Phytate-Calcium Interactions with Soy Protein

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Solubilities of mixtures of soy protein isolate, calcium and phytate were determined as a function of pH and molar ratio of the components. Below the isoelectric point, phytate and protein solubility profiles paralleled each other, indicating some type of protein-phytate interaction. Addition of phytate shifted the isoelectric point and the minimum solubility to lower values. Between the isoelectric point and pH 6.5, the complex apparently dissociates; addition of phytate results in an increase in the maximum solubility of the phosphorus and the protein, as well as a shift in their solubility profiles. Calcium has no apparent effect on protein solubility in this pH region. Higher pH (>6.5) results in the formation of ternary protein-calcium-phytate complexes and a significant drop in calcium and phosphorus solubility, probably due to formation of insoluble calcium phytate salts.

Soybeans, like most oilseeds and legumes, represent a great potential as a protein source, especially if consumed with other complementary plant proteins. Most plant proteins, however, contain several undesirable or antinutritional components that must be removed or destroyed to maximize the utilization of the protein. Phytate is one such component that has been implicated as a potential problem in mineral bioavailability, especially for calcium, iron and zinc (1, 2). Although there have been many in vivo studies over the past 50 years showing that phytate interferes with mineral bioavailability, there is little understanding about the mechanism of phytate interactions in plant foods, especially the solubility of phytate-mineral complexes in the presence of plant proteins. This is important because it appears that the complexed mineral cannot be absorbed through the intestinal wall. In addition, phytate binding may have an effect on the functional properties of the protein, causing slight changes in the pH-solubility profile (3).

The solubility of phytate and the complexed mineral appears to be affected dramatically by the presence of proteins. It is well known that calcium phytate and magnesium phytate complexes are soluble at acidic pH (below pH 5), and insoluble above pH 6 (4, 5). The solubility also is a function of the mineral:phytate molar ratio. In contrast, the solubility of phosphorus compounds in soybeans parallels protein solubility over much of the pH range of interest (6–8). This indicates a strong interaction between phytate, mineral and protein. Similar results have been observed in oats (9), cottonseed (6) and peanuts (6, 10).

In order to elucidate the nature of these phytate-mineral-protein interactions, the solubility behavior of phytate and a selected mineral (calcium) in a model protein system (soy protein isolate) was studied as a function of pH and the concentrations of these components. When compared with our earlier studies on the solubility behavior of pure calcium phytate and magnesium phytate complexes as a function of pH and molar ratio (4, 5), these experiments should provide a better understanding of the nature and extent of the interactions occurring in such systems and a basis for optimizing the removal of phytate from foods.

EXPERIMENTAL PROCEDURES

Sodium phytate (97% pure) was purchased from Sigma Chemical Co., St. Louis, Missouri. All other chemicals were reagent grade, unless otherwise mentioned.

Soy protein isolate was prepared by the ultrafiltration method of Nichols and Cheryan (11) using a 30,000 mol wt cut-off membrane. The starting material was a high NSI (nitrogen solubility index) defatted soy flour (Nutrisoy 7B, A.E. Staley Co., Decatur, Illinois). The retentate from the ultrafiltration was freeze-dried and stored refrigerated until needed. The proximate analysis of these products is given in Table 1.

Solubility studies. The following stock solutions were used for this study: $CaCl_2$ (7.739×10^{-2}M) and sodium phytate (2760 ppm phosphorus = 1.48×10^{-2}M phytate). Seven g of the soy isolate were added to about 10 ml of water and the appropriate amount of $CaCl_2$ solution and phytate (PA) solution added to obtain the desired Ca:phytate ratio (the protein concentration was kept constant) and the volume brought up to 250 ml.

Ten ml of the above reaction mixture were added to 2.5 ml of 1M NaCl (to minimize variations in ionic strength). The pH was adjusted with 1M NaOH or 1M HCl and the volume brought to 25 ml. This resulted in a 1% protein solution with varying Ca:phytate ratios. The solutions were flushed with nitrogen and the samples agitated mechanically in a shaker set at a

TABLE 1.

Proximate Analysis of Soy Flour and Soy Isolate (%), As-Is Basis

	Soy flour[a]	Soy isolate[b]
Moisture	14.75	3.13
Protein	46.84	87.52
Ash	6.65	2.67
Calcium	0.15	0.20
Total phosphorus	0.71	0.53
Phytate phosphorus	0.50	0.35
Phytate	1.79	1.27

[a]Nutrisoy 7B.
[b]Ultrafiltered soy isolate: Sample C in Table 2.

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TABLE 2

Concentration and Molar Ratios of Protein, Calcium (Ca) and Phytate (PA) in Reaction Mixtures

	Concentration[a]			Molar ratio[c]		
Sample	Protein	Calcium[b]	Phytate[b]	Ca:PA	PA:protein	Ca:protein
A	1	0.23	7.6	0.5	11.50	5.78
B	1	0.23	3.8	1.0	5.78	5.78
C	1	0.23	1.5	2.6	2.20	5.78
D	1	0.35	1.5	4.0	2.20	8.80
E	1	0.58	1.5	6.0	2.20	13.20

[a]Concentration in the solution (% w/v).
[b]Expressed on a protein basis (g/100 g protein).
[c]Mol wt of protein assumed to be 100,000 g/mol.

moderate speed for 110 min at room temperature (24-26°C). The pH was readjusted if necessary during the incubation period. The solutions were then centrifuged at 17,300 G for 10 min. The supernatant was analyzed for soluble protein, calcium and phosphorus. All experiments were replicated at least twice. Standard deviations were calculated for each data point (19) and are shown in the figures with bars. In most cases, however, the bar is smaller in size than the data point.

Analytical methods. Total phosphorus was measured by the method of Bartlett (12). Inorganic phosphorus was measured by extracting the samples with 12.3% trichloroacetic acid (TCA) for one hr, centrifuging the extract at 12,100 G for 15 min and measuring the phosphorus content in the supernatant.

Total solids and ash were measured by gravimetric analysis (13). Protein is expressed as N × 6.25. Nitrogen was measured by the Kjeldahl method. During the solubility studies, protein was measured by the Biuret method using bovine serum albumin as the standard. Stock solutions used for these studies were standardized using the Kjeldahl method.

Phytate was estimated by the $FeCl_3$ precipitation method as described by Thompson and Erdman (14). The samples were first extracted with 1.2% HCl + 10% Na_2SO_4 and centrifuged. Ferric chloride was added to the supernatant, and the mixture was centrifuged again. The phosphorus content in both supernatants was determined. Phytate acid phosphorus is expressed as phosphorus extracted in the first supernatant minus phosphorus in the second supernatant.

Calcium (Ca) was measured by atomic absorption spectroscopy or by the titration method of Ntailianas and Whitney (15). Both methods gave identical results.

RESULTS

Table 2 lists the model solutions used in this study and the coding used in the figures. PA refers to the phytate anion in the tables and figures. Sample C is the UF soy isolate as is. Samples A and B are added-phytate samples, resulting in changes in the Ca:phytate and phytate:protein molar ratios. Samples D and E are added-calcium samples, resulting in changes in Ca:phytate and Ca:protein ratios. Note that the concentrations are expressed on a protein basis rather than a solids basis, assuming the mean mol wt of the proteins to be 100,000 g/mol (16).

The solubility profiles of protein, calcium and phosphorus are shown in Figures 1-3 as a function of pH and the Ca:phytate ratio. Solubility data at other Ca:phytate ratios are given elsewhere (19). An examination of these profiles suggests that the mechanism of interaction may be different depending on the pH and/or the calcium and/or phytate levels in the system. Thus, the discussion is divided into three sections according to the pH region.

1. Low pH (below the isoelectric point of the protein). An earlier study (4) had shown that in pure Ca-phytate systems no precipitation was observed below pH 4, regardless of Ca:phytate ratio. However, when soy protein is present the phytate-phosphorus starts precipitating at pH 1.0. The pH of minimum phosphorus solubility and the relative amount of insoluble phosphorus depends on the Ca:phytate ratio. As shown in

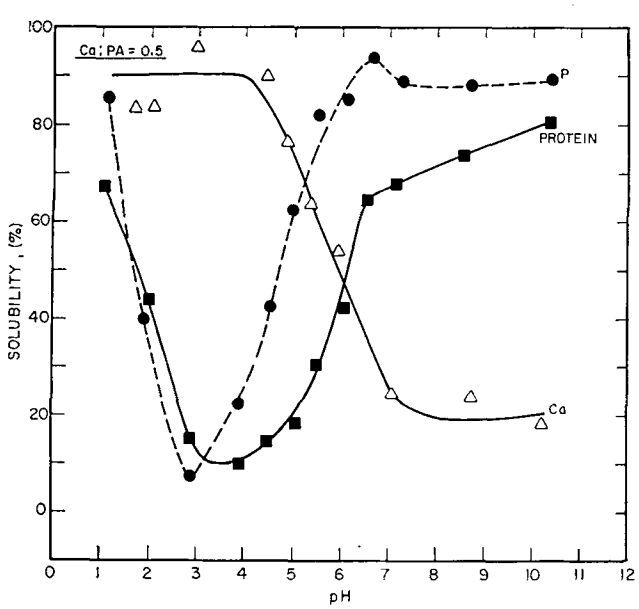

FIG. 1. Solubility of calcium (Ca), protein and phosphorus (P) in soy isolate reaction mixture at Ca:phytate molar ratio = 0.5 (sample A).

Figure 4, at low Ca:phytate ratios (0.5, 1 and 2.6) the pH of minimum solubility increased with Ca:phytate ratio. This could be related to the effect of added phytate on protein solubility profiles (see later). At higher Ca:phytate ratios (>2.6), there was little or no effect of Ca:phytate ratio on the pH of minimum phosphorus solubility.

The phytate profile at the low Ca:phytate ratios parallels the protein solubility profile quite closely [e.g., Fig. 1 and others in (19)]. This indicates strong protein-phytate interactions at these high phytate concentrations, as suggested by several authors (6, 7, 9, 17, 18). Below the isoelectric point, protein has a net positive charge and thus will interact with the negatively charged phytate anion at several sites such as the terminal amino groups, the amino groups of basic residues (e.g., lysine, arginine) and through hydrogen bonding with the carboxyl groups and the oxygens and hydrogens on the phytate molecule.

Calcium does not appear to be a factor in the insolubility of phytate (Fig. 4) or protein (Fig. 5) in this pH region. However, as the phytate concentration increases (Fig. 6), the protein precipitates out at a lower pH, and the isoelectric point also shifts to a lower pH. Increasing amounts of phytate were being bound to the protein, neutralizing its positive charges. Thus, zero charge will be achieved at a lower pH in the presence of phytate.

2. Intermediate pH (from the isoelectric point to pH 6.5). As pH increases, the protein becomes more soluble and phytate dissociates from it. The combination of these two factors results in a rapid increase in phosphorus solubility (Fig. 4). This is the opposite of phytate behavior in the absence of protein, when it would normally start precipitating out above pH 4 (4, 5). Moreover, as the phytate becomes more soluble, calcium becomes less soluble. Our data suggest the possible formation of a ternary (phytate-Ca-protein) complex as well as calcium binding to the protein. Thus, phytate will not exhibit the precipitation pattern observed when only calcium and phytate are present until another critical pH is reached at pH 6.5.

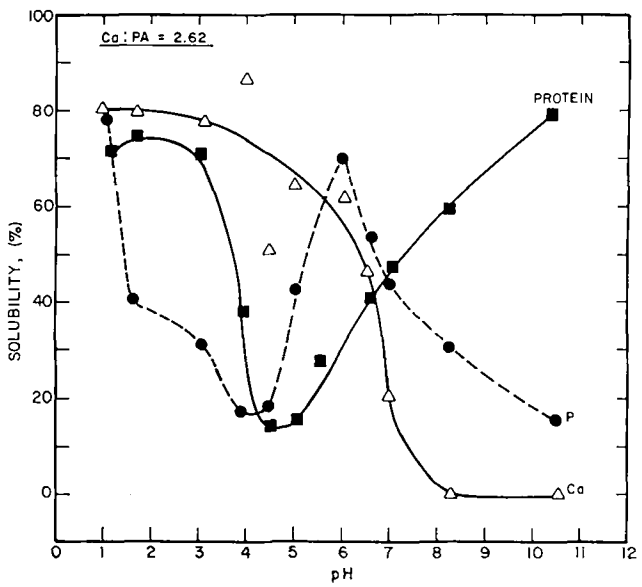

FIG. 2. Solubility of protein and phosphorus in sample C (Ca:phytate molar ratio = 2.6).

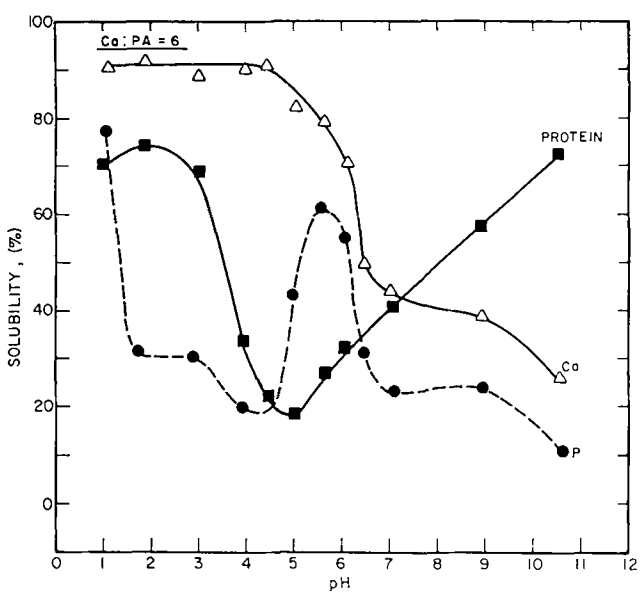

FIG. 3. Solubility of calcium, protein and phytate in sample E (molar ratio = 6).

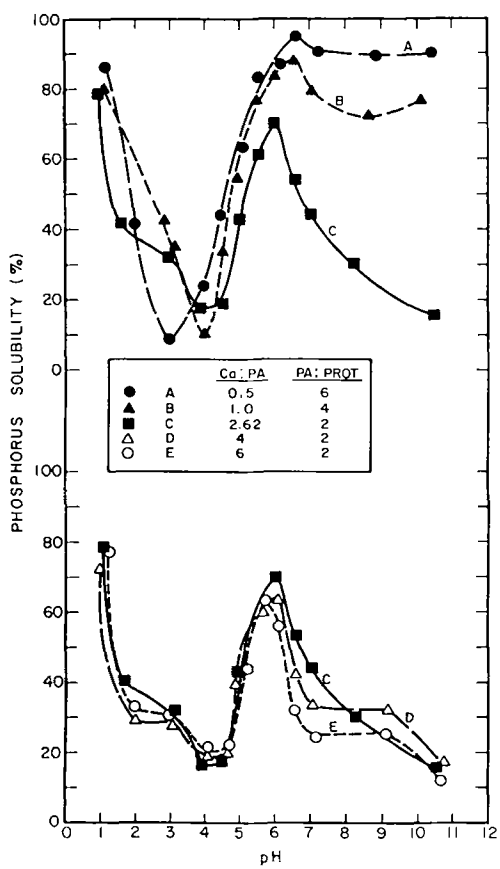

FIG. 4. Phosphorus solubility profile in the soy protein mixture as a function of added phytate (top) and added calcium (bottom).

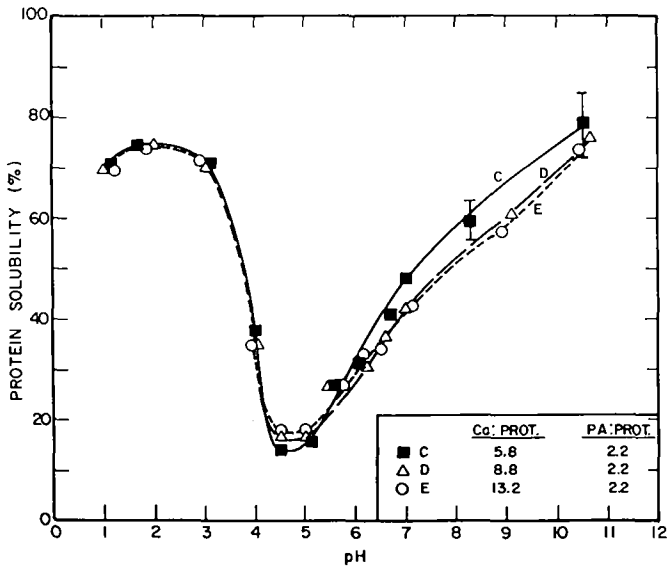

FIG. 5. Protein solubility profile as a function of added calcium. Bar represents two standard deviations.

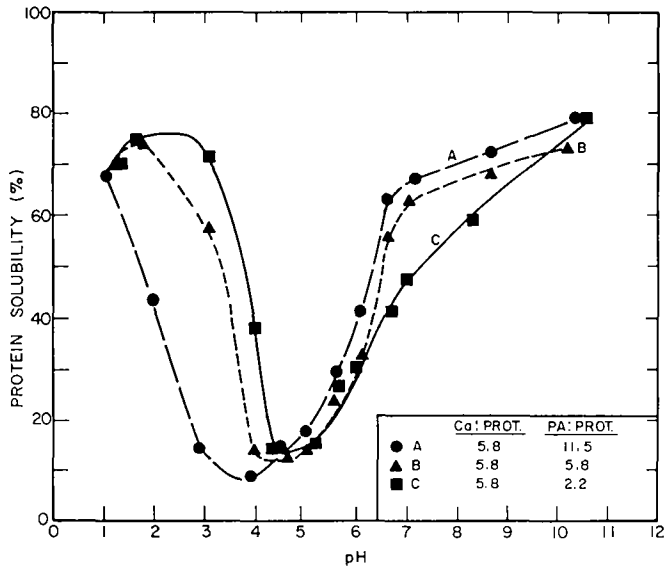

FIG. 6. Protein solubility profile as a function of added phytate.

The addition of phytate to the solution (samples A, B and C) produced two different effects in this intermediate pH region:

(i) Phosphorus solubility increased at any given pH with an increase in phytate concentration in the reaction mixture. However, at pH 6-6.6, the absolute amount of phosphorus in the precipitate is quite similar for all three samples (9-14 μmol phosphorus) despite their widely varying concentrations in the reaction mixture. This, as their calcium solubilities are not significantly different but the protein solubility shows trends similar to phytate phosphorus in this region, is a further indication of the importance of protein-phytate interactions.

(ii) There is a shift in the pH of maximum solubility to the right as the phytate:protein ratio increased, a trend opposite to that observed at the isoelectric pH region. This could be because the Ca:phytate ratio decreased as the phytate:protein ratio increased (Table 2). Lower Ca:phytate ratios result in higher phytate solubility at higher pH in the absence of protein (4), and this effect may contribute to the pH shift observed in this region.

The solubility of calcium decreases above pH 4 in almost all reaction mixtures due to its binding to insoluble protein and to phytate (that otherwise would have remained soluble). No significant changes in protein solubility profiles were observed upon addition of calcium (Fig. 5). This does not necessarily mean that calcium and protein are not interacting with each other. As the pH increases and the protein acquires a net negative charge, calcium can combine with the already precipitated protein without changing the protein's solubility. Moreover, calcium can also be bound to the soluble protein alone or as an alkaline-earth bridge between the protein and the phytate without causing the protein to precipitate (8). Higher phytate concentrations seem to produce a small but noticeable increase in protein solubility in this pH region (Fig. 6). This could be due to an increase in the net negative charge of the protein as a result of binding by phytate.

3. High pH (above 6.5). Above pH 6.5, protein solubility continues to increase until a maximum of 70-75% solubility is obtained at all Ca:phytate molar ratios. Phosphorus solubility also increased with higher phytate levels (Fig. 4, top), and so did the relative calcium solubility in the added-phytate systems. This suggests that the dominant reaction is probably the formation of the ternary complex. In contrast to the increasing solubility of protein with an increase in pH, both phosphorus and calcium decrease in solubility. This is due, perhaps, to the precipitation of calcium phytate salts. The Ca:phytate ratio, as well as the Na^+ concentration, seems to dictate the amount of calcium and phosphorus in the precipitate. De Rham and Jost (8) showed that the addition of 8.5% NaCl to the ternary complex at pH 7.5 was enough to precipitate calcium phytate.

DISCUSSION

A better understanding of the interactions between calcium and phytate in soy protein systems was obtained in these solubility studies. The interaction appears to be affected by the pH and the relative concentrations of the three components. At low pH, phytate associates with proteins to form insoluble coacervates with higher concentrations of phytate shifting the isoelectric point and the phosphorus minimum solubility to lower values. Calcium, on the other hand, does not seem to play an important role at this concentration and was largely soluble. However, when calcium is in a large excess (100-fold excess of calcium to cationic groups on the protein), the calcium can displace the phytate and render it soluble (16, 21).

At intermediate pH, the protein-phytate complex dissociates. The addition of calcium does not affect

protein and phosphorus solubility, but the addition of phytate (lowering the Ca:phytate ratio) results in an increase in, and a shift of, the maximum solubility of phosphorus. In this pH region, direct protein-phytate interactions apparently are not significant. This is also indicated by the gel chromatography work of Okubo et al. (16) and ultrafiltration studies (18, 21).

At high pH (>6.5), phytate solubility remains high at low Ca:phytate levels and calcium solubility decreases as predicted from our earlier studies with pure calcium-phytate (4). The increase in phytate solubility in this pH region could be an independent protein effect or it could be due to the formation of a ternary complex. With increasing concentrations of calcium (higher Ca:phytate ratios), the protein remains soluble and phosphorus precipitates as a result of the formation of calcium-phytate salts which are insoluble.

Our results at high pH disagree in some respects with the observations of Fontaine et al. (6), Saio et al. (7) and de Rham and Jost (8). However, these workers studied defatted soy flour or soybean meal, and our system used a purified soy protein isolate. Soybean meal or soy flour not only has other components known to bind minerals, but the calcium content and Ca:phytate ratio of their samples is unknown (except for de Rham and Jost, who also had a ratio of 2.6). Their sample-processing histories also are uncertain. For example, heating decreases solubility of calcium and phytate (7) and disrupts the ternary complex (21). Our results with defatted soy flour [not shown here; see (19)] were similar to those of the other workers and confirmed our theories of the mechanism of phytate-calcium interaction in soy proteins. Our conclusions are in general agreement with those of Prattley et al. (21), though they studied phytate-BSA interactions rather than phytate-soy protein interactions.

The synergistic effect of other minerals and the processing history of the product also are important determinants of solubility behavior. It also is apparent from this study that care should be taken when interpreting in vivo data from experiments where phytate or calcium were "added" to the food. In addition, protein-phytate complexes may be more resistant to proteolytic digestion than the native protein alone (1). Additional work using binding analysis methods such as equilibrium dialysis or ultrafiltration (20, 21) is needed to more fully understand the nature and extent of these interactions.

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