Phytic Acid-Metal Complexes.* (30559)

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The importance of phytic acid or its derivatives in reducing the availability of zinc for various species of animals has been well established in recent years. However, we are still not sure of the exact composition of phytic acid. Neuberg(1) obtained evidence for a formula of $C_6H_{24}O_{27}P_6$ with 18 acid hydrogens. Anderson proposed a formula of $C_6H_{18}O_{24}P_6$ with 12 replaceable hydrogens about the inositol-hexaphosphate nucleus(2). Hoff-Jorgensen(3) prepared the sodium salt of phytic acid and the sample dried at 120°C had the formula of $C_6H_6O_{24}P_6Na_{12}\cdot 3H_2O$ which indicates that phytic acid has the empirical formula proposed by Neuberg but with only 12 acid hydrogens. However, Brown *et al*(4) prepared sodium phytate which had the formula of $C_6H_6O_{27}P_6Na_{18}$.

Phytic acid is prepared from sodium phytate by passing it through a cation-exchange column of either Permutite(5) or Dowex-50 (4) but the free acid is quite unstable and decomposes to yield orthophosphoric acid (6). Formation of complexes between phytic acid and a number of metals by the changes in pH method(7) have been studied by Maddaiah *et al*(8) but their data on the titration of free phytic acid with NaOH differ from the data of Brown *et al*(4), or Barré *et al* (5). This has led us to reaxamine the problem of complex formation between phytic acid and certain metals.

Methods. Solutions of sodium phytate of known molar concentration were titrated against solutions of metal ions or standard HCl using a "Photovolt" pH meter equipped with a glass-calomel (saturated) electrode pair. The pH meter was standardized against a Beckman buffer of pH 7.00 ± 0.02 and could be read to ± 0.02 units. Nitrogen was bubbled through the solutions which were stirred with a magnet during the titrations at an ambient temperature of 22°C.

Sodium phytate (or phytic acid) samples were digested with HNO_3 and $HClO_4$; and the phosphoric acid was determined colorimetrically(9). Pure phytic acid samples gave no color with molybdovandate reagent but free phosphoric acid or its salts gave color. Sodium was determined by using a flame photometer. The reagent grade chemicals were used to prepare the following solutions: $ZnSO_4\cdot 7H_2O$, 0.0262 M; $CuSO_4\cdot 5H_2O$, 0.0252 M; $NiSO_4\cdot 6H_2O$, 0.0248 M; $CoSO_4\cdot 6H_2O$, 0.0249 M; $MnSO_4\cdot H_2O$, 0.0254 M; $CaCl_2$, 0.0246 M; $Fe(NO_3)_3\cdot 9H_2O$, 0.0165 M; $NiSO_4\cdot 6H_2O$, 0.0249 M.

Results and discussion. Sodium phytate obtained commercially was found to be free from orthophosphate and contained 10.9% moisture. A comparison of its P and Na content with the theoretical values based on various formulae is as follows:

	Molecular wt	% P	% Na	Moles Na /Moles P
Found		19 ±.1	27.8	1.97
Anderson's $C_6H_6O_{24}P_6Na_{12}$	923.8	20.11	29.95	2
Neuberg's $C_6H_6O_{27}P_6Na_{18}$	1109.8	16.7	37.3	3
Hoff-Jorgensen's $C_6H_6O_{24}P_6Na_{12}\cdot 3H_2O$	977.8	19.02	28.2	1.99

The analysis corresponds to the formula proposed by Hoff-Jorgensen(3) rather than that by Anderson(2) or by Neuberg(1). A sample of phytic acid free from phosphoric acid was prepared by passing sodium phytate through a Dowex-50 column. On titration with standard NaOH solution it gave 2 points of inflection, one of which was well defined at a molar ratio of NaOH/phytic acid of 5.0 (pH 5.0), and the second rather poorly defined corresponding to the molar ratio of 7.5 (pH 8.75). Our results differ from those of Brown *et al*(4) who obtained 2 well-defined points of inflection at molar ratios of 8 and 12; or those of Maddaiah *et al*(8) who give the molar ratios of 8 and 12 for the points

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of inflection but their data indicate the values as 6 and 12; or of Barré *et al*(5) who obtained inflections in the curve at molar ratios of 6 and 8. Brown *et al*(4) and Maddaiah *et al*(8) used a formula weight of 714 while Barré *et al*(5) used a formula weight of 660 for phytic acid. Hoff-Jorgensen(3) was able to determine easily only 8 of the 12 possible dissociations constants of phytic acid, and at a molar ratio of 5.5 obtained a pH of 5.1.

We have carried out titrations of sodium phytate against standard HCl and against various standard metal solutions. The data are presented as curves in Fig. 1, which gives the relationships between the changes in pH and the molar ratios of metals/sodium phytate in which molecular weight of 977.8 has been used for sodium phytate. As HCl is added to sodium phytate solution, the pH drops and the slope is quite steep with points of inflection at molar ratios of 5.0 and 7.0, beyond which no points of inflection could be obtained even when 12 moles of acid had been added per mole of sodium phytate (not shown). In the pH zone of 11.2 to 7.5 about 5.0 equivalents of HCl are titrated per equivalence of sodium phytate. The equivalents of HCl titrated between the pH zones of 7.5 to 4 is 2.25. These values and the shape of the curve resemble the one obtained by Barré *et al*(5). The titration corresponds to the following reactions:

$$\begin{aligned} Na_{12}Phy &+ 5HCl = Na_7H_5Phy + 5NaCl \\ Na_7H_5Phy &+ 2HCl = Na_5H_7Phy + 2NaCl \\ Na_5H_7Phy &+ 5HCl = H_{12}Phy \quad + 5NaCl \end{aligned}$$

The pH of the sodium phytate solution is high due to the hydrolysis of the phytate anion, and the reaction of metals with it leads to the formation of complexes, some of which appear to be quite insoluble (Fe^{++} and Zn^{++}). This results in a decrease in pH. The reaction is represented as follows: $Phy^{-x} + y\ H_2O = H_y\ Phy^{-(x-y)} + y\ OH^-$.

If the solubility product of the metal phytate which is precipitated is small, the change in phytate ion (indicated by pH) at the equivalence point will be large. This implies that a sharp drop in the pH of the solution is an indication of a high stability of the metal-phytate complex. For less stable metal-phytate complexes, the pH drop will be less and the end points will be poorly defined.

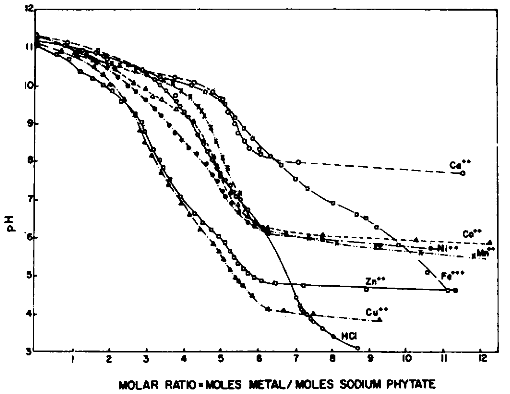

FIG. 1. Titration of sodium phytate with various metal and hydrogen ions.

TABLE I. Titration of Sodium Phytate with Metal Ions.

Sodium phytate, mmole	Titrant, molar	Molar ratio* at point of inflection	pH	Titratable hydrogens
.109	.0246, Ca^{++}	2.0 5.0	10.6 9.6	4 10
.106	.0249, Co^{++}	2.5 5.0	10.15 7.75	5 10
.106	.0254, Mn^{++}	5.0	8.25	10
.105	.0262, Zn^{++}	1 3 5	10.5 8.75 6.0	2 6 10
.116	.0252, Cu^{++}	2.7 5.0	9.0 5.6	5.4 10
.109	.0248, Ni^{++}	5.0	7.5	10
.109	.0165, Fe^{+++}	4 9	9.75 6.5	12.0 27

$$* \text{ Molar ratio} = \frac{\text{mmoles of metal ion}}{\text{mmoles of Na-phytate}}.$$

The results of the titration of sodium phytate against Ca^{++}, Co^{++}, Ni^{++}, Mn^{++}, Zn^{++}, Fe^{+++}, and Cu^{++} are also shown by the respective curves in Fig. 1. The data are summarized in Table I. The capacity of sodium phytate to form a complex with a metal is dependent on the pH of the solution. For example at a pH of 7.4 sodium phytate binds metals in the following decreasing order: Cu^{++}, Zn^{++}, Ni^{++}, Co^{++}, Mn^{++}, Fe^{+++}, Ca^{++}. In each titration, a distinct break in the curve takes place when the ratio between the moles of metal/moles of sodium phytate is about 5.

We also studied complex formation between metals and freshly prepared phytic acid (not shown). In this procedure, the phytic acid to which a slight excess of metal ion had been added was titrated against a standard base. Free phytic acid titration had 2 inflection points in the curve when

$$\frac{\text{moles NaOH}}{\text{moles phytic acid}}$$

values were 5 and 7.5. Beyond that there was no break in the curve. About 5 and 10 equivalents of the base were required for the 2 inflection points in the titrations of Ni^{++}, Co^{++}, and Mn^{++} complexed phytic acid at pH values of about 4.5 and 6.5. The Zn-complexed phytic acid titration curve had points of inflection at molar ratios of 3 and 7.5 at pH values of 3.2 and 5.0. Fe^{+++}-complexed phytic acid gave an inflection at a molar ratio of 6 at a pH of 5.5. These titrations were complicated by the fact that metal-phytates were insoluble and it was difficult to differentiate when metal hydroxides started precipitating along with phytates. At a pH of 7.4, the order in which phytic acid appeared to complex with metals is the same as given above, and is in reasonable agreement with the one reported by Maddaiah *et al*(8).

Gosselin and Coghlan(10) feel that phytate may exist in a polymeric form which may explain the finding of combining ratios which seem unusual for single molecular species. These authors also determined the formation constants for complexes of calcium with phytic acid by an ion-exchange method at a pH of 7.4. However, any attempts to determine the formation constants for zinc-phytic acid complexes by this procedure have been uncuccessful in our hands due to precipitation of zinc phytate at this pH.

The pH values at which other metal phytate complexes precipitated from sodium phytate and phytic acid solutions are given in Table II.

TABLE II. pH Value for Precipitation of Metal-Phytate Complexes from Sodium Phytate and Phytic Acid Solutions.

	pH values for precipitation from	
	Sodium phytate	Phytic acid
Ca^{++}	10.4	?
Fe^{+++}	7.33	from start
Mn^{++}	10.03	3.9
Co^{++}	9.4	4.4
Cu^{++}	9.10	3.0
Ni^{++}	9.0	5.2
Zn^{++}	6.3	3.2

Summary. Sodium phytate corresponding to a formula of $C_6H_6O_{24}P_6Na_{12} \cdot 3H_2O$ (formula weight 977.8) was titrated against metal ions and in each case an inflection in the titration curve was obtained when 5 moles of a divalent metal ion had been added per mole sodium phytate. At a pH of 7.4, sodium phytate formed complexes with metals in the following decreasing order: Cu^{++}, Zn^{++}, Ni^{++}, Co^{++}, Mn^{++}, Fe^{+++}, and Ca^{++}.

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