INVESTIGATION OF THE FORMATION OF PHYTATE–METAL COMPLEXES

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Abstract

The complex formation of phytic acid with calcium-, magnesium-, zinc- and copper ions were studied in model solutions using potentiometric titration. A constant ionic back concentration ($2M/dm^3$) was assured by $NaClO_4$. The phytic acid-metallic ion ratio was 1:1. The pH-ranges investigated were the following ones Ca: 4,0—8,0; Mg: 2,0—7,5; Cu: 3,0—5,5; Zn: 3,0—5,0. Stability constants of the phytic-acid-metal complexes were calculated from the experimental data.

The long discussions connected with the elucidation of the structure of myo-inositol 1, 2, 3, 4, 5, 6,-hexakis (dihydrogen phosphate) or phytic acid were ended after general acceptance of the structural formula proposed by R. J. Anderson in 1914 [1]. Among the many factors supporting the structure of Anderson the X-ray crystallographic and NMR measurements played a decisive role [2—6]. From the structure of phytic acid represented in the Fig. 1 it can be seen that the molecule contains 12 dissociable hydrogens. In earlier investigations the process of stepwise dissociation and the equilibrium constants belonging to the different steps of dissociation were studied using potentiometric titration [7—11]. On the basis of the evaluation of measured data it was possible to calculate three average dissociation constants. It was clear studying the inflection points of the titration curves that the first calculated dissociation constant is an average value of the dissociation constants of first six dissociating hydrogens having practically the same constants of dissociation. The next calculated dissociation constant belonges to the following two hydrogens and the last one represents the average value of the dissociation constants of the last four hydrogens.

Using nuclear magnetic resonance spectroscopy (with ^{31}P isotope) Johnson and Tate [6] determined all the 12 constants of equilibria. The published pK_d values were the following ones:

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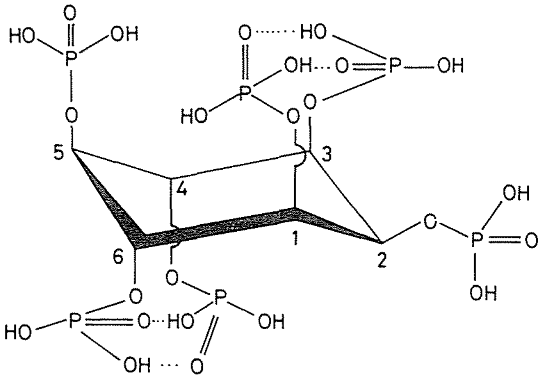

Fig. 1. Structural formula of the phytic acid

1,1	for the phosphate group located at carbon atom number 2 (C-2)
1,5	for the phosphate groups located at C-1 and C-3
1,7	for C-5
2,1	for C-4 and C-6
	average value: 1,67
6,85	for the phosphate group located at the carbon atom C-2 (second hydrogen)
7,60	for C-5 (second hydrogen)
	average value: 6,28
5,70	for the phosphate group located at the carbon atom C-1 (second hydrogen)
10,0	for C-4 and C-6
12,0	for C-3
	average value = 9,9

The knowledge of the all dissociation constants and also protonation constants (the reciproc values of dissociation constants) gives a possibility to calculate the molar proportions of all the dissociation products and the graphical interpretation of their distribution at different pH in per cents.

The molecular proportion of the i-th species (dissociation products)

$$\phi_i = \frac{(H)^i K_1 K_2 \ldots . K_i}{1 + (H)K_1 + (H)^2 K_1 K_2 + \ldots . + (H)^{12} K_1 K_2 \ldots . K_{12}}$$

where i = the number of bounded protons

(i = 1, 2, 3, . . . 12)

Naturally according the definition the sum of molar proportions is 1

$$\phi_0 + \phi_1 + \phi_2 + \ldots + \phi_{12} = \sum_{i=1}^{12} \phi_i = 1$$

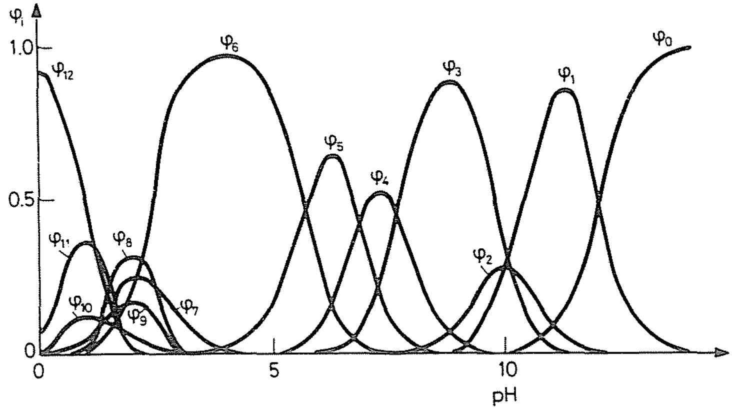

Fig. 2. Distribution of the different species of phytic acid anions at different pH values

The knowledge of the molar ratios of different species and their distribution curves at different pH (see Fig. 2) may be very helpful in the explanation of the structure of metal-phytic acid complexes (chelates).

The potentiometric measurements made by different authors and also in our laboratory [7—11] gave very similar results. (See Table 1.) The average values published are in good agreement with those determined by NMR technique. As it was mentioned above, it was not possible to determine separately all 12 dissociation constants using potenciometry.

The investigation of the complexes (chelates) of phytic acid formed with cations of metals occuring in living organisms started much later and most of the results published by different authors is of qualitative character. Mainly potentiometric titration was used in experiments. Only some authors [11—14] published semi-quantitative data making a comparison between the stabilities of some phytic acid-metal complexes.

Table 1

Comparative values of dissociation constants of phytic acid measured with potenciometric titration by different authors

Authors	$pK_d(1-6)$	$pK_d(7-8)$	$pK_d(9-12)$
Andrews et al. (10)	2.13	6.16	9.0
Barré et al. (11)	2.15 1.84	6.15 5.84	9.5 9.73
Costello et al. (9)	1.67	6.28	9.9
Own measurements	1.80	4.9	8.1

The main difficulty in the investigation of phytic acid-metal complexes is connected with the fact that at the same time in the solution there are present different species of phytic acid anions having different degree of protonation. As a consequence the metal ions interact parallelly with more and different anions. In addition side reactions are also possible. So we must calculate—at given conditions—with the formation of hidroxides (as result of hydrolysis) and also with precipitation of insoluble phytic acid-metal salts.

The purpose of experiments made in our laboratory was the investigation of formation of phytic-acid-metal complexes in modell solutions and on the basis of the results quantitative calculation of the stability of the complexes.

Experimental

Materials and methods

Model solutions were prepared using phytic-acid prepared from sodium phytate (SIGMA pa) by ion exchange, and $CaCl_2.H_2O$ (REANAL), $Mg(ClO_4)_2.6H_2O$ (MERCK), $Cu(NO_3)_2.3H_2O$ (REANAL), $ZnCl_2$ (REANAL)

The constant ionic back concentration ($2M/dm^3$) was assured by $NaClO_4$ (MERCK). For potentiometric titration a solution of 0,1 M/dm^3 NaOH was used. The concentrations of the model solutions were the following ones

Ca-phytates:	phytic acid	$5{,}68.10^{-3}M/dm^3$
	$CaCl_2$	$6{,}12.10^{-3}M/dm^3$
Mg-phytates:	phytic acid	$5{,}36.10^{-3}M/dm^3$
	$Mg(ClO_4)_2$	$5{,}91.10^{-3}M/dm^3$
Cu-phytates:	phytic acid	$2{,}73.10^{-3}M/dm^3$
	$Cu(NO_3)_2$	$3{,}27.10^{-3}M/dm^3$
Zn-phytates:	phytic acid	$1{,}61.10^{-3}M/dm^3$
	$ZnCl_2$	$5{,}00.10^{-3}M/dm^3$

An automatic RADIOMETER apparatus was used for potenciometric titration.

Results and discussion

From the titration curves the curve of complex formation was calculated using the following formula

$$\bar{n} = \frac{C_L - (L)}{C_M}$$

where $\bar{n}$ = the average number of coordination
(L) = concentration of the free ligand
C_L = the initial (total) concentration of the ligand
C_M = the initial (total) concentration of the metal ion

The pH ranges and the calculated maximal $\bar{n}$ values were as follows:

Ca phytates:	pH 4,0—8,0	$\bar{n}=0{,}43$
Mg phytates:	pH 2,0—7,5	$\bar{n}=0{,}72$
Cu phytates:	pH 3,0—5,5	$\bar{n}=0{,}82$
Zn phytates:	pH 3,0—5,0	$\bar{n}=1{,}37$

No hydroxid formation was observed during potenciometric titration in the pH ranges mentioned above.

On the basis of the knowledge of the coordination numbers—which values are lower than 1—except Zn-phytates—it can be stated that complexes with more than one center were not formed and that only the first stability constant may be calculated from the measured data (except Zn ions).

On the basis of the knowledge of average coordination numbers and free ligandum species at the different pH values

$$\bar{n}=\frac{K_1(HL)^{(12-i)-}}{1+K_1(H_iL)^{(12-i)-}} \qquad \text{and}$$

$$K_1=\frac{\bar{n}}{(1-\bar{n})(H_iL)^{(12-i)-}}$$

The concentrations of the free ligandum phytate anions protonated at different degree were calculated on the basis of the molar ratios of different species depending on their distribution at different pH values. For the calculations the data of Costello et al. [5] were used. The results are summarized in the Table 2.

The calculations were facilitated by the fact that overlapping between different species is low which decreases the error made calculating only the

Table 2

Stability constants of some phytic acid metallic ion complexes (in brackets the logarithm of the values)

$H_iL^{(12-i)}$	Ions			
	Ca^{2+}	Mg^{2+}	Cu^{2+}	Zn^{2+}
$i=6$	–	$5.95\cdot10^1(1.77)$	$2.51\cdot10^2(2.40)$	$1.84\cdot10^3(3.26)$
$i=5$	$8.15\cdot10^1(1.91)$	$4.12\cdot10^2(2.62)$	$2.00\cdot10^5(5.30)$	$1.15\cdot10^5(5.06)$
$i=4$	$7.74\cdot10^2(2.89)$	$1.67\cdot10^3(3.22)$	–	–
$i=3$	$1.44\cdot10^3(3.16)$	$9.65\cdot10^3(3.98)$	–	–

average coordination number. Practically at a given pH only two species are present in a quantity more than 10%.

Further investigations are in preparation with the purpose to determine the degree of participation of all the individual species in the forming the average coordination number.

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