Magnesium-Phytate Complexes: Effect of pH and Molar Ratio on Solubility Characteristics[1]

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ABSTRACT

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Solubility characteristics of magnesium phytate complexes as a function of pH and molar ratio of magnesium (Mg) to phytic acid (PA) in pure (in vitro) systems were studied. Complexes were soluble below pH 5 at molar ratios studied (0.5–12). Above pH 5, solubility decreased rapidly. The higher the Mg:PA molar ratio, the lower the pH at which the solubility drop was observed. At Mg:PA ratios of 4 or less, phytate phosphorus was relatively more soluble than magnesium at pH >5. However, at Mg:PA ratios greater than 6, the magnesium was more soluble than phytate. Our data suggest that the pentamagnesium form of phytic acid probably predominates when magnesium is in excess.

Studies have shown that phytic acid and insoluble carbohydrate (fiber) may be possible causes for depressed mineral bioavailability from plant-based diets (as compared to animal-based diets), even after accounting for the lower protein quality of most plant-based diets (Erdman 1979). Phytic-acid interactions in food systems were reviewed by Cheryan (1980), and the insolubility of mineral-phytate complexes at physiologic pH is considered to be the main cause for poor bioavailability of the complexed mineral. Thus, it is surprising that so few studies on the solubility characteristics of mineral-phytate complexes—especially as a function of pH and relative concentrations of mineral and phytic acid, ie, molar ratios—are available in the literature.

We report a study done on the solubility characteristics of magnesium-phytate complexes. Magnesium is an essential element in human and animal nutrition because it is an activator of numerous enzyme systems that control many body functions and is a component of soft tissue and bone. Average diets around the world, even those in the United States, may be below the RDA in magnesium (Seelig 1982). Certain stress conditions and dietary components may increase magnesium requirements, especially when vegetable protein sources form a major component of the diet.

Jackman and Black (1951) were probably the first to report on the solubility characteristics of magnesium phytate as a model system for soils. Oberleas and Moody (1981) determined the amount of precipitate formed in a reaction mixture containing a 1:1 molar ratio of Mg:PA in the pH range of 3–9. Tangkongchitr et al (1982) reported solubility of phytate phosphorus using a 3.9:1 molar ratio at pH 5–8 to demonstrate the possible role of magnesium phytate complexes in fermenting wheat doughs. Evans and Pierce (1981) determined the composition of the precipitate at pH 6.0, using a 6:1 molar ratio in the reaction mixture.

Our study covered a much wider range of Mg:PA molar ratios (0.5–12) and a pH range of 2–9. Most food products, ingredients, raw materials, diets, or processing procedures used should fall within these ranges. Furthermore, unlike some of the studies mentioned, we measured the solubility characteristics of both phytic acid and magnesium under these experimental conditions, because the degree of insolubility of the phytic acid phosphorus does not necessarily indicate the degree of insolubility of the mineral and vice versa, especially in the critical range of pH 6–7. The true mechanism of interaction of minerals and phytic acid in a complex system such as a real food product would be difficult to ascertain, however, because of the multiplicity of complex reactions occurring simultaneously. Thus, to avoid confounding the interpretation of the data, our studies were done with pure systems that may serve as models for future studies done with real systems.

MATERIALS AND METHODS

Sodium phytate (>98% pure, Sigma Chemical Company, St. Louis, MO) and reagent grade magnesium chloride were used. Stock solutions of magnesium chloride (0.3 m*M*) and sodium phytate of either 15 m*M* or 24 m*M* were used, depending on the ultimate molar ratios required. Ten milliliters of sodium phytate solution was mixed with the appropriate amount of $MgCl_2$ solution to result in the desired molar ratios of Mg:PA in the reaction mixture. Ten milliliters of 1*M* NaCl was added and the pH adjusted to the desired value, using 1*M* NaOH or 1*N* HCl. Deionized water was added to raise the total volume to 100 ml. The reaction mixture was flushed with nitrogen and incubated overnight at 30°C. The pH was measured during the incubation period and readjusted when necessary. The mixture was centrifuged at 12,000 × *g* for 15 min to separate the insoluble complex. An aliquot of the supernatant was taken for magnesium and phosphorus analysis. A minimum of two replications of this procedure was made for all molar ratios and all pH values.

Total phosphorus was measured by the method of Bartlett (1959), which is a modification of the Fiske-Subbarow method. Standard curves for phosphorus were run for each batch of samples because of day-to-day variation. Phytic acid concentration of samples was calculated (after accounting for dilutions and purity) based on 6 mol of phosphorus per mole of phytic acid and a molecular weight of phytic acid of 660.13. Thus, concentrations of phytic acid in the reaction mixtures were typically $1.5–3.0 \times 10^{-3} M$. Magnesium was analyzed by the back-titration method of Ntailianas and Whitney (1964). All assays for P and Mg were done in duplicate for each sample.

RESULTS AND DISCUSSION

Addition of a salt to a solution of sodium phytate will change the pH of the solution because of the displacement of hydrogen ions and the formation of complexes. Because no buffers were used to prevent confounding the interpretation of the data due to possible interaction of magnesium, phytic acid, or both, with buffer components, the pH of the reaction mixture usually changed by a few tenths of a pH unit during incubation. The magnitude of the pH change was proportional to the pH and the molar ratio. In all cases, we measured the pH of the reaction mixtures during and after incubation and readjusted them to the initial value. The NaCl was added to the reaction mixture to minimize variations in ionic strength. We estimated ionic strengths of our reaction mixture to be 0.11–0.17, which is the approximate range of ionic strengths in the human gut (Rendleman 1982).

Preliminary experiments showed that the mineral-phytate complexes sometimes existed in such a fine colloidal state, that normal *g* forces in ordinary laboratory centrifuges were not enough to sediment all of the insoluble complex. This was also referred to by Cheryan (1980) and by Gillberg and Tornell (1976). We found

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that consistent and reproducible supernatant turbidity readings and mineral assays were observed only when centrifugation was done at 12,100 × *g* for 15 min, or at 17,300 × *g* for 10 min.

Controls were also run to test for insolubility of the $MgCl_2$ and sodium phytate solutions themselves. No precipitates were observed in the controls. The random scatter observed in low-pH samples was traced to problems in ascertaining the precise indicator color change in the magnesium assay.

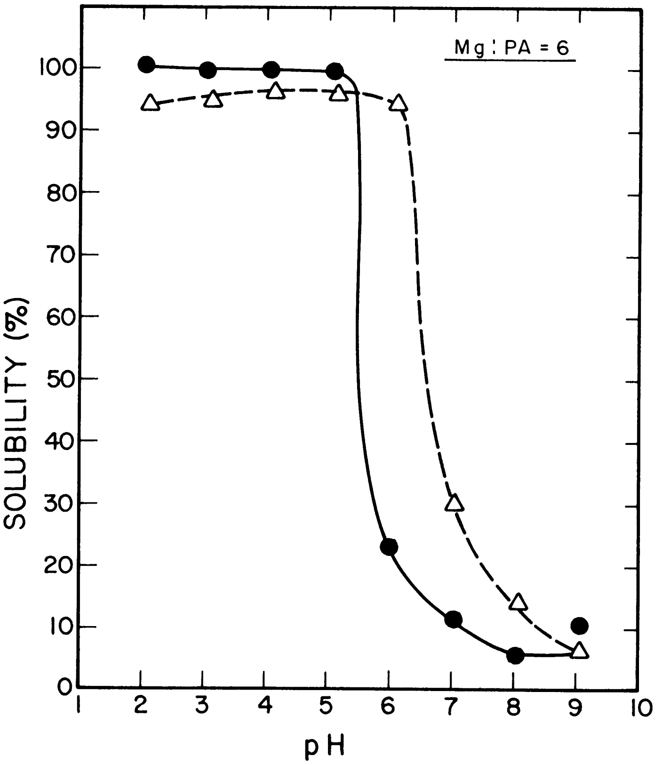

Fig. 1. Solubility profile of magnesium and phytic acid phosphorus in pure magnesium-phytate mixtures at a Mg:PA molar of 6:1. ●——● = P, △——△ = Mg.

The supernatants instead of the precipitates were analyzed, mainly because the precipitates were more difficult to handle. Our preliminary studies with several different metal phytate complexes suggested that when the precipitates were washed to remove occluded or adsorbed reaction solution, the washing process selectively dissolved the precipitate, thus changing the metal–phytate ratio. This phenomenon was demonstrated by Thompson and Erdman (1982), and it is not known whether other investigators were aware of this problem. Weighing such small quantities of precipitate also introduced fairly large errors in the final results, and obtaining crystals of the precipitate was fairly difficult (Evans and Pierce 1981). Thus, we believed that a better description of the solubility phenomenon would be obtained from assays of the supernatant. The precipitation of $Mg(OH)_2$ is not expected to be a problem, because its onset of precipitation occurs only at pH 10 and above.

Solubility Characteristics

Figure 1 shows typical solubility data as a function of pH at a Mg:PA molar ratio of 6:1. The complex is almost completely soluble below pH 5, drops rapidly in solubility between pH 5 and 7, and appears to be essentially insoluble above pH 8. The magnesium is always relatively more soluble at any pH at which complex formation is apparent (>pH 5.5), perhaps indicating that at the molar ratio of 6:1, magnesium is slightly in excess.

The effect of Mg:PA molar ratio and pH on solubility of magnesium is shown in Fig. 2. Figure 3 shows the corresponding behavior for phosphorus (ie, phytate) solubility. Our data show agreement with the data of Jackman and Black (1951), Tangkongchitr et al (1982), and Oberleas and Moody (1981) over the comparatively narrow ranges they studied.

At the low molar ratios (0.5 and 1.0), very little insoluble complex is formed throughout the pH range, except for a small amount at pH 8–9. The complexes remain soluble at all pHs below 5 at all molar ratios, but the pH of diminishing solubility of magnesium (Fig. 2) and phosphorus (Fig. 3) decreases as molar ratio increases. Higher magnesium concentrations relative to phytic acid result in greater complex formation and thus relatively

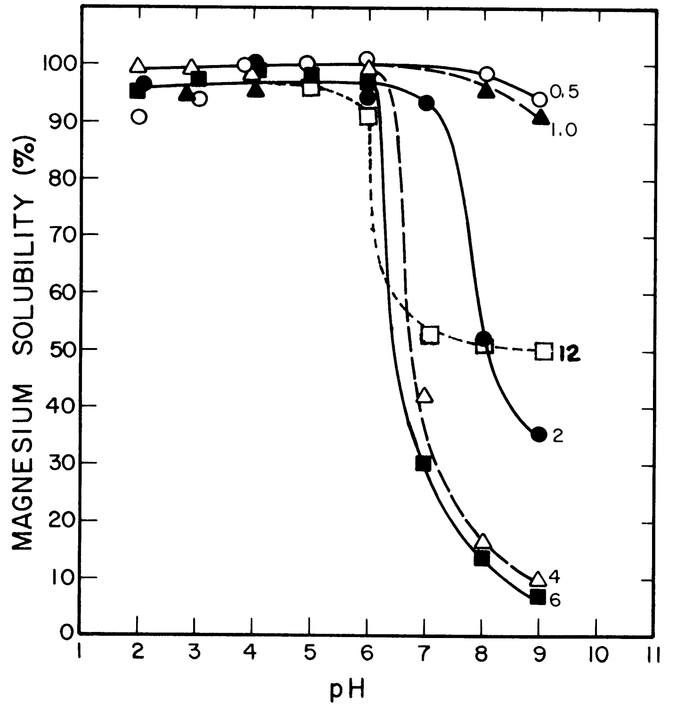

Fig. 2. Effect of pH and Mg:PA molar ratio on solubility of magnesium in magnesium-phytate mixtures.

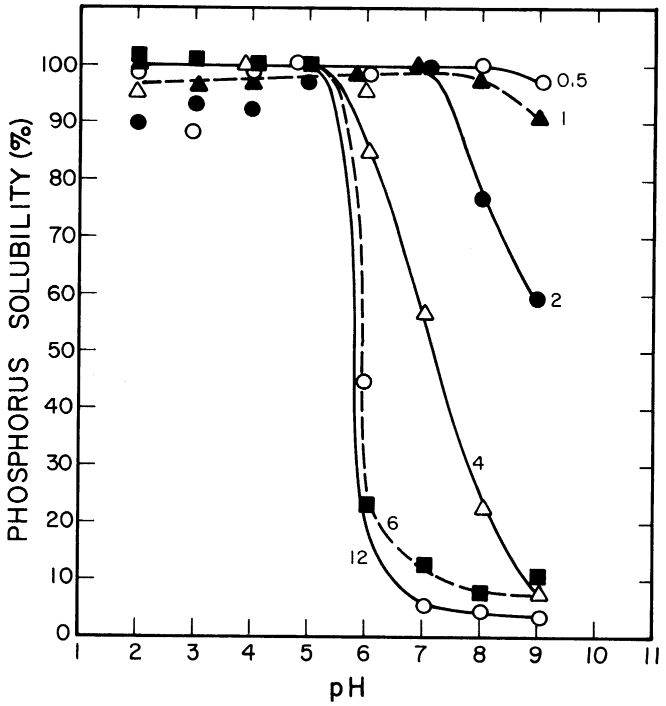

Fig. 3. Effect of pH and Mg:PA molar ratio on solubility of phytic acid phosphorus in pure magnesium-phytate mixtures.

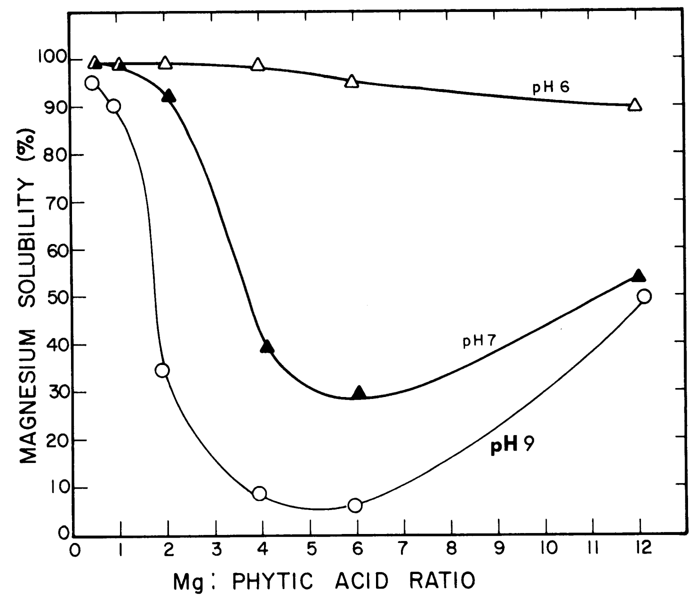

Fig. 4. Effect of Mg:PA molar ratio on solubility of magnesium in magnesium phytate mixtures at pH 6, pH 7, and pH 9.

greater insolubility of the complex. This phytate solubility phenomenon may be explained by the law of mass action, which states:

$$x\,Mg^{+2} + H_n(PA)^{-12+n} \rightleftarrows Mg_xH_{n-2x}(PA)^{-12+(n-2x)} + 2x\,H^+$$

Higher Mg concentrations drive the reaction to the right, competing with the hydrogens for the negative charges in the PA molecule. The displacement of the hydrogen ions cause the precipitation of the phytate salt at a lower pH.

The maximum precipitation of phytic acid phosphorus was observed at >pH 8 for the 6 and 12 molar ratios. In contrast, the maximum precipitation of the magnesium appeared to occur at molar ratios of 4–6 (Fig. 2). Additionally, at all molar ratios below 4:1, magnesium is generally less soluble than the phytic acid, whereas at molar ratios above 6:1, the magnesium is relatively more soluble. From these two sets of observations, the optimum Mg:PA molar ratio for formation of insoluble complexes appears to be 5:1, ie, the penta-substituted magnesium salt is favored when magnesium is not limiting.

This is also shown in Fig. 4, where the magnesium solubility at various pH is plotted versus the Mg:PA molar ratio. The data were fitted mathematically to a polynomial and the minima in the curves obtained from the derivatives. This, plus a preliminary analysis of the precipitate formed at the 6 and 12 molar ratios at pH 8–9, indicated that the pentamagnesium salt of phytate would probably predominate under these conditions. However, the composition of the insoluble complex is a function of the Mg:PA molar ratio in the reaction mixture and, of course, the pH. This can be seen in the data of Tangkongchitr et al (1982), who observed that the Mg:PA molar ratio of the precipitate increases steadily from 4.02 to 4.73 as pH is increased from 5.0 to 8.0, even though the reaction mixture molar ratio was kept constant at 3.9:1. Evans and Pierce (1981) reported a 4.4 Mg:PA molar ratio in the precipitate, but they considered only one pH (6.0) and one molar ratio in the reaction mixture (6:1). No conclusion can be made about the nature of the soluble complexes from these data.

Relative solubility of individual minerals and phytic acid is affected by the presence of other minerals and polyionic compounds such as proteins and fiber in the diet as well as by the processing history of the product. Nevertheless, pure in vitro system studies such as those reported here can serve as a model for future studies with actual food products or diets, especially to correlate bioavailability data with physicochemical interactions.

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