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# Structure Studies of Phytate–Zinc Ion Complexes: X-Ray Diffraction and Thermal Analysis

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Products from the reaction of phytic acid with zinc sulfate in aqueous solution in the pH range of 5–6 were studied by means of powder X-ray diffraction, differential scanning calorimetry (DSC), and thermogravimetric analysis (TGA) in order to obtain structural information. Complexes were synthesized under a variety of conditions that yielded products of various compositions. As the Zn:phytic acid ratio in solution increased, the Zn:P ratio in the isolated products also increased up to a maximum of 1.0. Most of the isolated materials were non-crystalline, non-stoichiometric mixtures of oligomers. Under certain experimental conditions, products were obtained that appeared to be hydrated complexes containing 5 moles of Zn per mole of phytic acid and 6 moles of water per mole of complex. Potassium ions, from the supporting electrolyte, were also bound to the molecules. The number of moles of potassium bound per mole of product was concentration dependent.

Phytic acid (myo-inositol hexaphosphate) is the major source of phosphorus in most grains and legumes. Its presence in dietary plant foods and its ability to bind certain physiologically essential mineral ions have been the focus of many studies [1–5]. The formation of these metal complexes is pH dependent and most are water insoluble at or near intestinal pH [6]. This insolubility results in reduced bioavailability of the essential metal ions and the attendant medical problems and diseases associated with deficiencies of these ions. These include anemia from lack of iron, rickets from lack of calcium, and immune system damage from zinc deficiency. This last item has been a topic of intense research interest in recent years [4, 7–9].

Considerable controversy has existed in the literature for some time regarding the exact structure of phytic acid: *i.e.* Anderson model [10] *vs.* Neuberg model [11]. Only recently was this alleviated by the X-ray diffraction work of Blank *et al.* [12] who published the single crystal structure of sodium phytate. Later NMR studies [13, 14] indicated that the solution structure and ring conformation of phytic acid are pH dependent.

Definitive information regarding the structure of metal complexes of phytic acid has been even more uncommon. Because of the physiological importance of dietary mineral bioavailability, we have undertaken a study of the reaction of phytic acid with various mineral ions at or near intestinal pH. This paper reports the results of some of our attempts to ascertain the structure of these complexes.

## Experimental

### *Materials and Methods*

Dodecasodium phytate (97–98%), Lot No. 107C-0066, Sigma Chemical Co.* was used for the preparation of phytic acid. Analysis by an independent commercial laboratory gave the following values for phosphorus and sodium: 17.07% P and 23.94% Na. These values yield a sodium to phosphorus ratio of 1.9 indicating 12 $Na^+$ per mole.

Phytic acid was prepared from sodium phytate by passage through cation exchange columns of Rexyn 101, research grade. The sodium content of the phytic acid, as measured by atomic absorption spectroscopy, approached that of the deionized water used for dilution purposes and preparation of other reagents, *i.e.* 0.2 ppm. The concentration of phytic acid was determined by measuring the phosphorus content by the Fiske-Subbarow method [15].

For the preparation of the zinc–phytate complex, 20 to 30 ml of approximately 0.05 M phytic acid were mixed with 100 ml of 1 M potassium chloride in a 250 ml beaker. To this solution was added a quantity of approximately 1.5 M zinc sulfate so that, in the reaction mixture, the zinc:phosphorus ratio varied from 1:1 to 6:1. This solution was then adjusted to pH 5–6 by slow addition of 1 M potassium hydroxide with stirring. Stirring was allowed to continue for an additional hour. The solution was filtered and the precipitate washed with three 20 ml portions of boiling, deionized water. The precipitate was allowed to air dry overnight and then was dried at 120 °C under vacuum for 1 h.

Powder X-ray diffraction patterns were obtained on a General Electric XRD-6 diffractometer employing Cu-K$\alpha$ radiation. Samples were ground (0.1–10 $\mu$) and evenly applied to a microscope slide coated with petroleum jelly. Scans were run from a 2-$\theta$

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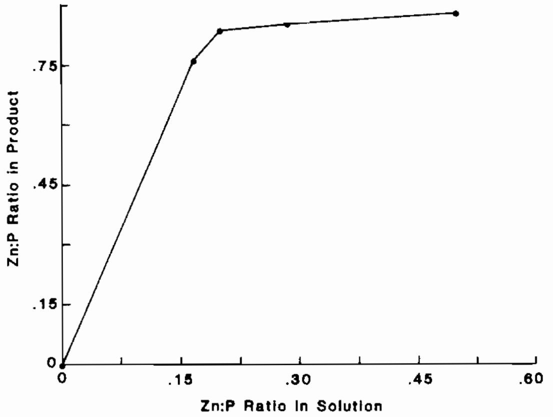

Fig. 1. Zinc:Phosphorus Molar Ratio in isolated products as a function of Zinc:Phosphorus molar ratio in solution.

angle of 3° to 63° at a scan rate of 2°/min and a take-off angle of 4°.

The DuPont 990 Thermoanalyzer was used with a 951 thermogravimetric module to measure the DSC and TGA curves. Sample weights were approximately 10 mg Heating rate was 10 °C/min in a nitrogen atmosphere. The temperature range covered in both DSC and TGA analyses was ambient to 500 °C.

## Results and Discussion

The presence of six *ortho*-phosphate moieties in the phytic acid molecule renders this compound a potential twelve coordinate ligand for complexing with metal ions. With monovalent cations, it is possible to isolate simple, crystalline, stoichiometric salts; and several of these, with different degrees of hydration, have been reported, for example, the X-ray diffraction work of Fennessey and Nowacki [16] on the hydrated dodecylsodium salt, $C_6H_6Na_{12}P_6O_{24}\cdot 38H_2O$. Truter and Tate [17] reported salts with 31, 33, 35, and 44 waters of crystallization, respectively. With polyvalent cations, however, the isolation of simple salts becomes much more difficult. The possibility of both inter- and intramolecular bonding can lead to the simultaneous formation of oligomeric, as well as monomeric species. These polymeric products usually have high molecular weights which render them insoluble in aqueous solution, and co-precipitation of non-stoichiometric mixtures results.

In an attempt to synthesize zinc complexes of phytic acid, solutions were prepared which contained various molar ratios of zinc to phytic acid. Potassium chloride was added to maintain a constant ionic strength and the solutions were adjusted to pH 5–6 by the addition of KOH. Figure 1 is a plot of the zinc:phosphorus molar ratio in the synthesis mixture *vs.* the zinc:phosphorus molar ratio in the isolated product.

The plot shows that as the Zn:P molar ratio is increased in the solution, the Zn:P ratio in the products is also increased, although not linearly. If the ratio in the solution is increased sufficiently, products can be obtained which contain one zinc ion for each available phosphorus atom in the molecule. However, in spite of numerous attempts to vary the synthesis parameters, most of the products isolated were insoluble, non-stoichiometric powders. If one assumed a certain molecular formula based on Zn:P ratios, the rest of the chemical analysis did not balance, thereby indicating that the isolated precipitates were mixtures of products. This is in agreement with previous work on the attempted isolation of phytate complexes of polyvalent cations [18, 19].

Exact structural determination of these products proved difficult due to their insolubility in any known solvent. Solvents strong enough to dissolve the complexes would also cause dissociation. Previous X-ray diffraction studies on crystalline hydrated salts of phytic acid have indicated unit cell dimensions from approximately 12 Å to 32 Å, with typical monoclinic dimensions of 23 X 23 X 12 [12, 16, 17]. Generally, intensities of reflections for metal complexes, particularly axial reflections, are greatest at low 2-$\theta$ angles. X-ray diffraction patterns of isolated zinc-phytate complexes were obtained using Cu-K$\alpha$ radiation ($\lambda$ = 1.54051 Å) from 3° to 63° in 2-$\theta$. This corresponds to a lattice spacing range of approximately 1.5 to 29.5 Å. No peaks (standard deviation $> 2\sigma$ of background) were observed in this entire range for any sample. A 'halo' above the baseline was generally apparent from approx. 10°–35° for most samples indicating the complexes were amorphous solids, or mixtures of non-crystalline products.

In two specific instances what appeared to be stoichiometric complexes were obtained from the reaction of zinc sulfate with phytic acid. In the first case, 25 ml of 0.05 M phytic acid was mixed with an amount of 1.5 M zinc sulfate sufficient to give a final solution with a Zn:P ratio of 1:1. Enough potassium chloride was added to make the final solution 1.0 M in KCl. The pH of the solution was adjusted and the precipitate collected and washed as indicated above. Although the solution contained a 6:1 molar ratio of Zn:phytic acid, analysis of the product showed it to be a 5:1 complex. Elemental analysis also showed that two mol of potassium ion and 6 mol of water were bound per mol of phytate giving an empirical formula $C_6H_6P_6O_{24}Zn_5K_2\cdot 6H_2O$. The analytical data are given in Table I.

The isolation of apparent 5:1 complexes of metal ions with phytic acid has been observed before.

TABLE I. Analysis of Phytate Complex, $C_6H_6P_6O_{24}Zn_5K_2 \cdot 6H_2O$.

|  | Experimental | Calculated |
|---|---|---|
| %C | 6.21 | 6.19 |
| %H | 1.56 | 1.53 |
| %P | 16.01 | 16.25 |
| %Zn | 28.15 | 28.29 |
| %K | 6.73 | 6.20 |

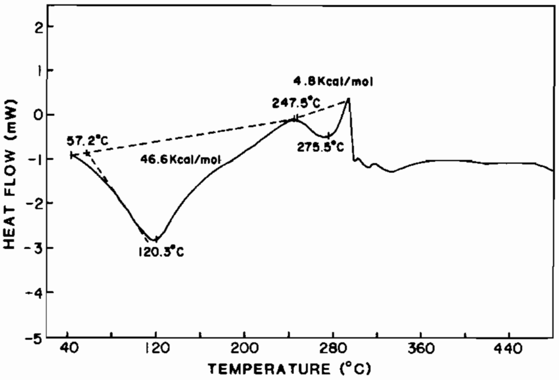

Fig. 2. Differential Scanning Calorimeter curve for 7.84 mg sample of $C_6H_6P_6O_{24}Zn_5K_2 \cdot 6H_2O$. Heating rate was 10 °C/min in nitrogen atmosphere.

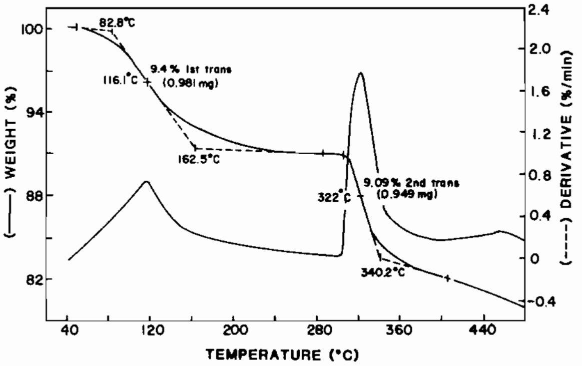

Fig. 3. Thermogravimetric Analysis curve for 10.44 mg sample of $C_6H_6P_6O_{24}Zn_5K_2 \cdot 6H_2O$. Weight loss (left scale) and first derivative of weight loss (right scale) as a function of temperature. Heating rate was 10 °C/min in nitrogen atmosphere.

Hoff-Jørgensen [20] reported the isolation of a 5:1 complex with calcium ion. Earlier work by Hay [21] has shown that a 6:1 complex is also possible with calcium. Studies by Evans and Pierce [19] reported that complexes approaching 5:1 stoichiometry are possible with other divalent metal ions.

The empirical formula suggested the presence of six coordinated water molecules. This was confirmed by differential scanning calorimetry and thermogravimetric analysis of the complex. Figure 2 is a DSC plot of the decomposition of 7.84 mg of the complex in a nitrogen atmosphere.

Differential scanning calorimetry indicated three regions of endothermic activity; one at approximately 116–120 °C, another centered at about 272–275 °C, and a third at 320–340 °C. However, when 10.44 mg of the same sample were subjected to thermogravimetric analysis (Fig. 3), only two regions of decomposition were observed; one at approximately 116 °C and another at about 322 °C.

The DSC endotherm at 120° is attributed to the loss of bonded water of crystallization. The experimental weight loss from the TGA data (Fig. 3) accounted for 9.4% of the complex. This is in good agreement with the calculated value of 9.3% for $C_6H_6P_6O_{24}Zn_5K_2 \cdot 6H_2O$. The coordinated water molecules can be bound into the complex ion one of two ways: either by hydrogen bonding to a phytate oxygen, or by oxygen coordination to a vacant metal orbital. Based on the transition enthalpy value obtained in DSC analysis, it would appear that the former mode is operative. The dissociation energy of most hydrogen bonds is in the range of 5–10 kcal/mol, while most oxygen to metal bond energies are somewhat higher. The bond dissociation energy calculated from the DSC data was 7.7 kcal/mol of water. The second endotherm observed in the DSC data centered at 275 °C was not accompanied by any weight loss. The exact interpretation of this activity is unknown at present. The third endotherm centered at 322 °C is also accompanied by a significant weight loss, and is attributed to thermal decomposition and charring of the complex.

The second molecular complex was isolated under conditions similar to the first. The only parameter altered was the concentration of the supporting electrolyte, KCl. The number of moles of potassium bound per mol of complex is apparently dependent upon the concentration of $K^+$ in solution. When 1.0 M electrolyte was replaced by 0.2 M KCl, the isolated salt had an empirical formula of $C_6H_7P_6O_{24}Zn_5K \cdot 6H_2O$. Again the presence of 6 moles of coordinated water was confirmed by thermogravimetric analysis and differential scanning calorimetry.

The present investigation also indicated that the Zn:P molar ratio obtained in the final products may be dependent upon the overall concentration of the reagents. For example, in two separate experiments, products with different Zn:P ratios were obtained even though the relative ratios in solution were constant. One solution contained 0.5 molar $Zn^{+2}$ ion and 0.5 molar phytic acid, the other contained 0.005 M $Zn^{+2}$ ion and 0.005 M phytic acid. We are currently investigating the reaction of phytic acid with a variety of metal ions under different pH conditions. Preliminary results from these studies indicate that the composition of the products obtained is also strongly dependent upon the pH of the reaction mixture.

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