

Equilibrium Studies of Phytate Ions. 1. Equilibria Between Phytate Ions and Protons in 3 M (Na)ClO₄ Medium

Neng Li,^{a,*} Olof Wahlberg,^{a,§} Ignasi Puigdomenech^b and Lars-Olof Öhman^c

^aInstitute of Physical, Inorganic and Structural Chemistry, University of Stockholm, S-106 91 Stockholm, ^bDepartment of Inorganic Chemistry, The Royal Institute of Technology, S-100 44 Stockholm and ^cDepartment of Inorganic Chemistry, University of Umeå, S-9087 Umeå, Sweden

Li, N., Wahlberg, O., Puigdomenech, I. and Öhman, L.-O., 1989. Equilibrium Studies of Phytate Ions. 1. Equilibria between Phytate Ions and Protons in 3 M (Na)ClO₄ Medium. – Acta Chem. Scand. 43: 331–339.

The protonation of phytate has been studied in the concentration interval $-12 < \lg[H^+]/M < 0.5$ and $0.001 M \leq C \leq 0.032 M$, where C = the analytical concentration of phytate. High precision emf methods have been used for the determination of the equilibrium constants, while nmr methods have been used to characterize the different ions and to tentatively establish structures. Dinuclear phytate ions were found in solutions with $C > 10$ mM. For the first three protonation steps the following pK_a values were obtained: $pK_{a12} \pm 3\sigma = 8.29 \pm 0.02$, $pK_{a11} + 3\sigma = 8.65 \pm 0.03$ and $pK_{a10} + 3\sigma = 8.06 \pm 0.02$, where σ is the standard deviation. Thus, the second step has the largest pK_a value. This phenomenon is discussed.

The protonation of myo-inositol hexaphosphate, or phytate for short, has been studied previously by a number of investigators^{1–10} (cf. Table 1). The dissociation constants for phytic acid are strongly dependent on the counter-ion concentration. This is mainly due to complex formation with metal ions, but also to variations in activity coefficients. In this study, we have kept the concentration of sodium ion high and constant. In a preliminary study,^{11a} with the sodium ion concentration less than 0.12 M, we found that the average charge of the phytate ion is -5 for $C = 10$ mM, indicating that the species Na_5Phyt^{5-} predominates in solutions with $pH > 11$. Strictly, the complex species formed should be written $Na_qH_pC_r(H_2O)_n^z$. However, since the Na^+ and H_2O concentrations are constants in our experiments, the values of q and n cannot be determined from our present data. The complexes are therefore simply denoted here as H_pC_r , and the apparent equilibrium constants have been determined. A separate study will be devoted to the sodium complexes.^{11b} The equilibrium reactions studied in this work can thus be written as:



The choice of a 3 M NaClO₄ medium makes it possible to vary the concentrations of C and H within large intervals while keeping the activity coefficients constant within the limits of error. The activity coefficients in this work are defined so that they approach unity when the solutions approach the pure medium, i.e. 3 M NaClO₄(aq). The emf

experiments could be extended to cover the concentration intervals $1 \text{ mM} \leq C \leq 32 \text{ mM}$ and $-12 < \lg h/M < 0.5$.

The structure of the phytate ion in solution has been studied by several authors.^{4,8,10,12,13} Two different conformations have been suggested, one in alkaline solution and one in neutral solution. The conformation in alkaline solution has one equatorial phosphate group and five axial groups, while the other conformation has one axial and five equatorial groups. The conformation in alkaline solution is probably stabilized by sodium ion bridges, as in solid sodium phytate.¹⁴ Space-filling models have been used to illustrate the structures of the phytate ions in solution (cf. Ref. 6a p. 28 and Ref. 6b p. 53).

Symbols

The most common symbols are H and C for H^+ and for $Phyt^{12-}$, respectively, H = the analytical concentration of hydrogen ions in excess of H_2O and $Phyt^{12-}$. The total analytical concentration of C is written as C . The concentrations of free H and C are denoted h and c , respectively. The symbol for the concentration of the complex species H_pC_r is c_{pr} . The formation constant of the complex H_pC_r is denoted β_{pr} . The average number of H^+ bound per C is written as Z . The average number of protons bound to a specific phosphate group on the phytate ion per C is written as z_i . The charge of a species "i" is written as z_i . The ³¹P chemical shift with 85% phosphoric acid as reference is written as δ . V and E are the measured volume and emf, respectively. The symbols are used to denote physical quantities.

*Permanent address: Department of Chemistry, Peking University, Beijing, China.

§To whom correspondence should be addressed.

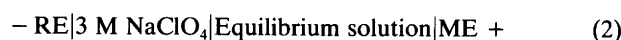
Table 1. Previously reported pK_a values for phytic acid.

Ref	Method	Medium	Temp./°C	pK_{a12}	pK_{a11}	pK_{a10}	pK_{a9}	pK_{a8}	pK_{a7}	pK_{a6}	pK_{a5}	pK_{a4}	pK_{a3}	pK_{a2}	pK_{a1}
1	Hydrogen el.	0.2 M NaCl	37	9.83	9.61	8.82	7.96	6.39	5.15	3.24	2.20				
2	Hydrogen el.	1.0 M NaCl	37	8.95	8.78	8.19	7.34	5.71	4.59	2.68	1.75				
3	Glass el.	0.01 M Na ₁₂ Phyt	25	9.7	9.7	9.7	9.7	6.3	6.3	1.8	1.8	1.8	1.8	1.8	1.8
3	Glass el.	0.1 M KNO ₃	25	10.11	9.80	9.58	8.0								
3	Glass el.	0.5 M KNO ₃	25	9.255	9.060	8.892	7.482	7.482	4.686	2.2					
4	Nmr-pH	0.12 M (butyl) ₄ N	28	12.0	10.0	10.0	7.60	6.85	5.70	2.1	2.1	1.7	1.5	1.5	1.1
5a	Glass el. (+calorim.)	0.2 M KCl	25	9.68	9.68	9.68	9.68	7.79	6.18	3.13	1.51	1.51	1.51	1.51	1.51
5b	Glass el. (KOH-titr.)	0.2 M KCl	25	9.52	9.52	9.52	8.44	6.40	5.42	3.28	1.50	1.50	1.50	1.50	1.50
5b	Glass el. (HCl-titr.)	0.2 M KCl	25	9.59	9.59	9.59	8.63	6.36	5.58	4.97	2.19	2.10	2.06	2.06	2.06
8	Nmr-pH	0.1 M Na ₁₂ Phyt	25	9.6	9.4	9.2	8.6	6.0	5.2	1-2	1-2	1-2	1-2	1-2	1-2
9	Nmr-pH	0.0125 M Na ₁₂ Phyt	10.0	9.8	9.8	9.8	9.8	6.4	5.2						

Experimental

Chemicals and analyses. Strong acids and bases were prepared and analysed as described in Ref. 15. We used sodium phytate from BDH Chemicals Ltd, England. The commercially available salt was recrystallized as described in Ref. 12. Solid sodium phytate is hygroscopic, and the salt was therefore stored in a desiccator containing argon with 44% relative humidity. This material was analysed carefully by potentiometric titrations. The computer program LETAGROPVRID²⁰ was used to calculate the molecular mass of the sodium phytate to be (1122 ± 1) g/mol. The acid-base impurities were found to contribute less than +0.1% to H . About 0.2 g of sodium phytate was weighed in before each experiment with an accuracy of ± 0.00003 g.

Apparatus. The emf measurements were performed using an automatic titration system developed by Forsberg and Kierkegaard.¹⁶ We measured the emf of the following cell:



where RE = Ag(s), AgCl(s)1, 2.99 M NaClO₄(aq) + 0.01 M NaCl(aq) and ME = a hydrogen electrode freshly prepared for each titration. The temperature was kept at (25.00 ± 0.02) °C. We calculated $h = [H^+]$ from the equation:

$$E = E_0 + E_Q \lg h + E_{H_2} + E_j \quad (3)$$

where $E_0 = 59.155$ mV and $E_{H_2} = 1/2 E_Q \lg (p_{H_2}/10^5 \text{ Pa})$. The constant $E_0 = -320.8$ mV was determined in separate experiments and refined in the least-squares data treatment. E_j is given by $E_j = (-16.8 h + 7 K_w h^{-1})$ mV, where $\lg K_w = -14.22$ (cf. Ref. 15). The activity coefficients were assumed to be constant within the accuracy of the measurements. They were defined to approach unity when the solution approaches the pure medium. For further details on the titration procedure, see Ref. 11a.

We have measured 1120 data points. A stable value of E , i.e. to within 0.05 mV, was obtained 20 min. after an addition from the burette, except in a few cases where we had to wait 40 min. to obtain a stable emf value. As seen in Fig. 1, all titrations, including forward and back titrations, agree very well with each other. Thus, the equilibria were found to be both reproducible and fully reversible.

The ³¹P nmr measurements were performed at (23 ± 1) °C using a Bruker WM-250 FT spectrometer. 85% H₃PO₄ was used as chemical shift standard, with positive shift values assumed for lower fields. The field frequency stabilization was maintained using a D₂O-filled 1.5 mm tube placed at the center of the 10 mm measurement capillary.

The operational parameters for obtaining a spectrum were as follows: 56 transients, 16 k data, 26 μs pulse duration (90°), 5000 Hz spectral width and 30 s relaxation delay.

A set of 19 spectra for solutions containing 10 mM phytate with $-12.2 \leq \lg (h/M) \leq 0.5$ were recorded (cf. Fig. 6).

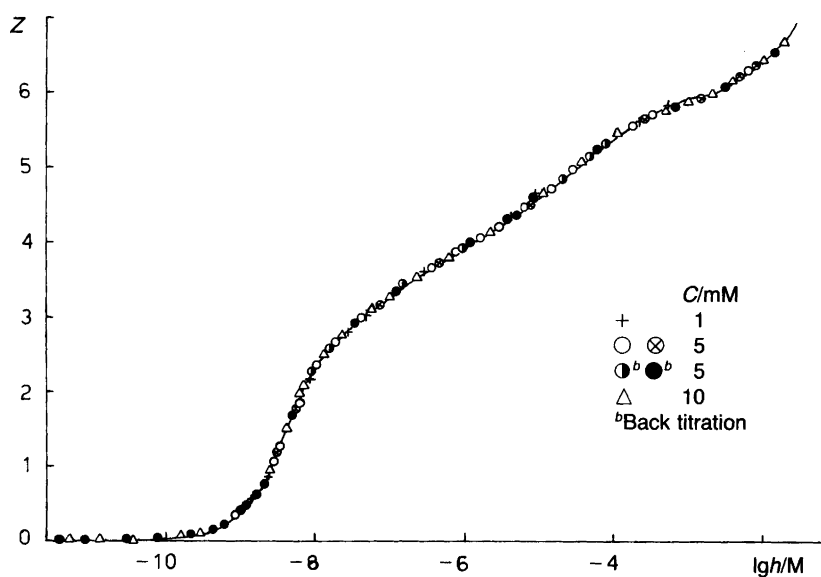


Fig. 1. The average number of bound H^+ per C, Z_{WC} , as a function of $\lg(h/M)$. The circles and triangles correspond to experimental points. The solid line was calculated using the final values of the equilibrium constants.

Most of the spectra were measured with broad-band proton decoupling. A few spectra in the interval $-8.5 < \lg(h/M) < -7$ were measured without proton decoupling. It was thus possible to see the coupling between ^{31}P and vicinal hydrogen atoms. The exact values of Z and $\lg(h/M)$, and the stoichiometric composition of each solution are known from the emf measurements. We also measured two spectra for solutions containing 100 mM phytate.

Computer programs. The following computer programs were used:

- (1) A microcomputer process was used for the data acquisition.¹⁶
- (2) Equilibrium models for the graphical treatment were constructed with GRAPE¹⁷ using a SORD computer.
- (3) The SOLGASWATER¹⁸ and HALTAFALL¹⁹ programs were used to construct the final theoretical models.
- (4) Finally, LETAGROPVRID²⁰ was utilized for the least-squares treatment. Most of the calculations were run on a VAX computer.

Equilibrium analysis. From the emf measurements were calculated $h = [H^+]$ [cf. eqn. (3)], and from the chemical analysis we obtained H and C . The equilibrium conditions corresponding to reaction (1) can be written as:

$$c_{pr} = \beta_{pr} h^p c^r \quad (4)$$

The mass balance and equilibrium equation used in our calculations are:

$$H = h + \sum p c_{pr} \quad (5)$$

$$C = c + \sum r c_{pr} \quad (6)$$

The average number of H^+ bound per C is obtained from:

$$Z = (H - h + K_w h^{-1})/C \quad (7)$$

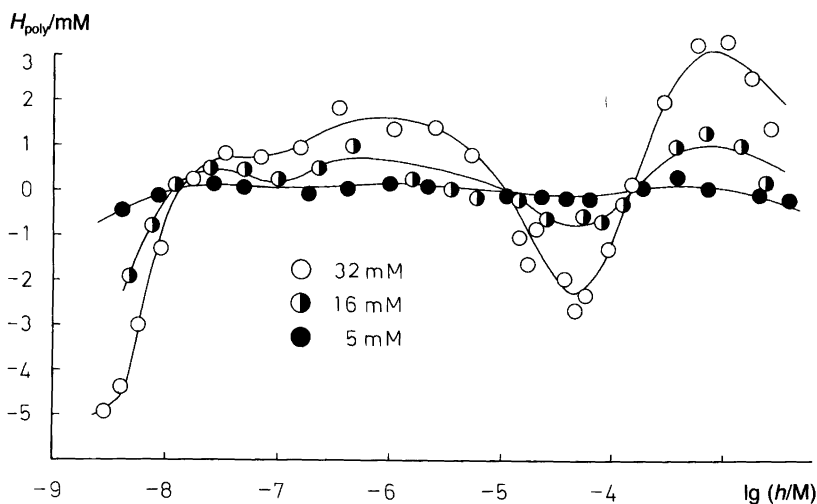


Fig. 2. The measured excess of H^+ over the calculated H_{mono} , $H_{poly} (= H_{tot} - H_{mono})$, as a function of $\lg(h/M)$. The solid lines were calculated using the final values of the equilibrium constants.

Table 2. Results of least-squares refinement of an equilibrium model for mononuclear phytate ions ($C \leq 10$ mM). σZ = the standard deviation of Z , $U = \sum(Z_{\text{calc}} - Z_{\text{obs}})^2$ has been minimized.

Number of points	lg h -interval	σZ	lg ($\beta_{pr} \pm 3\sigma$); σ = standard deviation
77	$-11 < \lg h < -8$	0.006	lg $\beta_{11} = 8.285 \pm 0.014$; lg $\beta_{21} = 16.949 \pm 0.006$; lg $\beta_{31} = 25.000 \pm 0.007$
78	$-8 < \lg h < -3$	0.017	lg $\beta_{41} = 31.622 \pm 0.022$; lg $\beta_{51} = 36.691 \pm 0.028$; lg $\beta_{61} = 40.571 \pm 0.032$
71	$-3 < \lg h < -1$	0.035	lg $\beta_{71} = 42.105 \pm 0.039$; lg $\beta_{81} = 43.2 \pm 0.2$

Treatment of data

1. *Emf Data.* Some of the data for $C \leq 10$ mM are shown in Fig. 1. All the data with ≤ 10 mM fall on the same $Z(\lg h)_C$ graph and thus indicate mononuclear complexes in this concentration interval. Data for $C = 16$ nM and $C = 32$ mM deviate from the mononuclear models. Fig. 2 shows the data for $C = 16$ mM and 32 mM represented as $H_{\text{poly}}[\lg(h/M)]$, which means the measured excess of H^+ over H_{mono} , i.e. the value of H calculated assuming only mononuclear complexes:

$$H_{\text{poly}} = H_{\text{tot}} - H_{\text{mono}} \quad (8)$$

For practical reasons it was necessary to treat different data ranges separately. The mononuclear data in Fig. 1 were subdivided into three different lg (h/M) ranges:

- (i) $-11 \leq \lg(h/M) < -8$
- (ii) $-8 \leq \lg(h/M) < -3$
- (iii) $-3 \leq \lg(h/M) \leq -1$

First, each part of the data was treated separately, and finally, all the data were treated together. The polynuclear data in Fig. 2 were treated in two parts: (i) $-8.5 \leq \lg(h/M) < -6.0$ and (ii) $-6.0 \leq \lg(h/M) \leq -2.5$. In the mononuclear region we started by using normalized plot techniques to construct models by graphical methods.¹⁷ The polynuclear data were treated directly by the least-squares program LETAGROPVRID.²⁰ All data were used for the refinements of the models by means of LETAGROPVRID.²⁰

Data for $C \leq 10$ mM. A model involving eight complexes was fitted to the data, and we examined whether a small proportion of phytate is decomposed to orthophosphate during the experiment. Orthophosphoric acid of unknown concentration was included in the model, and the total concentration of phosphoric acid was used as a variable parameter. We found that at most 0.1% of the phytate had decomposed. Since six phosphate groups may be dissociated, this is a sensitive test. The results are shown in Table 2.

Data for $C \geq 10$ mM. The data in the acidic region could be fitted well assuming one dinuclear complex, $H_{11}C_2$, together with the mononuclear complexes. The equilibrium constants for the mononuclear species agree very well with those determined in the $C \leq 10$ mM range. Several complexes were tested and the results for three of them are given in Table 3. In the alkaline region a single complex did not suffice to explain the experimental data. We therefore held the formation constants for the mononuclear species fixed (equal to the values obtained for $C \leq 10$ mM) and varied the parameters for the polynuclear complexes. In this way we established the presence of a series of dinuclear complexes.

Refinement of the equilibrium model. We have varied the equilibrium constants together with the systematic errors δH_0 and δE_0 . For $C \leq 10$ mM we minimized $U = \sum(Z_{\text{calc}} - Z_{\text{exp}})^2$ and for $C > 10$ mM we minimized $U = \sum(H_{\text{calc}} - H_{\text{exp}})^2$.

For $C \leq 10$ mM we tried incorporating H_6C , $H_{10}C$, $H_{11}C$ and $H_{12}C$ into the model; however, no significant improvement of the fit was obtained.

For $C > 10$ mM we tried incorporating H_8C_2 , H_9C_2 and

Table 3. Test of models with polynuclear complexes in the concentration intervals $-6.2 < \lg(h/M) < -2.5$, $C = 8$ mM, 10 mM, and 32 mM. σE_0 and σH_0 have been adjusted. The least-squares sum $U = \sum(H_{\text{calc}} - H_{\text{obs}})^2$ was minimized. σ = the standard deviation. Several complexes were tested and rejected.

p	r	lg ($\beta_{pr} \pm 3\sigma$)	lg ($\beta_{41} \pm 3\sigma$)	lg ($\beta_{51} \pm 3\sigma$)	lg ($\beta_{61} \pm 3\sigma$)	U	H/mM
—	—	—	31.40 ± 0.16	36.75 ± 0.09	40.57 ± 0.07	49.0	1.1
17	3	121.28 ± 0.25	31.56 ± 0.11	36.78 ± 0.06	40.55 ± 0.07	21.8	0.74
11	3	117.22 ± 0.22	31.62 ± 0.09	36.70 ± 0.07	40.57 ± 0.07	15.7	0.62
11	2	79.50 ± 0.11	31.63 ± 0.07	36.75 ± 0.04	40.56 ± 0.07	7.9	0.45

Table 4. The equilibrium constants for phytic acid valid in 3 M (Na)ClO₄ at 25 °C. σ = the standard deviation.

p	r	j	$\lg(\beta_{pr} \pm 3\sigma)$	$\text{p}K_{aj} \pm 3\sigma$
1	1	12	8.29 ± 0.02	8.29 ± 0.02
2	1	11	16.94 ± 0.01	8.65 ± 0.03
3	1	10	25.00 ± 0.01	8.06 ± 0.02
4	1	9	31.63 ± 0.03	6.63 ± 0.04
5	1	8	36.69 ± 0.03	5.06 ± 0.05
6	1	7	40.57 ± 0.04	3.88 ± 0.05
7	1	6	42.11 ± 0.04	1.54 ± 0.07
8	1	5	43.7 ± 0.1	1.6 ± 0.2
3	2		26.85 ± 0.17	
4	2		35.81 ± 0.05	8.96 ± 0.18
5	2		43.90 ± 0.05	8.09 ± 0.07
6	2		51.42 ± 0.09	7.52 ± 0.11
7	2		58.34 ± 0.07	6.92 ± 0.12
8	2		64.20 ± 0.20	5.86 ± 0.22
9	2		69.68 ± 0.07	5.48 ± 0.22
10	2		74.23 ± 0.14	4.55 ± 0.16
11	2		79.18 ± 0.03	4.95 ± 0.15

H₁₀C₂ in the acidic region. Although the data could be fitted well on the basis of H₁₁C₂ alone, the fit was improved by the additional assumptions. The inclusions of the species H₁₂C₂ did not improve the fit. The final model is given in Table 4. The discrepancy between calculated and experimental values of H is shown in Fig. 3. The distribution of phytate among different species in the solutions is shown for $C = 5$ mM in Fig. 4 and for $C = 32$ mM in Fig. 5.

2. *Nmr Data.* The nmr analysis can be carried out in great detail, since we know the precise stoichiometry and the distribution of the species present in the solutions studied (Figs. 4 and 5). We recorded two spectra with $C = 100$ mM and nineteen spectra with $C = 10$ mM. In the solutions with $C \leq 10$ mM we could neglect polynuclear complexes. First, we have carefully evaluated the peak areas (Fig. 6). The following ratios were found (to within 10% accuracy) for the ³¹P peaks in Fig. 6:

$$1:2:2:1, 2:4, 2:4, 2:2:2, 1:1:4, 1:2:2:1, 1:2:2:1, 3:2:1 \quad (9)$$

These various ratios reflect the changing equivalence of the R-OPO₃H_{*p*} groups in the different species. We have used

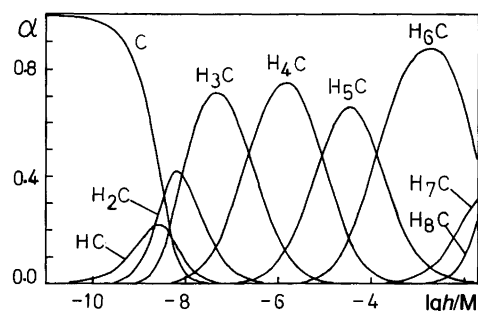


Fig. 4. Distribution of H⁺ among different mononuclear complexes as a function of $\lg(h/M)$. $C = 5$ mM.

the information from the crystal structure of solid sodium phytate¹⁴ to assign the nmr peaks of the most alkaline phytate species, viz. Na₇Phyt⁵⁻ (Fig. 8). If an aqueous solution of Na₇Phyt⁵⁻ is acidified, a conformation change will take place.^{4,8} The two conformers have different symmetries, the alkaline species being pseudo-centrosymmetric and the neutral species having a mirror plane. The assignments of the peaks for the two conformers are indicated in Fig. 8. The assignments were chosen so that A always refers to an equatorial and D to an axial phosphate group. Due to the symmetry change, B and C refer to different phosphate groups in the two conformers. The chemical shifts of the individual phosphate groups are plotted as

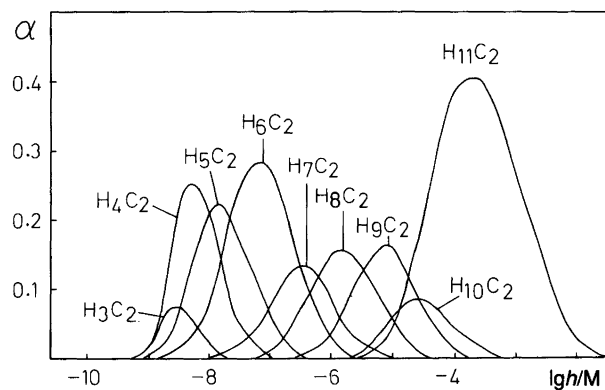


Fig. 5. Distribution of H⁺ among different polynuclear complexes as a function of $\lg(h/M)$. $C = 32$ mM. The mononuclear species are not shown in this figure.

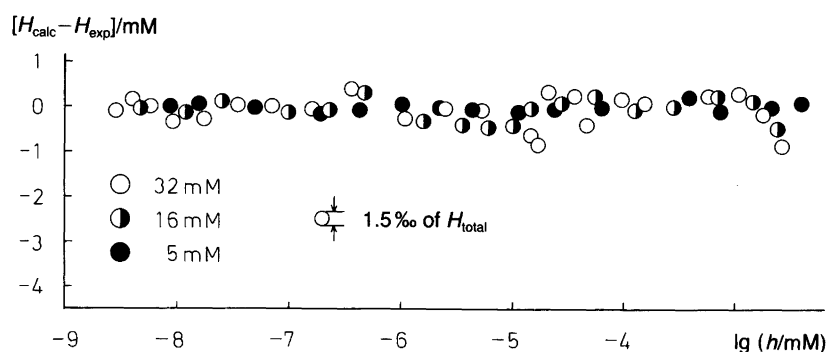


Fig. 3. The error obtained in the refinements of the equilibrium model, $H_{\text{calc}} - H_{\text{exp}}$, as a function of $\lg(h/M)$.

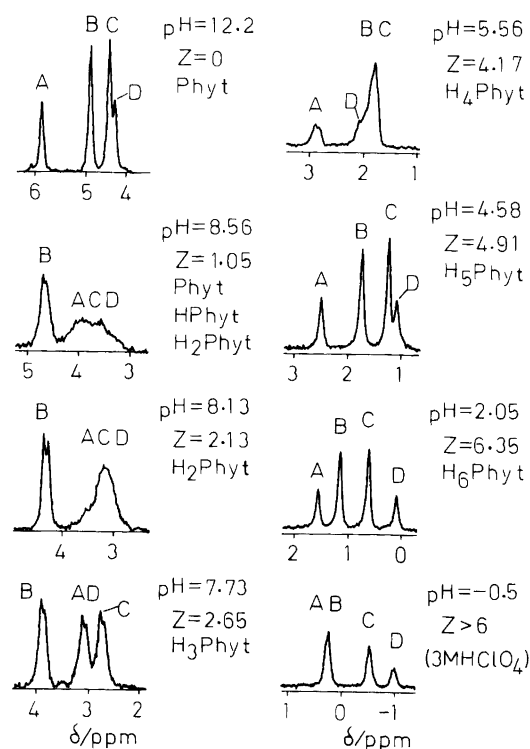


Fig. 6. ^{31}P spectra of phytate species. All spectra, except those in the interval $-10 < \lg(h/M) < -7$, were recorded with proton decoupling. Eight of the 19 recorded spectra are shown.

functions of $\lg(h/M)$ in Fig. 7. These data could be fitted very well by theoretical models, except for two points in the alkaline region. In that pH region, the corresponding peaks are very broad and difficult to measure. The theoretical δ values were calculated from:

$$\delta = \delta_0 + \sum \delta_i z_i \quad (10)$$

The summation in (10) is made over the relevant protonation steps. δ_i is the change in the chemical shift caused by the uptake of one proton by the phosphate group "i", and z_i is the formation function for the corresponding protonation step. Table 5 lists the determined parameters.

As discussed above, the nmr measurements give information about the different individual phosphate groups and thus about the microscopic equilibrium constants. From the treatment of the emf data, on the other hand, we have obtained the corresponding macroscopic equilibrium constants. Since some of the pK_a values for phytic acid are very close together, the macroscopic and microscopic constants will differ appreciably. These differences can be calculated by a statistical method,²¹ but in this article we have chosen an algebraic method to calculate the pertinent relationships.²²

3. Tentative Structures of Phytate Ions in Aqueous Solution. The information obtained from the emf and nmr measurements was used to derive the structures we propose for the

phytate ions. As indicated in Fig. 8, two different conformations of phytate occur in aqueous solution, as already suggested by Isbrandt *et al.*⁸ Some comments concerning the deduction of the structures are summarized below:

(i) *The ions C, HC, H₂C and H₃C.* The nmr spectra indicate that one pair of phosphate groups is independent of the others (Figs. 6 and 8) and that four phosphate groups are interrelated, giving a broad peak. This broadness has been studied by Zuiderweg *et al.*¹⁰ The macroscopic pK_a values given in (11) below could be calculated using the following assumptions: (1) Two protons are distributed statistically on the four interrelated phosphate groups, represented by two pairs each having a microscopic constant $pK_a = 8.4$. (2) The remaining proton is distributed over two phosphate groups which are independent of the others and correspond to one pK_a value, *viz.* $pK_a = 8.3$.

$$\begin{aligned} pK_{a12} &= 8,3 \pm 0,2, & pK_{a11} &= 8,7 \pm 0,2, \\ pK_{a10} &= 8,1 \pm 0,2 \end{aligned} \quad (11)$$

The constants agree very well with those calculated from emf data (Table 4). In H_2C , four phosphate groups are most likely connected by two hydrogen bonds, as indicated in Fig. 8. The spectrum of H_3C is completely different from that of H_2C (Fig. 6), and this can be explained by rapid exchange of one proton within each of the three pairs of equivalent phosphate groups of H_3C , as indicated in Fig. 8.

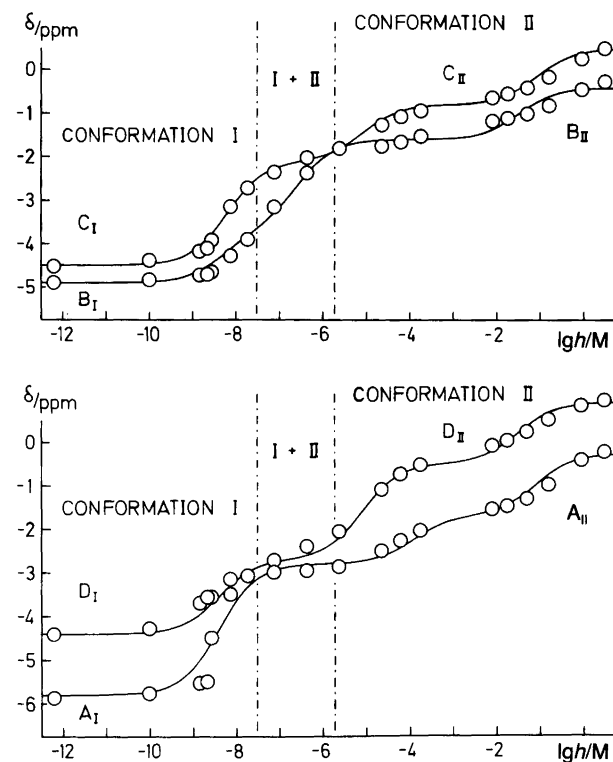


Fig. 7. The ^{31}P chemical shifts of phytate species as functions of $\lg(h/M)$. Circles represent experimental points. The solid lines were calculated using pK_a values and δ values from Table 5.

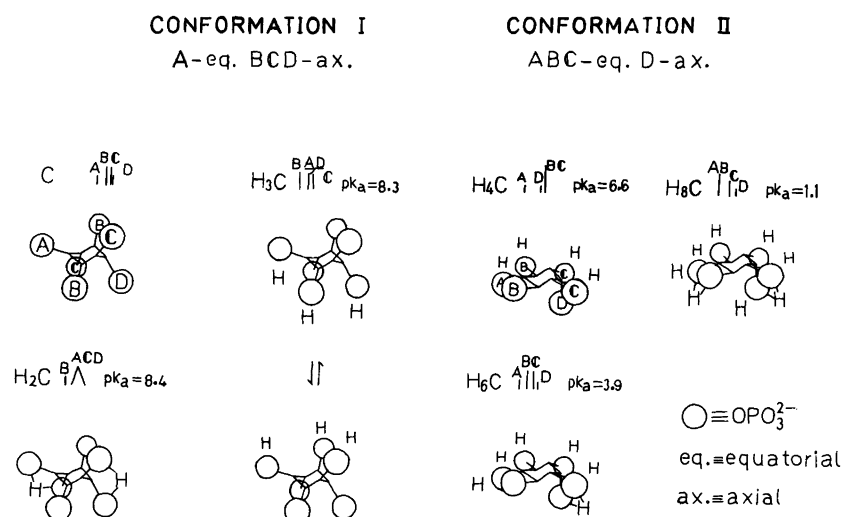


Fig. 8. Proposed models for phytate species based on our nmr and emf data. Note that A, B, C and D correspond to different phosphate groups in the two conformations. Conformer II has been rotated 180° in comparison with conformer I.

It is possible that both conformers exist as H_3C in equilibrium with each other. The alkaline conformer is stabilized by high concentrations of sodium ions.

(ii) *The ions H_4C , H_5C and H_6C .* The spectrum of H_4C shows four equivalent phosphate groups with higher δ values than the remaining two phosphate groups. We therefore suggest that the four equivalent phosphate groups are protonated (Fig. 8). The protonation of H_4C to H_5C gives two inflexion points, one on C and one on D (cf. Fig. 7). This has been interpreted as arising from the formation of a hydrogen bond or possibly an exchange of protons, thus involving two phosphate groups. The next protonation step, with $pK_a = 3.9$, gives one inflexion point on plot A. Thus, one proton is attached to the corresponding phosphate group in H_6C . This ion also contains one sodium ion¹¹ and should be written as $\text{NaH}_6\text{Phyt}^{5-}$. Thus, besides the possible hydrogen bonds there may also be one sodium bridge.

(iii) *The species H_7C , H_8C , H_9C and H_{10}C .* These ions do not contain sodium ions,¹¹ and all have pK_a values of 1.5 or less. The limiting spectrum is a very simple one, indicating

that the protons are evenly distributed over the phosphate ion. Exchange phenomena may occur, and hydrogen bonds almost certainly exist. Two possible hydrogen bonds have been indicated in Fig. 8. We have examined two possible explanations for our data: these involve (1) eight protonation steps and (2) ten protonation steps. The two most acidic protons of H_8C could be exchanged between different phosphate groups to give rise to the four inflexion points, one on each curve in Fig. 7. However, the four inflexion points, i.e. one on each curve at $\text{pH} \approx 1.5$, could also correspond to four different protonation steps in H_{10}C . Our nmr data did not give a clear answer to this question. Two models, one with eight protonation steps and one with ten protonation steps, were tested by fitting them to the emf data, but it was not possible to differentiate between them.

Results

We propose the equilibrium constants in Table 4 for the protonation of the mononuclear phytate species. These constants are valid in 3 M (Na)ClO₄ medium at 25 °C. We also found evidence for a series of dinuclear phytate ions.

Table 5. Characteristic parameters for the six phosphate groups of the phytate ions. σ_i = chemical shift; pK_i = microscopic acid dissociation constant.

Labels of PO ₄ groups (Number of groups)	σ_0	σ_1	pK_1	σ_2	pK_2	σ_3	pK_3
A (one)	-5.8 ± 0.2	3.0 ± 0.2	8.4 ± 0.2	1.1 ± 0.2	3.9 ± 0.2	1.4 ± 0.2	1.1 ± 0.3
B (two)	-4.9 ± 0.2	1.2 ± 0.2	8.3 ± 0.2	2.1 ± 0.2	6.6 ± 0.2	1.2 ± 0.2	1.5 ± 0.3
C (two)	-4.5 ± 0.2	2.4 ± 0.2	8.2 ± 0.2	1.3 ± 0.2	5.1 ± 0.2	1.3 ± 0.2	1.1 ± 0.3
D (one)	-4.4 ± 0.2	1.7 ± 0.2	8.4 ± 0.2	2.2 ± 0.2	5.1 ± 0.2	1.4 ± 0.2	1.5 ± 0.3

Their equilibrium constants are given in Table 4. The distribution of phytate among different species for $C = 5$ mM is shown in Fig. 4. Only a few per cent of the phytic acid exists as polynuclear species in this case. For $C = 32$ mM, about 40% of the phytate is present as dinuclear species (Fig. 5). We have also recorded two nmr spectra for $C = 100$ mM. We found the same peaks as in the mononuclear case, but with slightly shifted positions.

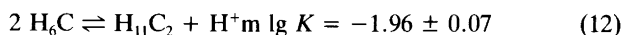
We have proposed tentative structures for the phytate species (Fig. 8). Hydrogen bonding, sodium bridges and rapid exchange are essential features of these structures, which occur in two different conformations. The inversion of the hexane ring is probably hindered by the sodium and hydrogen bridges between the phosphate groups. In this respect, the results of the present investigation are in agreement with those of earlier studies. However, due to our powerful equilibrium analysis we have been able to extract further information on the phytate species: (1) The order of the pK_a values is reversed in alkaline solutions. One reason for this is the high stability of H_2C compared to HC , which depends on the sodium ion concentration. (2) The compositions and the equilibrium constants of the polynuclear complexes have been determined. (3) Tentative structures are given for the phytate species.

Discussion

The acid-base equilibria of the phytate ions are very complicated. However, it was necessary for us to characterize these equilibria in detail before starting to investigate the zinc(II) and calcium(II) complexes of the phytate ions. The mixed sodium-proton complexes will be treated separately. It might be worthwhile to study the structures of the phytate species in greater detail by sodium and proton nmr.

The systematic errors. We have planned our experiments so that δH_0 and δE_0 account for the main systematic errors, and these parameters have been adjusted in the LETAGROPVRID²⁰ calculations. We could neglect all other analytical errors in comparison with δH_0 and δE_0 .

The polynuclear species. We tried incorporating $H_{12}C_2$ into our model, but this species was always rejected in the LETAGROP calculations. We can write the following equation for the formation of the most acidic dinuclear species:



The two phytate residues in $H_{11}C_2$ are probably held together by one hydrogen bond. This acidic dimer exhibits several protolytic steps, and the corresponding pK_a values are given in Table 4. The present investigation was not planned as a detailed examination of the polynuclear complexes; however, such a study could possibly be made by the self-medium technique.²³ The influence of the polynuclear complexes on our data is rather small. Thus, for $C \leq$

10 mM we can neglect the dinuclear complexes. The polynuclear complexes only caused a small shift in the nmr data. The number of protons and their symmetry around the phosphorus atoms probably do not change significantly when dimers are formed from the monomers.

The most acidic species. Some authors have reported pK_a values for $H_{12}C_2$, $H_{11}C$, $H_{10}C$ and H_9C (Table 1). We have not been able to determine pK_a for these species in the present study. More information on the acidic species could probably be obtained by emf measurements on more concentrated phytic acid solutions in acidic perchlorate medium. However, there are several difficulties involved in such studies, e.g. the establishment of activity coefficients, and complications due to liquid junction potentials and polynuclear species.

Chemical explanations of the nmr spectra of phytate species.

The conformation of the phytate species in alkaline solution contains from one to three protons and from four to seven sodium ions. The ion with one proton is not very stable, while that containing two protons is stabilized by two symmetric hydrogen bonds (Fig. 8). At least four sodium ions seem to be necessary to stabilize the alkaline conformation. In aqueous solution two of those ions probably bind to the ester oxygens, while the other two connect ordinary phosphate oxygens, as they do in the solid state.³ The second conformation of the phytate species is less rigid than the first one, and the phosphate groups can more easily exchange the protons and sodium ions among each other. We have been able to follow the protonation of phytate by phosphorus nmr. Sodium and proton nmr could probably give further details on the bonding of the sodium ions and protons to phytate.

Acknowledgements. We wish to thank prof. Ingmar Grenthe for valuable discussions. A grant from the University of Stockholm to O. W. and a grant from the University of Stockholm and Peking University to N. L. made this work possible. Dr. Sven Westman helped us to correct the English of this article, for which we are very grateful. Ing. Karl-Erik Johansson and Dr. Kim Holmén are acknowledged for their help in developing the data acquisition system.

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Received May 20, 1988.