by many factors such as differences among cultivars, soil and climate conditions during fruit growth, the effects of storage and handling conditions after harvest, etc. One factor that could be measured, even though it could not be controlled, was the natural Ca²⁺ ion concentration in the cucumbers. Figure 4 shows the relationship between the natural level of calcium in the tissue and the rate of tissue softening for blanched tissue in 1.5 M NaCl without added calcium. It was found that, for cucumbers obtained from a variety of sources and climates over a 2-year period, the natural Ca²⁺ ion concentration in the tissue varied from 1.8 to 8.2 mM. Though the data showed greater scatter than Figures 2 and 3, the hyperbolic model still accounted for 97% of the observed variation. This result suggested that the natural Ca²⁺ level is a major factor in determining the softening rates observed in cucumber tissue without added calcium. Both the lower initial softening rate, r_m, and the higher $c_{0.5}$ calcium ion concentration observed for the experiment in Figure 2, as compared to Figure 3, may be due to the higher natural calcium level in the cucumber used in the
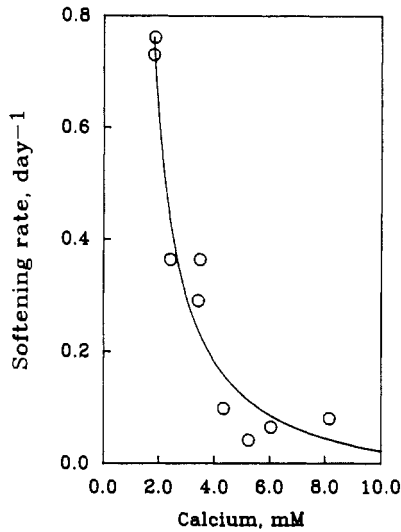
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Figure 4. Relationship between the natural concentration of calcium in different lots of cucumbers and the rate of tissue softening in the presence of 1.5 M NaCl. The line is the least-squares fit of the observed rates to a hyperbola. Parameters of the hyperbola: $r_m = 0.752$ day^{-1}; $r_s = -0.076$ day^{-1}; $c_{0.5} = 1.02$ mM; $R^2 = 0.9743$.

experiment shown in Figure 2. The presence of 3 mM equilibrated natural calcium would have lowered the softening rate without added calcium, r_m. Then, due to the hyperbolic pattern of calcium inhibition, higher concentrations of added calcium were required to reach $c_{0.5}$.

Considering the many instances in which Ca^{2+} ions have been found to inhibit softening of fruit and vegetables, including cucumbers (Buescher et al., 1981; Tang and McFeeters, 1983; McFeeters et al., 1985), it was not surprising that calcium ions inhibited cucumber mesocarp tissue softening. However, the quantitative aspects of this inhibition were quite interesting. The excellent hyperbolic relationship between the concentration of calcium and its effect on softening rates had not been previously demonstrated. This indicated that calcium ions were saturating a binding site that inhibited softening. Additionally, the observation that low levels of Ca^{2+} ions inhibit softening in the presence of high Na$^+$ concentrations led to an effort to estimate the frequency of calcium ion cross-links that would be expected if calcium had the same binding affinity for cucumber tissue pectin, as has been reported by Kohn and Furda (1967) for citrus or apple pectins with a similar degree of esterification.

To help make such an estimate, data on the calcium concentration required for half the maximum softening inhibition (1.5 mM), the sodium concentration, the degree of pectin methylation (62%), and pH (3.3) from the experiment shown in Figure 3 were used. From the literature on ion binding by pectin, a selectivity coefficient for calcium ion displacement of potassium ions (log $K = 0.46$ at 62% methylation) determined by Kohn and Furda (1967) and an estimated pK of 3.6 for pectin carboxyl groups (Cesaro et al., 1982) were used in the calculation. On the basis of these values, it was calculated that only one ionic cross-link per 2700 uronic residues should occur at 1.5 mM calcium, assuming a random distribution of methyl ester groups in the pectin (see Appendix). If on the other hand it were assumed that the nonesterified carboxyl groups were located in blocks with a low degree of esterification so that within these block regions the calcium/potassium selectivity coefficients were large (log $K = 2.19$ at 5.6% methylation), the frequency of calcium cross-links would be 1 every 40 uronic acid residues within the block regions
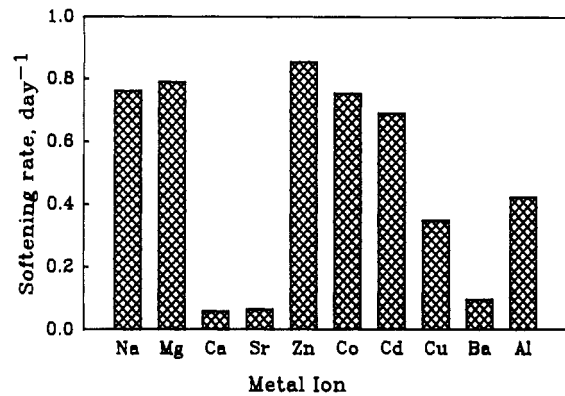

Figure 5. Comparison of cucumber mesocarp tissue softening rates in the presence of 1.5 M sodium ions and 10 mM concentrations of multivalent metal ions to the softening rate in the presence of 1.5 M sodium ions alone. With the exception of barium and aluminum, the multivalent ions are in the order of increasing binding affinity to pectin according to Kohn (1975, 1987).

or 1 every 110 uronic acid residues considering both the esterified and nonesterified residues. Anger and Dongowski (1985) reported that esterification in cucumber tissue is random. Therefore, the 1:2700 estimate for cross-link frequency is probably the better estimate. Regardless of the distribution of ester groups, these estimates clearly indicate that calcium cross-links must be relatively infrequent under low-pH and high-NaCl concentrations where calcium is an effective inhibitor of softening. This suggests, in turn, that the egg box model (Grant et al., 1973) is not an adequate model to explain the inhibition of softening by calcium ion in these experiments. Prior to the development of the egg box model to explain metal ion effects in dilute pectin solutions, it had been pointed out that isolated ionic cross-links between carboxyl groups would not be sufficient for gelation of pectic substances (Deuel et al., 1950). Doesburg (1965) pointed out the problems of explaining calcium effects in vegetables in terms of pectin cross-linking. However, in the absence of either clear evidence to the contrary or alternative explanations for the textural effects of calcium, the tendency has been to explain the effect of metal ions on texture in terms of the egg box model (Selvendran, 1985; Jarvis, 1984; Demarty et al., 1984; Bartley and Knee, 1982), even though the esterification problem was recognized.

The ability of calcium ions to inhibit softening at low concentrations in the presence of a high concentration of NaCl at low pH raised the question whether other ions, some of which bind to pectin with greater affinity than calcium (Kohn, 1975), would also inhibit softening under these conditions as well or better than calcium. Figure 5 shows a comparison of softening rates for a number of ions at a 10 mM concentration in the presence of 1.5 M NaCl. The greatest inhibition was caused by Ca^{2+}, Sr^{2+}, and Ba^{2+}. Little or no inhibition was seen with Mg^{2+}, Zn^{2+}, Co^{2+}, and Cd^{2+}. Aluminum and copper ions were special cases. Both ions showed a moderate inhibition of softening rates, but the cucumber tissue stopped softening with a higher residual firmness than the other ions (<0.7 N). Tissue with added copper ion stopped softening with a firmness of 4.3 N. For aluminum, softening stopped at 1.5 N. There was considerable softening of the cucumber tissue during equilibration at 17 °C only with Al^{3+} ion. Reasons for these different effects of Cu^{2+} and Al^{3+} ions are not known. As a result of the occurrence of softening during equilibration with aluminum ion, the equilibration temperature was reduced to 4 °C in subsequent experiments.
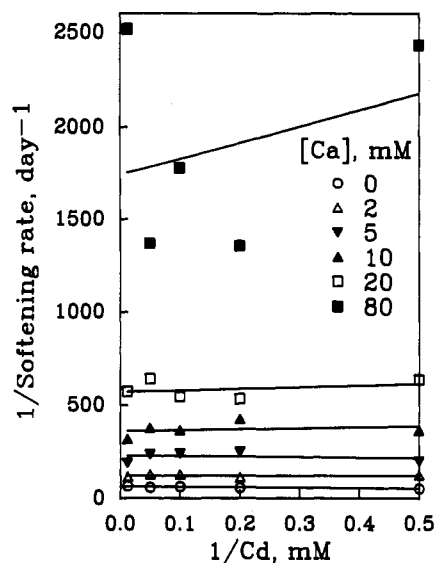
Figure 6. Double-reciprocal plots of cadmium ion concentration versus the softening rate of cucumber tissue pieces at different calcium ion concentrations.

Figure 7. Inhibition of softening rates of cucumber tissue pieces by lanthanide ions. The inhibition by a 10 mM concentration of each ion is related to the concentration of calcium ion required to give an equivalent softening rate. The ionic radii are from Shannon (1976) for octacoordinate cations.

On the basis of the work of Kohn (1987), the relative affinity of the divalent ions used in this study for pectin is Cu ≫ Cd > Co > Zn = Sr = Ca > Mg. The relative inhibition of softening was Ca ~ Sr ≫ Cu. Cd, Co, Zn, and Mg had little or no effect on softening rates. Barium and aluminum were not investigated for their affinity to pectin. Thus, there appeared to be little correlation between the affinity of ions for pectin and the ability of the ions to inhibit tissue softening. If inhibition was primarily due to egg box type cross-links, it would be expected that ions with greater binding affinities should create more frequent bridges and, therefore, be better inhibitors for softening. If there were blocks of free carboxyl groups in the cell walls, this effect should be accentuated. Thibault and Rinado (1986) found that Co, Cd, and Cu ions did cause gelation of deesterified pectin but that cooperatively of chain association was less for these ions than Ca, Sr, and Ba. Thus, the gel-forming capabilities of Co, Cd, and Cu may be less than the alkaline-earth ions, even though their binding affinities to pectin carboxyl groups may be greater. These results suggest either (1) softening inhibition by calcium and strontium is due to ion binding to pectin, but the electronic or size characteristics of Zn, Co, Cd, and Cu ions prevent this effect from occurring when they interact with pectin; or (2) the textural effects of calcium and strontium ions are due to binding at specific texture modification sites other than pectin carboxyl groups, but other ions are not sufficiently similar to calcium ion to bind at these sites.

The inability of cadmium ion to inhibit softening was especially interesting. Cadmium has nearly the same ionic radius as calcium, and Kohn (1987) has found that the affinity of cadmium for galacturonic acid oligomers is considerably greater than calcium ions. These factors suggested that it would be useful to do a competition experiment in which the concentrations of cadmium and calcium ions would be varied in the presence of 1.5 M NaCl. Then, if case 1, described above, were true, cadmium ions should competitively reverse the ability of calcium ions to inhibit softening. If case 2 were true and calcium was having its effect by binding to sites where cadmium could not bind, the presence of cadmium ions should have no influence on the inhibition of softening by calcium. The results of this experiment are shown as a double-reciprocal plot in Figure 6. If the texture-modi-
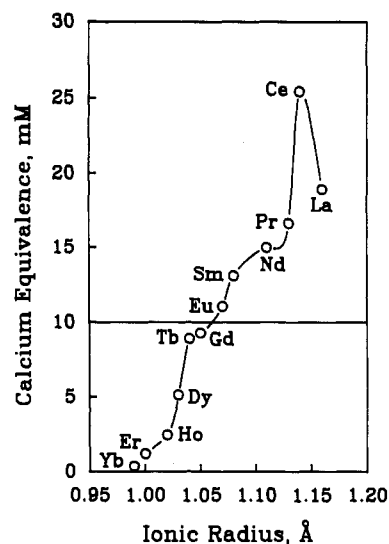
fying sites for calcium were pectin carboxyl groups, cadmium ions should competitively displace calcium ions such that the lines at different calcium concentrations should all intersect on the y-axis at the point where the 0 mM calcium line intersects the y-axis. If there was no competition between calcium and cadmium, the lines should be parallel to the x-axis. Since statistically the slopes of the lines were not different from 0 and, therefore, parallel to the x-axis, the conclusion was that cadmium did not compete with calcium at the site responsible for inhibition of softening. The large scatter of points at 80 mM calcium resulted from taking reciprocals of very small softening rate constants. The results in Figure 6 suggest that calcium has its textural effect by binding at sites other than pectin carboxyl groups.

The fact that strontium and barium were the best inhibitors of softening other than calcium suggested that ions that can bind to sites specific for calcium ions might be the best possibilities as softening inhibitors. There has developed over the years extensive literature in which lanthanide ions have been shown to bind specifically and strongly to calcium binding sites in proteins (Hurrocks, 1982; Martin, 1983; O'Hara, 1987). Calcium substitution by lanthanides has not been investigated for carbohydrate polymers, though Angyal (1981) has shown that La³⁺ can bind to some neutral sugars in a way similar to calcium, strontium, and barium. Based upon this ability of lanthanide ions to substitute for calcium ions, a series of lanthanide ions were compared to calcium for their ability to inhibit the rate of cucumber mesocarp tissue softening. Figure 7 shows that all these ions inhibited softening to some degree. Ions of atomic number <62 had a greater degree of inhibition than calcium on an equimolar basis. Thus, it appears that ions that can substitute for calcium in proteins with very high selectivity can also inhibit tissue softening. This result contrasts with the lack of correlation for the metal ions discussed above between affinity for pectin carboxyl groups and their ability to reduce softening rates. It cannot be determined from this experiment whether softening inhibition is due to the interaction of lanthanide ions with polysaccharides, proteins, or other components of the cucumber cell wall.

There has been a general acceptance of the egg box model as a way to look at the effect of multivalent cations,

particularly calcium, on the structure of plant tissues whether fresh or processed. However, Tepfer and Taylor (1981) concluded that their data did not support the idea that divalent cation inhibition of acid-induced cell wall loosening was caused by pectic gel formation in the cell wall. McFeeters et al. (1985) suggested that calcium might influence the texture of cucumber tissue by mechanisms other than the egg box model, based upon the fact that calcium inhibited softening, regardless of the degree of pectin methylation in the tissue. The present results also strongly suggest that another mechanism of calcium action on texture exists in cucumber tissue. The chemical basis for this mechanism is not yet known. Considering the fact that the rate of softening is reduced rather than that firmness is increased, it appears that calcium must act by inhibiting the degradation of cell wall polymers, presumably by acid hydrolysis, considering the low pH at which experiments were conducted. The inability of cadmium ion, with the same size and charge as calcium, to have any effect on softening rates indicates that the site of binding is quite specific for calcium. The ability of strontium, barium, and a series of lanthanide ions to inhibit softening also suggests a high specificity for binding of ions that act as calcium analogues in other biological systems. It will be of interest to see whether a similar effect of calcium can be observed in other plant tissues.

SUMMARY AND CONCLUSIONS

It has been found that low concentrations of calcium ions were able to inhibit softening of blanched cucumber tissue in the presence of high salt concentrations. The relationship between calcium concentration and inhibition of softening rates was hyperbolic. Among a variety of other metal ions tested, only ions that are known to substitute for calcium ions in biological systems were comparable to calcium in their ability to inhibit softening rates. These included strontium, barium, and a series of lanthanide ions.

The pattern of metal ion effects on tissue softening rates and the conditions in which inhibition occurred led to a reevaluation of the ability of the egg box model of calcium binding to pectin to explain the observed textural effects. Three lines of data were difficult to explain by the egg box model. First, under conditions where calcium was an effective inhibitor of cucumber tissue softening, calcium cross-links would be very infrequent. Second, there was no apparent relationship between the affinity of divalent ions for pectin and the ability of these ions to reduce softening rates. Third, cadmium ions, which did not inhibit tissue softening, also did not reverse the effect of calcium even though cadmium ions bind more strongly to pectin than calcium. This suggested that the textural effects of calcium are a result of calcium binding at sites other than pectin carboxyl groups. Therefore, efforts should be directed to look for calcium interactions with plant cell walls, in addition to egg box type cross-linking. The fact that lanthanide ions caused textural effects similar to that of calcium may make it possible to use certain lanthanides as spectroscopic probes of the location and function of calcium ions in cell walls in ways similar to what has been done for calcium binding proteins.

APPENDIX

Estimation of the Frequency of Calcium Cross-Links. The number of uronic acid residues per calcium ion cross-link (N_f) was estimated from

$$N_f = 2 \times \frac{1}{(1.0 - F_e)F_c X_{Ca}}$$

where F_e = fraction of uronic acid residues esterified, F_c

= fraction of negatively charged carboxyl groups at the experimental pH, and X_{Ca} = equivalent fraction of calcium ions in pectin. The factor 2 is required because two charged carboxyl groups are required per cross-link. F_e is obtained by measuring the pectin esterification in cell wall samples. F_c is calculated from the Henderson Hesselbach equation and an estimated pK of 3.6 for pectin uronic acid residues (Cesaro et al., 1982). X_{Ca} is calculated by using the selectivity coefficients from Kohn (1967) and solving the equation defining the selectivity coefficient for the equivalent fraction of calcium ions in the polymer.

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