Mineral Binding Properties of Soy Hull. Modeling Mineral Interactions with an Insoluble Dietary Fiber Source

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Dietary fiber may influence essential mineral adsorption from the gastrointestinal tract. Soybean seed coat (soy hull) cell wall tissue was employed as a substrate for modeling the ion-exchange properties of insoluble dietary fiber. Equations developed to model ion equilibria in plant cell walls were utilized to predict the extent of cation binding to soy hull. Methods were developed to accurately evaluate the cation-binding parameters required by this model, including the concentration of fixed anionic cation-binding sites and the dissociation constants of these sites for H^+, Ca^{2+}, and Zn^{2+}. The model predicted the extent of cation binding in divalent/monovalent, divalent/divalent, or triangular divalent/divalent/monovalent cation-exchange systems. However, the extent of anion association was greater than expected. This work suggests that soy hull may not bind minerals to a significant degree under the low pH or high ionic strength conditions found in the gastrointestinal tract.

The response of essential trace mineral absorption processes in the human body to the presence of dietary fiber is not well characterized. Dietary fiber, the portion of the plant cell wall that is not digested in the gastrointestinal tract, includes pectic substances, hemicelluloses, cellulose, lignin, and other wall components resistant to enzymatic hydrolysis. Dietary fiber components may be broadly divided into two categories, soluble and insoluble, on the basis of their solubility characteristics under simulated digestive tract conditions. The mineral-binding attributes of soluble dietary fibers, generally pectins and neutral hemicelluloses, and purified fiber components have been studied (Camire and Clydesdale, 1981; Rendleman and Grobe, 1982; Garcia-Lopez and Lee, 1985; Lee and Garcia-Lopez, 1985), although their influence on trace mineral absorption has not been determined precisely. Studies of the mineral-binding properties of the insoluble cell wall matrix have shown that it has a substantial cation-exchange capacity and may affect trace mineral absorption (Thompson and Weber, 1979; McBurney et al., 1983; Allen et al., 1985; Ward and Reichert, 1986).

Although the mineral-binding properties of dietary fiber, as such, are poorly understood, the interaction of cations with plant cell walls in general is a highly studied process. Numerous models have been developed that describe the cation absorption capacity and selectivity of cell walls. These models range from those that are rigorously precise in their thermodynamic treatment of the ion-exchange process (Demarty et al., 1976, 1980; Marinsky, 1980; Van Cutsem and Gillet, 1983) to those that rely on empirical observations (Ritchie and Larkum, 1982; Amory and Dufey, 1984). Models of the former type usually require knowledge of parameters experimentally inaccessible, particularly in complex biological systems, while the latter model types do not provide insight into the physical processes involved in cation binding nor provide adequate predictions outside a narrow set of ionic conditions. However, the equations developed by Sentenac and Grignon (1981) provide a model that bridges this gap by invoking physical phenomena that may be expressed exactly (Donnan potential, chemical equilibria, conservation of electrical neutrality). From these equations, simplifying assumptions are made, and the result is a set of equations

requiring the measurement of a limited number of parameters. These simplified equations provide a physical or conceptual sense of the cation-binding process and at the same time produce a quantitative estimation of the extent of binding even in solutions of relatively great ionic complexity.

The equations of Sentenac and Grignon have been adopted herein to predict ion binding to the soybean seed coat. Soy hull was selected as a model tissue for examining mineral associations with dietary fiber because it is composed almost entirely of primary and secondary plant cell wall material (Mitaru et al., 1984). Its polysaccharide composition is well characterized (Aspinall et al., 1966; Aspinall and Jiang, 1974). Furthermore, soy hull lacks phytic acid and has a very low content of lignin, mineral-binding components commonly present in other legume- or cereal-based sources of dietary fiber. This report describes procedures for accurately assessing the mineral-binding parameters, i.e. the total number and concentration of fixed anionic sites within the cell wall, and the cation dissociation constants of these sites necessary to predict the extent of cation association with soy hull, by utilizing the Sentenac and Grignon model.

MATERIALS AND METHODS

Materials. Calcium-45 (810 MBq/mg), zinc-65 (20.3 MBq/mg), and orthophosphate-32 (carrier free) were purchased from Amersham Corp. Solutions were made from distilled, demineralized water. Other reagents were analytical grade or better.

Soy Hull Preparation. Century soybean seed [*Glycine max* (L.) Merr.] was soaked in distilled water for 0.5 h to loosen the hull from the cotyledon. The hulls were removed by hand and the pigmented hilum discarded. Endogenous hull minerals were removed by extraction with 0.05 N HCl (three times with approximately 250 mL/g dry weight). This material will be referred to as "acid-washed" soy hull. For some experiments, hulls (without hilum) were dried by rinsing with acetone and storage under vacuum and then ground with a Wiley mill to pass a 40-mesh screen. The ground hull was acid treated, as above, and then suspended in 10 mM 4-morpholineethanesulfonic acid (Mes). The suspension was adjusted to pH 6.0 by the addition of tetramethylammonium hydroxide (TMAH) and stirred at room temperature for 2 h. The insoluble residue was collected by filtration, rinsed sequentially with water and acetone, and stored under vacuum. This material will be referred to as "buffer-extracted" soy hull.

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Cation Binding. *Method 1.* Two essentially different protocols were employed to determine the amount of minerals bound to soy hull material. Method 1 measured the amount of minerals bound after equilibration with a known free concentration of each ion, adopted as described by Sentenac and Grignon (1981). Approximately 50 mg of either acid-washed or buffer-extracted hull was suspended in 50 mL of buffered solutions of known ionic composition. The suspension was stirred at 25.0 °C for 20 min. The buffer was decanted, and the equilibration process was repeated twice. By this procedure, the hull material became equilibrated with a solution whose mineral concentration was essentially equivalent to the composition of the initial buffer solution. Buffered solutions consisted of 10 mM acetic acid for the range pH 4.40–5.20, 10 mM Mes for pH 6.00, or 10 mM 4-morpholine-propanesulfonic (Mops) for pH 7.20, adjusted to the indicated pH with either KOH or TMAH. The chloride salts (KCl or tetramethylammonium chloride) of the respective hydroxides were added to bring the solution to the appropriate total monovalent cation concentration. Divalent cations (Ca^{2+}, Mg^{2+}, Zn^{2+}) were added as their chloride salts. After the final equilibration step, the hull material was collected by filtration on a sintered glass (coarse) funnel. The material was washed successively with ethanol and acetone to remove extraneously adsorbed buffer and then dried overnight under vacuum. Finally, the weighed hull material was extracted with 100 mL of 0.05 N HCl. Particulate hull material was removed from the acid extraction medium by filtration through a 1.2-μm Millipore filter. The mineral content of the acid extract was determined by atomic absorption spectroscopy (Varian Techtron), as described by Garcia et al. (1972).

Method 2. This method measures the free concentration of each mineral after the hull material was equilibrated in a buffer of known total mineral composition. The bound mineral value was then obtained by subtraction of the measured free cation concentration from the total solution concentration. Use of method 2 was generally limited to the study of mineral binding to buffer-extracted soy hulls, for reasons to be explained in Results. Hull material was suspended in 100 mL of buffer (composition described above) at a concentration of 1.0 g of dry weight/L. The suspension was stirred for 1 h at 25.0 °C, and then varying concentrations of divalent cations were added and allowed to equilibrate for 0.5 h. Depending on the method employed to detect the free divalent cation concentration after eqilibration, the suspension was (a) measured as is with cation-specific electrodes (for Ca^{2+} and Mg^{2+} determinations), (b) centrifuged for 5 min before sampling 200 μL of the supernatant (for ^{45}Ca and ^{65}Zn determinations), or (c) filtered through a 0.2-μm filter and measured by atomic absorption (for Ca^{2+}, Mg^{2+}, and Zn^{2+} determinations). ^{45}Ca and ^{65}Zn were determined by scintillation and γ-ray spectroscopy, respectively. Bound K^+ concentrations were always determined on the buffer-extracted hull material following method 1 since the concentration of free monovalent cations never differed measureably from the total solution concentration under these experimental conditions.

Anion Binding. Acid-washed hull was ground to pass a 40-mesh screen. The ground hull material (35–100 mg) was suspended in 1.0 mL of 10 mM Mops buffer, pH 7.2, containing either trace amounts of $^{32}P_i$ or 0.5 mM K_3Fe-$(CN)_6$. The suspension was incubated for 1 h with frequent vortexing and then centrifuged for 5 min. The $^{32}P_i$ content of the supernatant was determined by scintillation. The $Fe(CN)_6{}^{3-}$ concentration of the supernatant was measured

by its absorbance at 420 nm.

Hull Hydration Volume. The rehydration volumes of acid-washed hull (ground to pass a 40-mesh screen) and buffer-extracted hull were measured following the general procedure of Pepper et al. (1952). Hull material (20–40 mg) was weighed into a Centrifree micropartition cartridge (Amicon Corp.) and suspended in 10 mM Mops buffer, pH 7.2. Following a 1-h incubation period, the cartridge was centrifuged at approximately $1000g$ for 30 min. The mass difference due to retained buffer was taken as the hydration volume of the hull material. A density of 1.00 mL/g (25.0 °C) was measured for the hydrated acid-washed hull with a Moore–Van Slyke specific gravity bottle.

Uronic Acid Content. The total uronic acid and uronate methyl ester content of the various soy hull fractions was determined by following the procedures of McFeeters and Armstrong (1984). Briefly, the total uronic acid content was measured colorimetrically with 3,5-dimethylphenol reagent following partial acid hydrolysis of 2–10 mg of hull material. The degree of methylation was estimated by measuring the amount of methanol generated by base treatment of 10–20 mg of hull material. Methanol was analyzed by GLC with a Bendix 2600 gas chromatograph equipped with a 2-m 5% Carbowax 20M glass column.

RESULTS

Theory. The plant cell wall may be viewed as a macromolecular structure containing numerous fixed anionic sites, in pectins or other acidic polysaccharides, that binds cations with a characteristic capacity and specificity. Two factors control the extent and specificity of cation (i.e., mineral) binding.

First, the anion concentration difference between the wall and solution phases creates a Donnan-type potential such that cations are accumulated in, and mobile anions are excluded from, the wall phase. The relationship expressing the ratio of the concentration of a particular unbound cation or anion with a charge of Z_i in the wall phase, $[\bar{M}_i{}^u]$, to its free solution concentration $[M_i]$ due to the Donnan potential, where r is an exponential term expressing the Donnan ratio, is given by

$$[\bar{M}_i{}^u]/[M_i] = r^{Z_i} \qquad (1)$$

The second factor influencing cation-binding specificity is the intrinsic affinity of the fixed anionic carboxyl groups for each of the cationic species present in solution. This intrinsic affinity is expressed by the dissociation constant (K_i) for each cation in the mass-action equation

$$K_i = [\bar{M}_i{}^u][R_{COO^-}]/Z_i[\bar{M}_i{}^b] \qquad (2)$$

where $[R_{COO^-}]$ represents the unassociated fixed anion concentration and $[\bar{M}_i{}^b]$ the cation concentration bound or complexed specifically to the anionic sites.

The overall electroneutrality of the wall phase is maintained by counterbalancing the $[R_{COO^-}]$ charges with unassociated cations:

$$[R_{COO^-}] = \sum_{i=1}^{n} Z_i[\bar{M}_i{}^u] \qquad (3)$$

The summation is carried out over all unbound cationic and anionic species in the wall phase.

Finally, the concentration of unassociated fixed anions is related to the total concentration of fixed anions, $[R_t]$, by

$$[R_{COO^-}] = [R_t] - \sum_{i=1}^{k} Z_i[\bar{M}_i{}^b] \qquad (4)$$

where the summation is carried out over all bound cationic

Table I. Removal of Minerals from Soybean Hulls by Extraction with 0.05 N HCl

	sample contents, μmol/g dry wt		
element[a]	control	acid-washed	mineral content decrease, %
Fe	6.6	2.8	58
Mg	60	2.2	96
Zn	0.47	0.17	66
Cu	0.14	0.002	99
Ca	100	0.0	100
K	310	0.0	100
total	477	5.2	99

[a] Mineral content was determined by atomic absorption measurements of wet-ashed hull samples (approximately 1.0 g).

species. With the appropriate substitutions of eq 1–3 into eq 4 for the quantities $[R_{COO^-}]$ and $[\bar{M}_i^b]$, an equation expressing the relationship of the free M_i concentration to $[R_t]$, as a function of the Donnan ratio, is derived:

$$[R_t] = \left(\sum_{i=1}^{n} Z_i r^{-Z_i}[M_i]\right)\left(1 + \sum_{i=1}^{k} \frac{r^{-Z_i}[M_i]}{K_i}\right) \qquad (5)$$

With known values for $[R_t]$ and K_i the polynomial, eq 5, may be solved for the Donnan ratio term for any given concentration of free solution M_i species, and then the ionic composition of the wall phase may be deduced from eq 1 and 2. The total fixed anion concentration $[R_t]$ and the individual cation dissociation constants (K_i) must be deduced independently. Equations 1–5 differ from those originally formulated by Sentenac and Grignon (1981) in that the ion activity coefficients have been assinged values of 1. This was done since the activity coefficient terms essentially cancel out in eq 5, and it greatly simplified the analysis. In addition, the contribution of anionic species to the electroneutrality of the wall phase (eq 2) was not included as it is generally negligible, and its omission further simplified eq 5. For the purposes of this work, cations accumulated in the cell wall due to complexation $[\bar{M}_i^b]$ and cations counterbalancing the fixed $[R_{COO^-}]$ groups are both considered to be bound species.

Justification of Experimental Protocols. The endogenous hull minerals were successfully removed by the 0.05 N HCl acid-wash procedure described in the Materials and Methods. Table I demonstrates that the residual mineral content of the acid-washed soy hull was only 1% that of the original hull tissue. Thus, hull-endogenous minerals did not complicate the analysis by contributing to the total mineral content of the system (i.e., the hull phase plus aqueous phase) nor mask any potential mineral-binding sites. Two protocols were employed to measure the extent of mineral binding. Method 1 is essentially identical with that originally developed by Sentenac and Grignon, in which the plant cell wall tissue is equilibrated with known free quantities of ions and then collected and washed free of adventitiously adsorbed solution (see Materials and Methods). A possible source of systematic error is introduced by the wash step since the wall phase is no longer at equilibrium with the aqueous phase, resulting in the loss of bound ions. The loss of bound ions during the wash step was minimized by utilizing organic dehydrating solvents. Method 2 avoids the equilibrium upset associated with the tissue-washing step by measuring, instead, the free solution concentration at equilibrium of the mineral of interest after adding a known total concentration to the system. The assumption inherent in method 2 is that the bound minerals can be adequately separated from the free minerals or that the technique employed actually measures
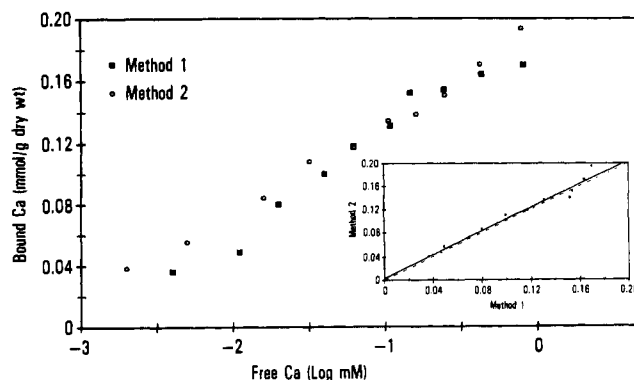

Figure 1. Comparison of methods 1 and 2 for determining the amount of a mineral bound to buffer-extracted soy hull. Buffer-extracted hull tissue (1.0 g/L) was equilibrated for 2 h with varying concentrations of calcium (0.040–1.0 mM, total concentration) in 10 mM Mops/TMA buffer, pH 7.2. Bound-calcium values were determined for method 1 (see Materials and Methods) by acid extraction of the recovered hull samples and the free-calcium values calculated by subtraction. Free calcium was determined on the filtered solutions for method 2 and the bound-calcium values calculated by subtraction. All calcium determinations were performed by atomic absorption. Inset shows bound values of data determined by methods 1 and 2: solid line, linear least-squares fit of bound values (method 1 vs. method 2); dashed line, ideal relationship of methods (see text).

"free" minerals, as opposed to a soluble but bound mineral species. This assumption was not found to be valid for measurements made on solutions containing acid-washed soy hull. Small but significant differences were found in the apparent free Ca^{2+} concentrations, as measured by atomic absorption spectroscopy vs. Ca^{2+}-specific electrode potentiometry, of solutions containing acid-washed soy hull tissue. This difference was attributed to the presence of a soluble mineral-binding moiety released from the acid-washed soy hull upon incubation in low-salt, neutral-pH media. A mineral-binding material was isolated from the aqueous phase by ethanol precipitation. Its high galacturonic acid content (4.3 mmol/g) indicated that the soluble mineral-binding substance was mostly pectin. The solubilization of pectin from the hull cell wall by the sequential acid and buffer treatments is to be expected because this procedure is similar to the ammonium oxalate method commonly employed to remove pectins from plant cell walls (Aspinall et al., 1966; Aspinall and Jiang, 1974).

Since mineral binding to soluble pectin is not subject to Donnan potential forces, as it is in binding to pectin within the wall phase, the presence of soluble pectin would complicate the analysis of the model described by eq 1–5. Method 1 does not suffer severely from this problem since the hull tissue is repeatedly equilibrated with fresh buffer solution and any soluble pectin is removed. Therefore, the use of method 2 was limited to measurements made on buffer-extracted hull tissue, from which the soluble pectin fraction had been removed.

By utilizing an additional method, which was a hybrid of methods 1 and 2, the equivalence of these two methods for mineral-binding analysis was verified. Buffer-extracted hull samples were equilibrated with a range of Ca^{2+} concentrations, foregoing the normal repeated replacement of the aqueous phase. The samples were analyzed for free Ca^{2+} concentration by method 2 (analysis of aqueous phase) and bound Ca^{2+} by method 1 (determined on recovered hull fraction). Figure 1 demonstrates that the two methods gave quite comparable results. The inset to figure 1 depicts an alternative way for comparing the results given by methods 1 and 2. The bound values deduced from methods 1 and 2 are plotted against each other for each

Laszlo

Table II. Mineral-Binding Parameters for Acid-Washed and Buffer-Extracted Soy Hull

parameter[a]	acid-washed hull	buffer-extr hull	parameter[a]	acid-washed hull	buffer-extr hull
$[R_t]$	135	100	K_{Mg}	3.2	3.2
K_H	0.37	0.37	K_{Zn}	0.60	0.60
K_{Ca}	0.80	0.80	K_{mono^+}	inf[c]	inf

[a] All parameters have units of millimolar. $[R_t]$ is the hull phase fixed anion concentration. The other parameters are the indicated cation dissociation constants. [b] All monovalent cations, except protons. [c] Infinity, or largest computer-manageable number.

sample. With a perfect correlation between the two methods, all points should fall on a line with a slope of 1.0 and an intercept of 0.0. Linear least-squares regression analysis of the data gave a slope of 1.005 ± 0.007 (intercept 0.003 ± 0.009), indicating a strong correspondence between the bound values determined by the two methods. The equivalence of results given by methods 1 and 2 indicated that the dehydration/wash step of method 1 did not significantly alter the mineral composition of the hull tissue from its equilibrated state.

Mineral-Binding Parameters. The term $[R_t]$ in eq 5, the concentration of fixed anions in the cell wall phase, was calculated from the measured total mineral-binding capacity (i.e., milliequivalents/gram dry weight) and the hydrated tissue volume (milliliters/gram dry weight). The mineral-binding capacities of the acid-washed and buffer-extracted hull tissues were determined by equilibrating the tissues in Mops buffer, pH 7.2, containing 10 mM potassium and various concentrations of calcium (0.04–2.0 mM total Ca^{2+}) and then analyzing the Ca^{2+} and K^+ contents of the tissues by method 1. The acid-washed and buffer-extracted hull samples bound a constant number of total milliequivalents, 0.54 ± 0.01 (eight samples) and 0.40 ± 0.02 (nine samples) meq/g dry weight, respectively. the number of fixed anionic sites determined by this method was somewhat higher than that predicted from the apparent uronic acid content of these tissues. The total uronic acid plus methyl ester content of the acid-washed hull sample was 0.79 ± 0.02 mmol/g, and the methyl ester content was 0.32 ± 0.05 mmol/g, indicating a total number of anionic sites of 0.47 mequiv/g. It is unknown whether the employed method of uronate analysis underestimated the actual hull composition or there are additional mineral-binding sites other than uronic acid residues. Selection of the higher anionic site value is also consistent with the original soy hull mineral content (Table I), although not all of the original endogenous minerals may have been bound specifically to the cell wall. Regardless, the additional cation-binding sites were assumed to be anionic in nature and thus contributed to the $[R_t]$ term of eq 5. The hydration volume (actually, the water regain mass) of both the acid-washed and buffer-extracted hull tissues was found to be 4.0 ± 0.4 mL/g dry weight. Therefore, the concentration of fixed anion sites was 135 mM for the acid-washed hulls and 100 mM for the buffer-extracted hulls.

Prediction of the bound cation content of a plant cell wall from the cation composition of the solution requires that the dissociation constants of the fixed anionic sites (presumably galacturonic acid residues in this case) be known for all the cationic species in solution. The derived K_i values for the monovalent and divalent cations studied in this work are given in Table II. The proton affinity, or pK_a, of the soy hull tissues could not be readily assessed by simple acid or base titration of the intact tissue since, as indicated by eq 1, the presence of a Donnan potential

would require the knowledge of the free H^+ concentration in the wall phase during the titration. Instead, the pK_a of free galacturonic acid was chosen. The pK_a of D-galacturonic acid titrated at 25.0 °C by TMAH was found to be 3.43 ± 0.02 (K_H = 0.37 mM; Table II). This compares favorably with the pK_a of 3.41 for soluble pectins (Kertesz, 1951) and the value (3.20) selected by Sentenac and Grignon (1981). The dissociation constants of all other monovalent cations were given a value of +infinity (i.e., no intrinsic affinity). No difference between K^+, Na^+, or TMA^+ was observed for Ca^{2+} titrations of acid-washed or buffer-extracted hull samples. From theoretical considerations, monovalent cations should have some intrinsic binding affinity for wall phase pectins (Manning, 1969; Thibault and Rinaudo, 1985), apart from the simple electroneutrality requirement of eq 3. This monovalent cation affinity must be very weak. In fact, any intrinsic monovalent cation affinity would be incorporated into the divalent cation dissociation constants in the form

$$K_{divalent} = K_{monovalent}^{divalent} \text{ exchange}$$

The zwitterion species of Mes and Mops were similarly considered not to have a significant affinity for the wall phase binding sites nor to contribute to the electroneutrality of the wall phase.

The K_{Ca} could not be deduced by simple titration of galacturonic acid with Ca^{2+}. Free galacturonic acid does not form Ca^{2+}–bis(galacturonate) complexes in aqueous solution (Gould and Rankin, 1970), yet this is the most likely form of the Ca^{2+} complex formed in the cell wall (Irwin et al., 1984). Furthermore, the K_i value for any of the divalent cations must reflect the structural or geometrical constraints imposed on the fixed uronic acid residues by the wall matrix. These constraints cannot be mimicked by free-solution conditions. Therefore, the K_i values for Ca^{2+}, Mg^{2+}, and Zn^{2+} were established by determining the values that minimized the root-mean-square error (rms) between the predicted and observed binding values over the entire data set. The minimum rms value given by

$$rms = \left[\frac{1}{n} \sum_i^n (obsd_i - pred_i)^2 \right]^{1/2} \qquad (6)$$

is also an estimate of the standard error of the measurement. The rms error was calculated from unweighted data.

The K_{Ca} value given in Table II was determined from the data shown in Figures 2 and 3. Figure 2 demonstrates the good agreement between observed and predicted values for Ca^{2+} and K^+ binding to buffer-extracted soy hull. The free K^+ concentration was varied from 10 to 100 mM and free Ca^{2+} varied from 0.003 to 1.0 mM, at a constant pH 7.2. No significant mineral-binding difference between pH 6.0 and 7.2 was observed. Only below pH 6.0 did H^+ ions noticeably reduce Ca^{2+} and K^+ binding in the hull phase. This is consistent with the predicted low pK_a of the mineral-binding sites. Similar good agreement between the observed and predicted values for Ca^{2+} binding was achieved at lower solution pH conditions (Figure 3). The given model (eq 1–5) also predicts the extent of H^+ binding, but as these values are experimentally inaccessible, the predicted bound H^+ values are not included in the presented data. Clearly, the employed model adequately predicts the mineral composition of the hull tissue under either simple Ca^{2+}/K^+ exchange (Figure 2) or triangular $Ca^{2+}/K^+/H^+$ exchange (Figure 3) conditions.

Magnesium bound to soy hull with an affinity significantly less than that of calcium. This lower affinity is reflected in its higher dissociation constant (Table II). The K_{Mg} value was determined from experiments in which both
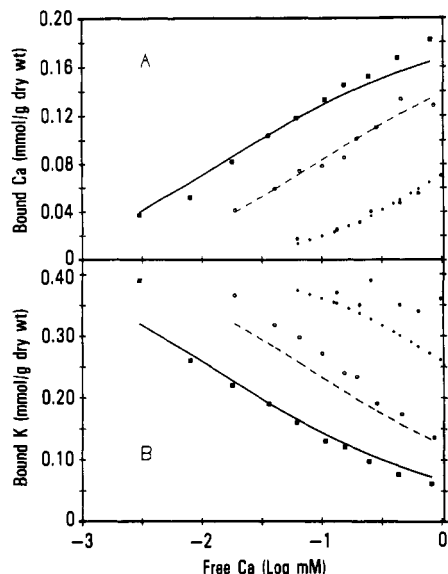
Mineral Binding Properties of Soy Hull

J. Agric. Food Chem., Vol. 35, No. 4, 1987 **597**

Figure 2. Comparison of observed and predicted values for (A) Ca^{2+} and K^+ binding to buffer-extracted soy hull. Buffer-extracted soy hull samples (1.0 g/L) were equilibrated with various concentrations of calcium in 10 mM Mops buffer, pH 7.2, containing either 10, 25, or 100 mM KCl. Mineral binding was measured following procedures described for method 1. Predicted mineral-binding values were obtained by employing eq 1–5 and the parameters given in Table II for buffer-extractd soy hull. Root-mean-square (rms) error values were calculated from eq 6. The rms differences between observed (■) and predicted (solid lines) for Ca^{2+} binding (panel A) and K^+ binding (panel B) in the presence of 10 mM KCl were 0.0087 and 0.0056, respectively. The rms values for observed (O) and predicted (broken lines) Ca^{2+} and K^+ binding in 25 mM KCl were 0.0059 and 0.0090, respectively. In 100 mM KCl, rms values for observed (*) and predicted (dotted lines) Ca^{2+} and K^+ binding were calculated to be 0.0027 and 0.0235, respectively.
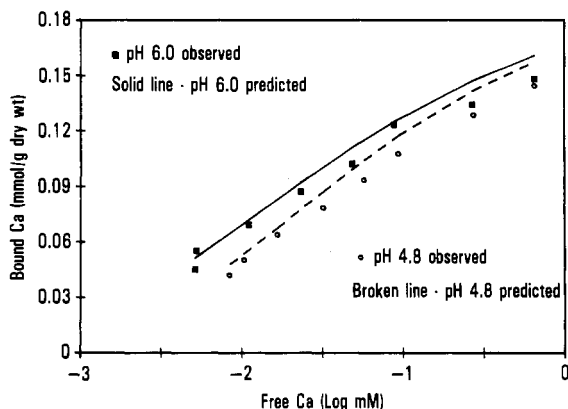

Figure 3. Influence of solution pH on calcium binding to soy hull. Buffer-extracted soy hull (1.0 g/L) was suspended in solutions containing either 10 mM Mes/TMA, pH 6.0 (■), or 10 mM acetate/TMA, pH 4.8 (O), and titrated with radiolabeled calcium. The extent of Ca^{2+} binding was determined by method 2. The rms error values for Ca^{2+} binding at pH 6.0 and 4.8 were 0.0160 and 0.0096, respectively. The observed and predicted Ca^{2+}-binding values at pH 7.2 are given in Figure 2 (panel A).

free Mg^{2+} and Ca^{2+} were present in solution (Figure 4) or in which free Mg^{2+} was the only divalent cation in solution (Figure 5). The K_{Mg} values determined for either data set were essentially the same, within the limit of error of the measurements.

Buffer-extracted soy hull bound Zn^{2+} with a slightly higher affinity than Ca^{2+} (Table II). Measurements of Zn^{2+} binding were limited to conditions in which the solution was kept below pH 5.2 in order to prevent the formation
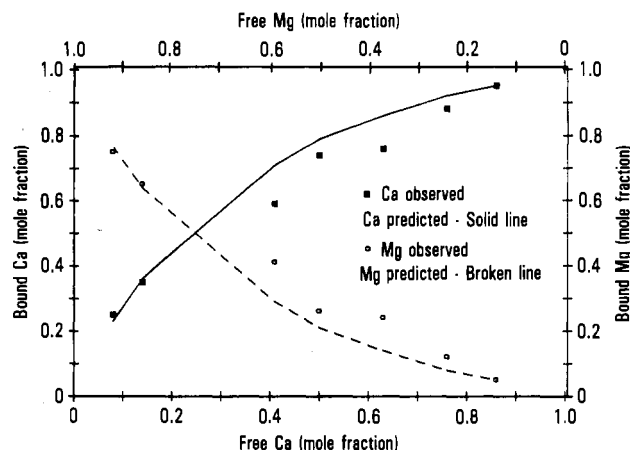

Figure 4. Ca^{2+}/Mg^{2+} exchange in buffer-extracted soy hull. Buffer-extracted hull (1.0 g/L) was equilibrated in buffer containing 10 mM Mops/TMA, pH 7.2, and varying concentrations of calcium (0.1–0.4 mM, total) and magnesium (0.05–0.8 mM, total). The extent of mineral binding was determined by method 1. The free and bound divalent cation quantities are expressed as mole fraction values, based on the ionic compositions of the solution and hull phases, respectively, at equilibrium. The rms error values for observed (■) and predicted (solid line) Ca^{2+} binding and observed (O) and predicted (broken line) Mg^{2+} binding, were 0.009 and 0.026, respectively. the rms values were minimized from the original individual ion binding values (i.e., from the predicted and observed bound values) and not the depicted mole fraction values, which necessarily reflect the error for both divalent cations.
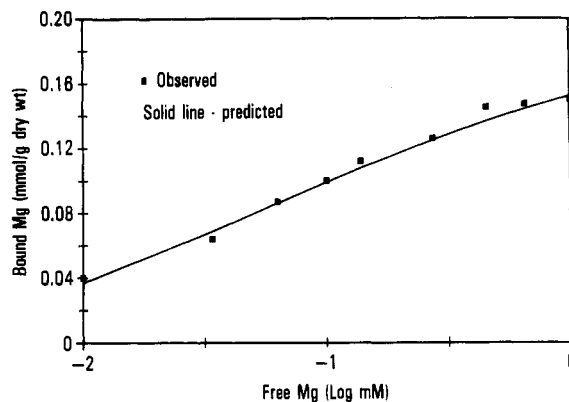

Figure 5. Mg^{2+} binding to buffer-extracted soy hull. Buffer-extracted hull was titrated with Mg^{2+} in a solution containing 10 mM Mops/TMA, pH 7.2. Mg^{2+} binding was determined following method 1. The rms deviation between observed (■) and predicted (solid line) binding values was 0.004.

of zinc hydroxides that would likely diminish the binding of Zn^{2+} to the soy hull cell wall. The K_{Zn} value given in Table II was determined, similarly to the K_{Mg} value, from both Zn^{2+}/monovalent cation exchange (Figure 6) and Zn^{2+}/Ca^{2+} exchange (Figure 7) experiments. Figure 6 demonstrates the fit obtained for Zn^{2+} binding over the free Zn^{2+} concentration range of 0.0065–0.65 mM, under conditions where Zn^{2+} ions were competing with H^+ (0.0158 mM) and TMA^+ ions (10 mM) for binding. Although Zn^{2+} affinity was greater than Ca^{2+} affinity, the maximum amount bound to soy hull was the same. Furthermore, Ca^{2+} was able to compete successfully with Zn^{2+} for binding sites (Figure 7). This indicates that there were no additional sites available for Zn^{2+} binding that do not bind Ca^{2+}, consistent with the assumption that the vast majority of mineral-binding sites in soy hull are carboxylic acid residues (but not necessarily only uronic acid residues). However, it is possible that if a pH range higher than pH
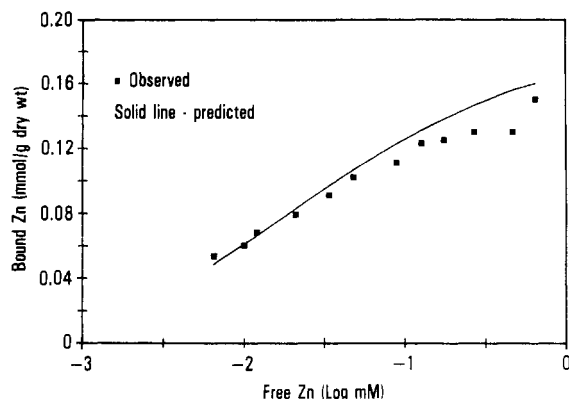
Figure 6. Zn^{2+} binding to buffer-extracted soy hull. Hull samples were suspended in 10 mM acetate/TMA buffer, pH 4.8, and titrated with radiolabeled Zn^{2+}. The extent of Zn^{2+} binding was determined by method 2. the rms error between observed and predicted Zn^{2+} binding was 0.011.
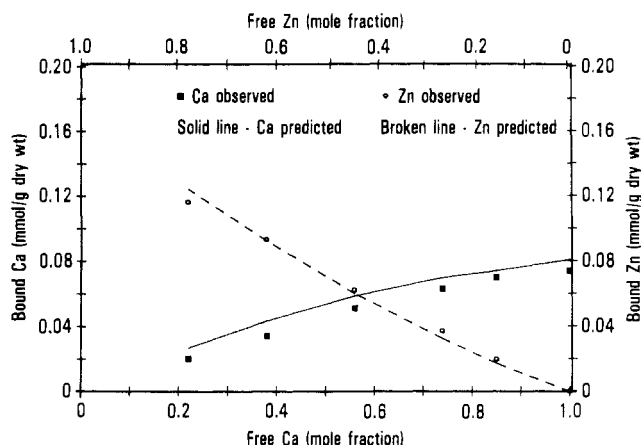

Figure 7. Ca^{2+} and Zn^{2+} binding to buffer-extracted soy hull. Hull material was equilibrated in 10 mM acetate/TMA buffer, pH 4.8, containing 0.1 mM Ca^{2+}, total, and varying concentrations of Zn^{2+} (0.0–0.4 mM, total). Mineral binding was measured following method 2, with radiolabeled Ca^{2+} and Zn^{2+}. The rms values for Ca^{2+} and Zn^{2+} binding were 0.007 and 0.004, respectively.

5.2 had been investigated, additional Zn^{2+}-binding sites would have been observed due to the presence of imidazole, amino, or sulfhydryl coordinating groups (Breslow, 1973).

The K_i values obtained from experiments performed on buffer-extracted soy hull were also useful in predicting mineral binding to the acid-washed hull samples, which have a higher pectin content. The only parameter that was changed was the [R_t] value (Table II). Figure 8 shows the correlation between the observed values for Ca^{2+}, Mg^{2+}, and K^+ binding and the values predicted from the model. The correlation was good for Mg^{2+} and K^+, but there was significant deviation from the predicted values for Ca^{2+}. The observed bound Ca^{2+} values were generally less than predicted, possibly due to the loss of pectin from the hull tissue during the equilibration steps of method 1.

Anion Binding. Just as the Donnan potential term of eq 1 predicts that cations will be preferentially accumulated in the wall phase, so too does it posit that anions will be excluded. The greater the charge on the anion, the more effectively the anion should be excluded. Attempts to demonstrate anion exclusion from the cell wall phase of acid-washed soy hull were unsuccessful. With either tracer quantities of radiolabled orthophosphate, which is predominantly a divalent anion at pH 7.2, or a 0.5 mM concentration of the $Fe(CN)_6^{3-}$ complex, soy hull reduced the free anion solution concentration. This indicated that soy
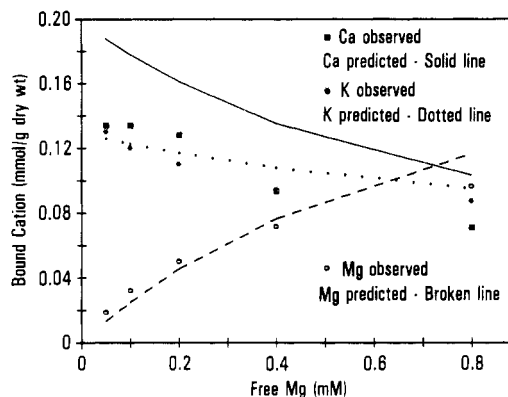

Figure 8. $Ca^{2+}/Mg^{2+}/K^+$ exchange on acid-washed soy hull. Acid-washed soy hull samples were equilibrated in 10 mM acetate buffer, pH 5.1, containing 10 mM K^+, 0.2 mM Ca^{2+}, total, and varying concentrations of Mg^{2+} (from 0.05 to 0.8 mM). The extent of ion binding was determined by method 1. The rms error for observed (■) and predicted (solid line) Ca^{2+} binding was 0.056, for observed (O) and predicted (broken line) Mg^{2+} binding 0.008, and for observed (*) and predicted (dotted line) K^+ binding 0.008.

hull has a finite anion-binding capacity. Under identical conditions, (carboxymethyl)cellulose, an ion-exchange gel with functional exchanger groups similar to those expected to be found in soy hull, excluded the phoshate anion from about 75% of the hydrated gel volume. The anion-binding capacity of acid-washed soy hull was estimated to be approximately 6–12 μequiv/g dry weight. This is probably an underestimation because the extent of monovalent anion (Cl^-) binding was not measured along with the tracer anion. The identity of the soy hull anion-binding sites was not investigated.

DISCUSSION

The work reported herein demonstrates that equations developed to predict mineral binding to plant cell walls in general (Sentenac and Grignon, 1981) may be successfully employed to model the mineral-binding properties of insoluble dietary fiber from sources such as soybean hull. This is not, necessarily, the only model that could have been applied to describe the cation-binding properties of soy hull [cf., Helfferich (1962)]. Rather, the utility of the presented model is derived from its relative simplicity and its ability to estimate the extent of binding from solutions of complex ionic composition. This capability is particularly important when addressing questions involving in vivo situations, such as the influence of dietary fiber on mineral absorption.

To predict the extent of mineral association with a particular cell wall tissue, utilizing eq 1–5, it is necessary to know the concentration of anionic charges in the hydrated tissue and the intrinsic affinity of the various minerals for these anionic sites. Sentenac and Grignon (1981), working with cell wall fractions of lupine and horse bean root, selected the value of the concentration of fixed anionic sites based on the maximal amount of cation equivalents found bound to the cell wall, as was done in this work, and a hydration volume estimated from the intact root cell wall volume (approximately 1.0 mL/g dry weight for both lupine and horse bean). The latter estimate being very imprecise, they used the hydration volume as a semiadjustable parameter in fitting their binding data. Their assumption that the isolated wall tissue maintains the same volume as the native cell wall is questionable. Furthermore, selection of the hydration value based on the morphology of the original tissue would be clearly inappropriate in cases where the plant sample has undergone significant mechanical or enzymatic disruption, such as in

Mineral Binding Properties of Soy Hull

J. Agric. Food Chem., Vol. 35, No. 4, 1987   **599**

food products. Instead, in this work, the volume of the cell wall phase was determined by measuring the mass of solution retained by the hydrated hull tissue. This property may be readily determined for insoluble cell wall or dietary fiber substrates.

Somewhat surprisingly, selection of the fixed-anion concentration term $[R_t]$ of eq 5, based on a gross macroscopic determination of the apparent hydration volume, provided the best fit value in predicting K^+ binding over a wide range of ionic strength and pH conditions (Figures 2 and 8). While the prediction of the extent of monovalent cation binding is sensitive to all of the binding parameters, it is the most critically dependent on the value of $[R_t]$. In fact, given an arbitrarily chosen value of the hydration volume, a different set of K_i values for the divalent cations could be deduced that predicted divalent-cation binding equally well, as long as monovalent-cation binding was not considered. Only the ratio of K_i values was needed to predict divalent-cation exchanges adequately (i.e., the relative selectivity, or the ratio of K_{Mg} to K_{Ca}, was independent of the selected values of K_{Ca} and $[R_t]$). Unique K_i values were obtained only when the extent of monovalent cation binding was evaluated as well. The best fit hydration volume value, selected only on the basis of minimizing the rms error for K^+ binding, over the entire presented data set of Figures 2 and 8 (and additional data not shown), was 4.0 ± 0.5 mL, well within the error limits of the independently evaluated, physical hydration volume values of the acid-washed and buffer-extracted soy hull tissues. The fixed anion concentration value $[R_t]$ of acid-washed soy hull (135 mM; Table II) is quite similar to the density of negative charges (138 mM) in the cell walls of soybean cell suspension cultures, estimated by observing the affect of the Donnan potential on the cell wall-bound phosphatase activity (Crasnier et al., 1985). The correlation between the "best fit" hydration volume and the grossly measured macroscopic quantity of the hydration volume was unexpected since the former is dependent on the microscopic environment of the hull and the latter is a bulk tissue property. Furthermore, the hydration volume of the hull tissue should have changed as the ionic composition of solution phase was altered (Ritchie and Larkum, 1982). Yet, no modification of the selected $[R_t]$ value was required to fit the binding data over an ionic strength range of 10–100 mM (Figure 2). This implies that the hydration volume was not influenced significantly by solution ionic strength. Wolterbeek (1987) similarly found that the hydration volume of tomato xylem cell walls was invariant under a wide range of pH and ionic strength conditions. Possibly, the effect of the aqueous phase ionic strength on hydration volume was obscured by its much greater influence on Donnan potential.

An alternative method for determining the $[R_t]$ term of eq 5 might have been to measure the anion-exclusion volume of the hull tissues, as this represents the volume over which the Donnan potential exerts its influence in the cell wall, commonly referred to as the "Donnan free space" (Dainty and Hope, 1961). The phenomenon of anion exclusion from plant cell walls has been well documented (Pitman, 1965a,b; Shone and Barber, 1966; Haynes, 1980). However, soy hull bound anions, thereby masking the anion-exclusion process. Thus, in the presence of soy hull, the free anion concentration of a solution at equilibrium may reflect the opposition of two forces: Donnan exclusion from the cell wall phase and specific anion binding to cell wall components. Anion adsorption in plant root cell walls has been observed by others (van Steveninck, 1964; Pettersson, 1961, 1966) and has been ascribed to the presence

of amino or imidazole groups in cell wall proteins (Baker and Hall, 1975; Lauchli, 1976). The presence of these anion-binding sites may be an influence in the interactions of dietary fiber with bile and fatty acids (Mongeau and Brassard, 1982; Tepper et al., 1984).

The dissociation constants for Ca^{2+} and Mg^{2+} employed in this work are considerably lower than those selected by Sentenac and Grignon (1981). This is reasonable since the selected $[R_t]$ values were considerably different, for the reasons mentioned above. The K_{Ca} value derived from minimizing the root-mean-square error of the data (0.8 mM; Table II) is similar to the affinity of apple pectin for calcium. Kohn and Furda (1967) determined the Ca^{2+} stability constants for a series of pectins having different degrees of esterification. Wild apple pectin, having a degree of esterification (38.6%) similar to that of the pectins in soy hull (approximately 40%), had an apparent stability constant for Ca^{2+} of $10^{3.15}$ M^{-1} [see Table II of Kohn and Furda (1967)], which is equivalent to a dissociation constant of 0.71 mM. Although this close similarity of K_{Ca} values may be somewhat fortuitous, in that the K_i values defined herein should be independent of the degree of pectin esterification, it strongly supports the selection of this K_{Ca} value rather then the value given by Sentenac and Grignon (1981), which is 2 orders of magnitude higher. A similar conclusion has been reached by others (Amory and Dufey, 1984). The observed selectivity order of divalent cations ($Zn^{2+} > Ca^{2+} > Mg^{2+}$) for soy hull is identical with that found for soluble pectins (Haug and Smidsrod, 1970; Thom et al., 1982) and other plant cell wall preparations (Van Cutsem and Gillet, 1982; Amory and Dufey, 1984).

Dietary fiber has been shown to bind nutritionally important minerals in vitro (Reilly, 1979; Thompson and Weber, 1979; Reinhold et al., 1981; Fernandez and Phillips, 1982a; McBurney et al., 1983; Rendleman, 1982; Rendleman and Grobe, 1982; Dintzis and Watson, 1984; Allen et al., 1985; Lee and Garcia-Lopez, 1985) and to impair mineral bioavailability (Fernandez and Phillips, 1982b). The presented model offers a qualitative understanding, in addition to its quantitative predictions, of mineral association with fiber sources rich in acidic polysaccharides. The Donnan potential term suggests that cations will bind to insoluble fiber generally in the order trivalent > divalent > monovalent (H^+ being the exception), while the intrinsic dissociation term confers selectivity among isovalent species. The mineral-binding properties of dietary fiber sources containing mineral-complexing or -chelating components other than pectins, such as lignins and tannins (Platt and Clydesdale, 1985), would not be expected to be adequately described by the given model.

Soybean hulls are a source of both soluble and insoluble dietary fiber, comprising approximately 15% and 65%, respectively, of the dry weight of untreated hulls (Schweizer and Würsch, 1979). The buffer-extracted hull material was depleted of water-soluble polymers. Therefore, buffer-extracted hulls must consist of at least 80% insoluble dietary fiber. It is this fraction of the hull that passes through the small intestine of nonruminants, where the major portion of the mineral absorption processes occur. When the hull-derived insoluble dietary fiber enters the colon, the fermentative activities of the fecal microbial population will drastically modify the composition of the hull fiber, probably greatly diminishing the fiber's mineral-binding capacity.

The present study suggests that for soy hull, under the high ionic strength or low pH conditions found in the gastrointestinal tract, the Donnan influence would be negligible and essential mineral binding minimal. Al-

600 *J. Agric. Food Chem.,* Vol. 35, No. 4, 1987

Laszlo

though the experimental conditions employed were not selected specifically to mimic in vivo conditions, Table I implies that the endogenous mineral compliment of soy hull is readily extracted under the pH 2.0 conditions of the stomach (Ovensen et al., 1986). Nor would the acid-extracted soy hull have a significant affinity for polyvalent cations under the approximate 0.15 M ionic conditions of the intestinal lumin (White et al., 1954). The relatively high bioavailability of minerals from soy hull diets corroborates this prediction (Meyer et al., 1983; Johnson et al., 1985; Ward and Reichert, 1986).

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