Stability and mode of coordination complexes formed in the silver(i)/nucleoside systems

Equilibrium study on the interaction of phytic acid with polyamines and metal ions

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Abstract

Interaction of phytic acid (*myo*-inositolhexakisphosphoric acid, IP) and polyamines (A = en, tn, Put, dien, 2,3-tri, 3,3-tri, Spd, 3,3,3-tet, spermine(Spm)) have been studied by potentiometric and ^{31}P-NMR techniques. The non-covalent interactions have led to formation of stable molecular complexes of (IP)H_n(A) type at the 1:1 molar ratio of the ligands, but of different numbers of protons. The IP protonation constants, stability constants of the molecular complexes and metal (Mg^{2+}) complexe have been determined. The structural and pH dependences of stability constants showed the interactions between IP and A have the acid-base character determining their effectiveness, although the IP structure (5ax1eq, 5eq1ax) in molecular complexes should be also taken into account. ^{31}P NMR study showed in the presence of Spm ^{31}P highfield shifts and high pH shift of signal broading due to chemial exchange between 5ax1eq and 5eq1ax. The preferable binding of Spm to IP over Mg^{2+} in neutral pH indicated the importance of polyamine as a stabilizer of phosphate compounds.

Keywords: Phytic acid, polyamines, metal ions, coordination, non-covalent interaction

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1. Introduction

Phytic acid IP6 (the abbreviation IP is used in the text for the sake of clarity) is an important component of human diet. It interacts with inorganic and organic cations and its complex formation ability influences properties of food. Phytic acid was first identified in 1855 and found to occur in high concentrations in legumes, cereals, nuts and other crops [1-3]. It is the main source of reactive phosphate groups for plants. Since zinc deficiency in human was reported at 1961 to be due to the chelate of phytic acid, it has been considered as antinutrient, because it can bind essential dietary elements, e.g., calcium, iron, and zinc, thus limiting their bioavailability in human organism [1,4,5]. Recent studies have demonstrated that the antinutrient effect on metal binding is only observed when large amounts of IP are consumed and the daily consumption of about 1-2 g does not affect the metal status in the human body [1]. The *in vivo* as well as *in vitro* studies have shown that phytic acid is an important antioxidant and the presence of IP in living cells has beneficial effects such as protection against cancer, heart diseases, diabetes and renal calculosis [6-8]. Anticancer and biological activity of this compounds is related to the IP interaction with nucleic acids [9-12], RNA editing enzyme [13] and kinase [14]. Moreover, phytic acid can be used as corrosion inhibitor, component of toothpaste, in dental cements and mouthwashes [4]. The function of IP in nucleus was also described and the interaction between IP and polyamines is important in the case of HIV attack [15].

A number of model systems have been investigated in the aspect of phytic acid reactions in order to obtain more information on the mode of interactions in the living organisms. However, no consensus on the role of phytic acid in physiological processes has been reached [16,17].

Phytic acid displays pH dependent conformational behaviour. There are five phosphate groups in equatorial positions and one axially oriented in 5eq/1ax conformation, whereas the 5ax/1eq structure has one equatorially and five axially oriented groups [18]. IP has twelve replaceable protons and can exist in various forms. A large number of phosphate groups provides with strong multicoordination chelating sites of the ligand in the reactions with such metals as Mg(II), Ca(II), Na(I), K(I), Fe(III), Mn(II), Cu(II) and Zn(II). Many studies have been performed on IP systems with metal cations in order to explain the significance of the interactions *in vivo* [15]. As regards the biological activity of the ligand, a number of amine polycations (putrescine, spermidine, spermine), occurring on the millimole level in cells, should be also taken into consideration, in addition to inorganic cations [19-21].

Polyamines are present in the physiological fluids as protonated species and in this form they interact with negatively charged fragments of other bioligands including IP. The mechanism of such an action on the molecular level has not been fully elucidated. The hypothesis that the only factor determining the character of the bioprocesses is the charge of components does not explain the structure dependence of the interactions. Our recent studies on the model systems including polyamines and nucleotides indicate that of key importance to formation of molecular complexes (along with charge) is the polyamine length [22,23].

It should be taken into regard that both IP and polyamines are present in the living cells so that their mutual interaction can modify their biological activity [24]. These modifications can be important in view of the fact that the studied compounds react with nucleic acids and show anticancer activity.

This paper presents results of the potentiometric equilibrium study of interactions of polyamines with *myo*-inositolhexakisphosphate (IP), and reports the first stage of investigation of reactions in the systems containing IP and cations (both inorganic and organic ones).

2. Experimental

Dodeca-sodium cation-containing (IP)Na_{12} salt extracted from rice was obtained from Aldrich. 5 g of (IP)Na_{12} was dissolved in about 25 cm^3 of deionised warm water and passed through a column packed with Amberlit IRN 77 (Supelco, 99.8% exchange, H^+ form). The process of passing through the column was repeated at least four times. The strongly acidic middle fraction pH<2 of the eluent was collected to prevent dilution of the acid. After each pass of IP, the column was carefully rinsed with deionised water, and then it was re-converted to acidic form by passing 0.1M HCl through it. None of the samples contained detectable amounts of potassium and for this reason only sodium determination was carried out. Concentration of Na^+ in IP after the fourth exchange was 0.9 ppm (the maximum level of Na^+ allowing further study has been estimated as 1 ppm).

The polyamines en, tn, Put, dien, 2,3-tri, 3,3-tri, Spd, 3,3,3-tet, Spm (Figure 1) in the form of nitrates were synthesised from free polyamines dissolved in methanol to which water/methanol solution of nitric acid was added at the ratio 1:2 for diamines, 1:3 for triamines and 1:4 for tetramines (for tn, polyamine nitrate precipitates when excess of ether was added). The purity of products was checked by performing elemental analysis and results were consistent with the calculated ones (±0.5%).

Potentiometric measurements were carried out on a Titrino 702 Metrohm instrument. An electrode with flexible sleeve diaphragm, inert filling (6.0255.100 Metrohm) was calibrated in terms of hydrogen ion concentration on titration of HCl with $(C_2H_5)_4NOH$. Calculations were performed for the points taken in the pH range of 2.5-3.5 [25]. The measurements in IP-polyamine systems (R.Z, L.L) were made in helium atmosphere at the ionic strength I=0.15M($(C_2H_5)_4NClO_4$), t=20±1^oC using CO_2-free tetraethylammonium hydroxide $(C_2H_5)_4NOH$ as a titrant. Mg-IP system was studied at I=0.10M($(C_2H_5)_4NClO_4$), t=25±0.05^oC (A.O.) [26]. Tetraethylamine perchlorate $(C_2H_5)_4NClO_4$, synthesised from tetraethylamine hydroxide (Aldrich) and perchloric acid (Merck), was used in order to adjust ionic strength. The reaction ($HClO_4$ + $(C_2H_5)_4NOH$ → $(C_2H_5)_4NClO_4$ + H_2O) was conducted in methanolic solution at 1:1 ratio using a small excess of the acid. The salt precipitates from the solution after addition of a large amount of diethyl ether with the yield about 85%. The purity of the product was checked by elemental analysis performed on a CHN 2400 Perkin-Elmer elemental analyser (calc. %N, 6.10; %C, 41.83; %H, 8.77; found %N, 5.95; %C, 41.59; %H, 9.53). The sodium content in the 0.2M solution of the titrant was 0.6 ppb. The determined ionic product for water was pK_w=13.78. No precipitate was observed in the pH range studied (pH 2-11.5).

The concentration of phytic acid was roughly estimated from differences in two inflection points in the titration curve using $(C_2H_5)_4NOH$ as a titrant, knowing that the first inflection point was observed after titration of six protons of IP and the second one after titration of two more protons. The estimated acid concentration was then introduced to the SUPERQUAD computer program [27], and the concentrations of IP as well as H^+ were accurately determined. When the exchange procedure on the column was correct, the ratio of concentrations of hydrogen ions to phytic acid were above 11.5 and finally near 12.

The protonation and stability constants β_{pqr} defined as following (charges are omitted as clarify) were determined with the SUPERQUAD program [27], and species distributions using the HALTAFALL program [28].

$$pA + qIP + rH \overset{\beta_{pqr}}{\rightleftharpoons} A_p(IP)_qH_r \qquad\qquad pM + qIP + rH \overset{\beta_{pqr}}{\rightleftharpoons} M_p(IP)_qH_r$$

$$\beta_{pqr} = \frac{[A_p(IP)_qH_r]}{[A]^p[IP]^q[H]^r} \qquad\qquad \beta_{pqr} = \frac{[M_p(IP)_qH_r]}{[M]^p[IP]^q[H]^r}$$

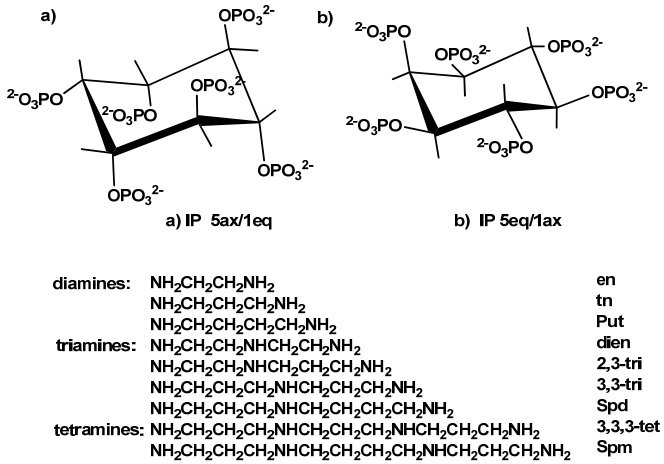
where A, IP, M and H refer to free polyamine, free IP, free metal ion and proton, respectively.

In determination of stoichiometric composition pqr of the complexe by using SUPERQUAD, the testing began with the simplest hypothesis and then in the following steps the models were expanded to include progressively more species, and the results were scrutinized to eliminate those species that were rejected by the computer refinement processes. The criteria used for verification of results are given in our earlier paper [29].

The ^{31}P NMR spectra at room temperature were taken with an NMR Unity-300 Varian Spectrometer (with H_3PO_4 in capillary as an external standard) in D_2O solution with 0.5-1 mM 1:1 IP/polyamine ratio. The presence of M(I) such as Na^+ and K^+ shifted ^{31}P signals of IP due to the cordination To pass the coordination problems we studied ^{31}P-NMR in IP-12H-$((C_2H_5)_4NOH)_n$ system (n=0-12) where ionic radii of $(C_2H_5)_4N$ cation is large. The values of pD were corrected according to the formula $pD = pH_{readings} + 0.4$ [30]. ^{31}P NMR spectra of IP showed 4 peaks involving 1:2:2:1 signal intensity due to the symmetrical IP structure of intramolecular racemic compound. ^{31}P NMR signals assignment was based on the crystal structure of IP-12Na·38H_2O of 5ax1eq conformation [46], and two signals in solid ^{31}P-NMR of 1:5 peak intensity involving with lowfield P2 and highfield P1,3,4,5,6 signals. At low pH (pH<1) two signals in solution ^{31}P-NMR of 5:1 peak intensity showed the reverse order and indicated 5ax1eq at high pH, 1ax5eq at low pH, and their chemical exchange at intermediate pH.

3. Results and Discussion

The structures of the ligands studied are presented in Figure 1.



diamines:	$NH_2CH_2CH_2NH_2$	en
	$NH_2CH_2CH_2CH_2NH_2$	tn
	$NH_2CH_2CH_2CH_2CH_2NH_2$	Put
triamines:	$NH_2CH_2CH_2NHCH_2CH_2NH_2$	dien
	$NH_2CH_2CH_2NHCH_2CH_2CH_2NH_2$	2,3-tri
	$NH_2CH_2CH_2CH_2NHCH_2CH_2CH_2NH_2$	3,3-tri
	$NH_2CH_2CH_2CH_2NHCH_2CH_2CH_2CH_2NH_2$	Spd
tetramines:	$NH_2CH_2CH_2CH_2NHCH_2CH_2CH_2NHCH_2CH_2CH_2NH_2$	3,3,3-tet
	$NH_2CH_2CH_2CH_2NHCH_2CH_2CH_2CH_2NHCH_2CH_2CH_2NH_2$	Spm

Figure 1. Formulae of the ligands

3.1. Determination of protonation constants of polyamines and phytic acid

Stepwise protonation constants K_n polyamine A (n stands for the number of protons in species) and phytic acid H_nIP (IP=deprotonated *myo*-inositolhexakisphosphate anion(12-)) refer to the equilibria of the following reaction:

$$H_{(n-1)}A^{(n-1)+} + H^+ \overset{K_n}{\rightleftharpoons} H_nA^{n+}$$

$$H_nIP^{(n-12)} + H^+ \overset{K_n}{\rightleftharpoons} H_{n+1}IP^{(n-11)}$$

The overall protonation constants β_{10r} of the polyamine A and β_{01r} of the IP were related with the stepwise protonation constants K_n as following equation: $\log \beta_{10r} = \log K_1 + \log K_2 + ... + \log K_n$, $\log \beta_{01r} = \log K_1 + \log K_2 + ... + \log K_n$. The successive constants of the polyamines and IP calculated are presented in Table 1.

Table 1. Overall protonation constant* log β_{10n} of polyamines A, log β_{01n} of IP and successive protonation constant log K_n

compound	log β_{101}**	log β_{102}	log K_2	log β_{103}	log K_3	log β_{104}	log K_4
en	10.15 (2)	17.45 (2)	7.30				
tn	10.70 (2)	19.64 (2)	8.96				
Put	10.83 (1)	20.51 (2)	9.68				
dien	9.94 (2)	19.04 (2)	9.10	23.34 (3)	4.30		
2,3-tri	10.47 (1)	19.90 (1)	9.43	26.16 (1)	6.26		
3,3-tri	10.48 (2)	20.22 (2)	9.74	28.03 (3)	7.81		
Spd	10.97 (1)	21.03 (1)	10.06	29.61 (2)	8.58		
3,3,3-tet	10.36 (2)	20.38 (3)	10.02	29.01 (3)	8.63	36.39 (4)	7.38
Spm	10.91 (2)	21.28 (2)	10.37	30.39 (3)	9.11	38.67 (3)	8.28
IP	log β_{012}	log β_{013}	log β_{014}	log β_{015}	log β_{016}	log β_{017}	
	23.73 (1)	34.30 (3)	43.64 (2)	50.83 (2)	56.72 (1)	59.34 (1)	
	log K_1 log K_2	log K_3	log K_4	log K_5	log K_6	log K_7	log $K_{8\text{-}12}$
	~12	10.57	9.34	7.18	5.89	2.63	<2

*values in the parentheses refer to standard deviations σ given by SUPERQUAD

** log β_{101} = log K_1

For the investigated series of amines, the enthalpy change ΔH of the protonation process is a result of changes in the interaction of charges (-NH^+) or dipoles (-NH_3^+) which become more negative with increasing length of the chain between individual donor atoms. Protonation of the primary amine group is more exothermic than that of the secondary one [31]. As follows from protonation constants presented in Table 1, the proton lability depends on the length of the methylene chain placed between donor nitrogen atoms. Such relations suggest the necessity of regarding the often neglected structural factor in analysis of non-covalent interactions, besides the charge of the component [23,32]. Protonation of spermidine (Spd) and spermine (Spm), the longest biogenic amines, occurs with negligible transmission of inductive effect through a tetramethylene group. Spermine, as we have recently found, reacts as two independent $NH_2CH_2CH_2CH_2NH$- moieties [33].

Investigation of interaction between bioligands requires the knowledge of protonation constants of bioligands due to the fact, that cations compete with proton in the reactions of molecular complex formation. Table 1 presents the protonation constants of phytic acid determined in this work. Although the protonation constants of phytic acids have been reported earlier by several laboratories, they referred to the systems studied at different conditions and – what is especially important – different supporting electrolytes were used [5,34-40]. Taking into consideration the fact that IP molecule contains 12 labile protons, the constants obtained from various sources are often significantly different mainly due to complicated character of equilibria. Moreover, majority of studies have been carried out in the presence of sodium and potassium ions, therefore, taking into account their considerable affinity to IP, these values can be regarded as apparent protonation constants only (even as meaningless). The discrepancy in values of these constants makes their redetermination necessary. Detailed analysis of factors responsible for the acidity of IP protons is presented in Refs. [35,39], however, in this work we are not interested in analysis of differences between particular constants, but we try to establish the mode of interactions and the character of possible IP reactions in the aspect of its role in biological systems.

Ranges of the presence of particular IP species, shown in the distribution diagram (Figure 2) implies that statistically at pH below 2.6, at least 7 protons are bound in the IP molecule. (The constants presented are macroscopic values and cannot be assigned to particular phosphate groups.) On the other hand, at least two protons are bound to IP anion at pH above 12. The low log $K_{7\text{-}12}$ ($pKa_{1\text{-}6}$) values of PO_3^{2-} group of IP listed in Table 1 indicate that protons dissociate very easily from subsequent phosphate groups. However, the dissociation of the seventh H^+ (log K_7 = 2.63) is more difficult as it can be concluded taking into account the pKa_1=1.1 and pKa_2 = 6.36 values for $CH_3PO_3H_2$ [41], and possible several hydrogen bonds formation between phosphate groups.

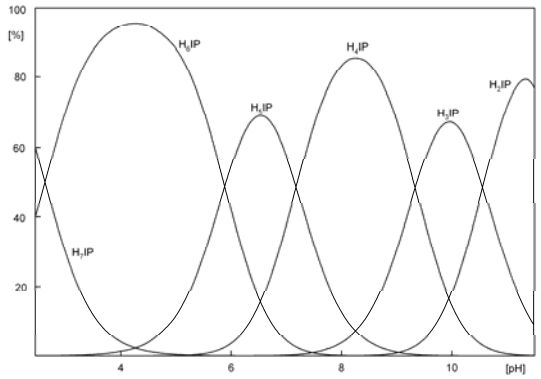


Figure 2 Distribution curves of particular species of IP/H^+. [IP]=0.001M

On the basis of analysis of the macroscopic constants and apparent microscopic constants, Brigando et al., suggest that the first four stages correspond to deprotonation of the groups P(2), P(5), P(1,3) and P(4,6) [18]. The result differs in the sequence of deprotonation with that reported by Costello et al. who suggest that the dissociation occurs successively on P(2), P(1,3), P(5) and P(4,6) groups [42]. Unfortunately, the presence of several IP species in narrow acidity ranges and the complex character of the equilibria with involvement of highly charged species make accurate determination of protonation constants in regions of high and low acidity practically impossible. This conclusion is consistent with that drawn in Ref. [5]. In the pH range studied (2-11.5) the most stable IP species are H_6L^{6-} and H_4L^{8-}. The range of their domination at pH close to 4.0 and 8.5, respectively (Figure 2) corresponds well to plateau regions in the curve illustrating the mean number of protons bound by one mole of IP *versus* pH, and also indicates relatively high stability of both above mentioned species [5]. Distribution diagrams obtained for the protonation constants measured at different ionic strength and temperature are shifted by about one pH unit, but also in this condition, the H_6L^{6-} and H_4L^{8-} are the main IP species in solution [36].

3.2. Equilibrium study of interactions in phytate/polyamine systems

As a result of non-covalent interactions of phytate anion (IP) with polyamine (A), the acid-base equilibrium in the system has been observed to change (the charges are omitted for the simplicity.) :

$$H_xIP + H_yA \rightleftharpoons (IP)H_{x+y-z}A + zH$$

Taking into regard the fact that the interactions between the system components bring about a release of protons, stability constants and stoichiometry have been calculated on the ground of analysis of potentiometric pH data using computer programs [23]. The overall stability constant β_{11r} of a complex $(IP)H_rA$, calculated in the computer procedure, can be expressed as:

$$A + IP + rH \overset{\beta_{11r}}{\rightleftharpoons} (IP)H_rA \qquad \beta_{11r} = \frac{[(IP)H_rA]}{[A][IP][H]^r}$$

The overall stability constants ($\log \beta_{11r}$) of molecular complexes as a result of their non-covalent interactions in the phytate/polyamine systems are presented in Table 2.

The typical titration curves for the IP/Spd system are shown in Figure 3. The difference between two titration curves is due to the adduct $(IP)H_rA$ formation. The occurrence of molecular adduct complex in the systems is confirmed by the superposition of experimental curves of pH-metric titrations with those obtained from computer simulations performed assuming the adduct formation and using determined stability constants of the complex. When the presence of adducts was not taken into account, the experimental and simulated curves were considerably divergent (example in supplemental Figure 1). The greatest differences observed in pH range

of 6-8 correspond to formation of the dominant species $IPH_7(Spd)$, whose deprotonation begins from pH of about 8.5 (Figure 4). As a result of this complex decomposition, the divergence in the both curves decreases to reappear from pH of about 9, which corresponds to formation of the $IPH_6(Spd)$ species that dominate at pH close to 9.5.

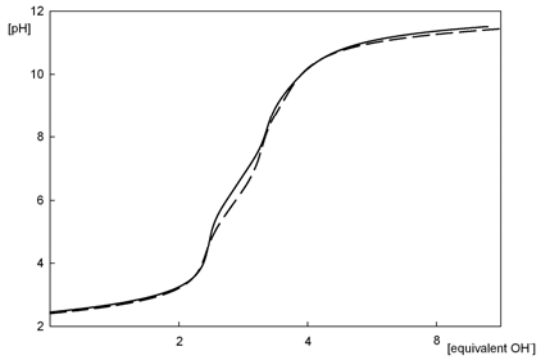


Figure 3 Titration curves of IP and IP/Spd systems. $c_{IP}=c_{Spd}=0.001M$
—— experimental titration curve of IP
– – – experimental titration curve of the IP/Spd system

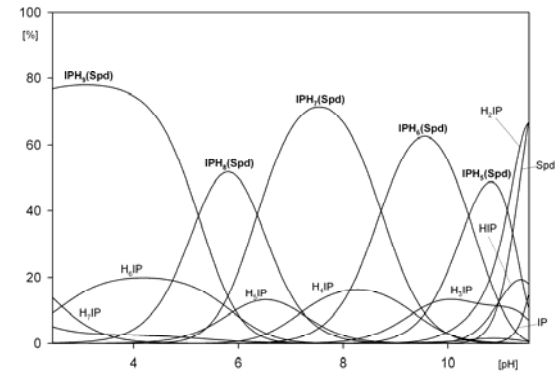


Figure 4 Calculated distribution diagram for IP/Spd system.
Percentage refers to total IP; [IP]=0.001M, [Spd]=0.001M

Table 2. The overall stability constants* (log β_{pqr}) and equilibrium constants (log K_e) for molecular complexes formed in the $A_p(IP)_qH_r$ systems

			log β_{pqr}								
pqr			en	tn	Put	dien	2,3-tri	3,3-tri	Spd	3,3,3-tet	Spm
112										27.58 (4)	28.70 (6)
113			38.88 (8)	39.28 (5)	39.42 (9)				39.39 (4)	38.61 (4)	40.17 (2)
114			48.99 (6)	50.16 (7)	50.63 (6)		50.32 (4)		49.14 (3)	49.77 (6)	51.25 (2)
115			58.50 (8)	60.44 (7)	60.89 (5)	58.60 (3)	59.87 (5)	60.06 (7)	59.72 (3)	60.25 (6)	61.85 (2)
116			66.93 (6)	68.90 (7)	69.23 (8)	66.76 (8)	69.42 (5)	70.03 (6)	69.11 (4)	70.25 (4)	72.29 (2)
117			73.45 (9)	74.96 (8)	75.77 (7)	73.62 (9)	77.51 (6)	78.10 (5)	78.72 (4)	79.69 (3)	81.27 (1)
118			79.08 (6)	79.83 (9)	80.62 (9)	79.94 (8)	83.02 (8)	83.60 (6)	84.62 (5)	86.96 (2)	88.34 (2)
119							87.31 (8)		91.64 (8)	92.27 (1)	93.84 (2)
			log K_e								
X(IP)	Y(A)	r	en	tn	Put	dien	2,3-tri	3,3-tri	Spd	3,3,3-tet	Spm
2	1	3	5.00	4.85	4.86				4.69	4.52	5.53
3	1	4	4.54	5.16	5.50		5.54		3.87	5.11	6.04
3	2	5			6.08				4.36		
4	1	5	4.71	6.10		5.02	5.76	5.94		6.25	7.30
4	2	6	5.84	5.62	5.08	4.08	5.88	6.17	4.41	6.22	7.37
4	3	7				6.64	7.71	6.43	5.47	7.04	7.24
5	2	7	5.17	4.49	4.43						
4	4	8								6.93	6.03
5	3	8				5.77	6.03	4.93	4.18		
6	2	8	4.90	3.47	3.39						
5	4	9								5.05	4.34
6	3	9					4.43		5.31		

*values in the parentheses refer to standard deviations σ given by SUPERQUAD

3.2.1. Formation of molecular complexes in the phytic acid/polyamine systems

As a result of non-covalent interaction, a several host-guest adducts are formed with different number of protons in the IP/A complexes. For the reaction of molecular complex formation:

$$H_xIP + H_yA \overset{K_e}{\rightleftharpoons} (IP)H_{x+y}A$$

their equilibrium constants (log K_e) were calculated by using r = x+y

$$K_e = [(IP)H_rA] / [H_xIP][H_yA]$$

$$\log K_e = \log \beta_{11r} - \log \beta_{01x} - \log \beta_{10y}$$

where β_{11r} β_{01x} , and β_{10y} are overall stability constant of $(IP)H_rA$ adducts, overall protonation constant of H_xIP, and overall protonation constant of H_yA species, respectively. The values of the equilibrium constant (K_e) correspond to adduct formation energy and indicate the tendency of ligands to form molecular complexes as well as allow to compare the character of adducts comprising different number of hydrogen atoms and different basicity of the ligands [43]. Among various combinations of x and y (2) assigned to each adduct, the x and y given smallest log *K*e values were cited. The calculated equilibrium constants are presented in Table 2.

With increase in the number of protons y from amine species (when the number of protons in IP is constant), the log K_e values increase as a result of amine positive charge increment (Table 2). For example, in (IP) H_r(en) systems log K_e : 4.71(y = 1) < 5.84 (y = 2). Moreover, with increase in the number of protons x from IP species (when the number of protons in polyamine is constant) the log K_e values decrease as a result of reduction in IP negative charge. For example, in $(IP)H_r$(2,3-tri) systems log K_e : 7.71(x = 4) > 6.03 (x = 5) > 4.43(x = 6*)*.

In the IP/en system, $(IP)H_4$(en), $(IP)H_5$(en), $(IP)H_6$(en), $(IP)H_7$(en) and $(IP)H_8$(en) adducts occur at pH<11, all with bioligands at the 1:1 ratio. The adduct formation begins already at low pH and at the physiological pH value the six-protonated complex binds over 80% of the substrates. Similar pattern of adduct formation is observed for other diamines, i.e. for tn and Put systems (supplemental Figure 2). At the physiological pH value, also the species $(IP)H_6A$ are dominant and bind almost 80% of the substrates. The pH ranges of formation of particular diamine complexes correspond to those of their practically complete protonation, and it indicates that diprotonated ligands with a charge +2 are involved in non-covalent interactions and adduct formation. At pH close to 11 the $(IP)H_4A$ adducts undergo decomposition, which corresponds to pH ranges of diamine deprotonation.

Molecular complexes of triamine start forming from pH below 2. Stability constants of adducts of the shortest triamine studied, i.e. dien, are lower than log β values obtained for other triamines of the same degree of protonation, which corresponds to lower basicity of dien. The lower efficiency of dien adduct formations is confirmed by the fact that adducts undergo decomposition at pH by about one unit lower than for other triamines. As we emphasized in conclusions concerning polyamine interaction with nucleoside, the structure (length) of particular polyamine is a significant factor which decides about the stability of a complex [44].

Molecular complexes of tetramines (3,3-tet and Spm) start forming from pH close to 2, however, the number of formed species is much greater than in the systems with di- and triamines. In the physiological medium, both phytic acid and polyamines are bound mainly in 5 – 7 H^+ complexes in 1:1 IP:A systems. The adducts of the lowest protonation degree $(IP)H_2$(tetramine) and $(IP)H_3$(tetramine) decompose at pH close to 12, which is, of course, a result of amine deprotonation.

The difference in the efficiency of the interactions depends on pH. Increasing pH increase the degree of polyamine protonation (positively charged centers) and decreasing pH increases phytic acid deprotonation (negatively charged centers). The plot of equilibrium constants log K_e (Table 2) vs. number of proton r in $(IP)H_rA$ showed the maximum (Figure 5). Large log K_e values for the $(IP)H_nA$ complexes are observed in the n range of 5 – 8 (5,6 : diamine, 7 : triamine, 5-8 : teramine) and it occurs at pH of about 7-9. The average value for $(IP)H_7$(triamine) adducts is by 2 units higher than that for $(IP)H_6$(triamine) and $(IP)H_8$(triamine). Most of highest values of log K_e are observed for those molecular complexes which are formed between H_4IP (the most stable conformation of IP) and fully protonated polyamine (en, triamines) except tn, Put and tetramines (Spm, 3,3,3-tet). The ionic form of the tetramines contain one NH which may bind H^+ of H_nIP to participate IP/polyamine adduct formation.

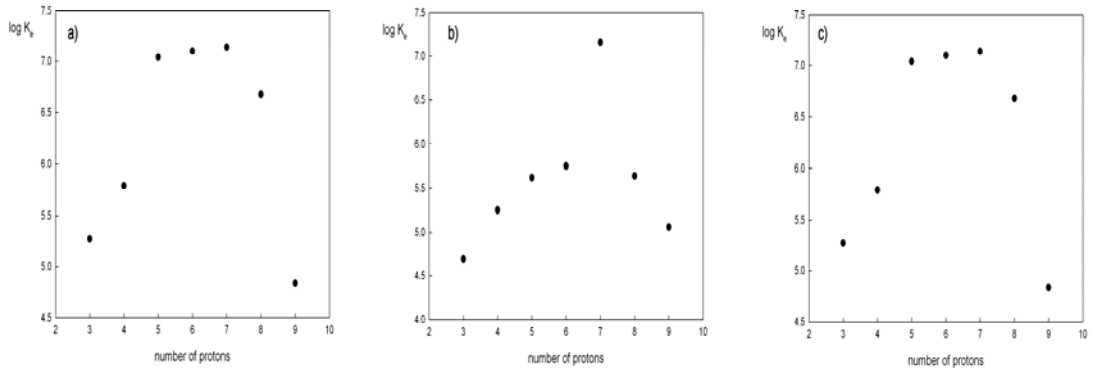


Figure 5 Average values of log K_e for IP/polyamine adducts *vs.* number of protons in the adducts.

a) diamines b) triamines c) tetramines

The values of the total basicity of the polyamine (ΣK_i) are linearly dependent on the number of amine groups in the molecule (only for complexes of the same component composition) (Figure 6). This correlation shows the adduct interaction is due to acid-base (base---H^+---base) interaction, that is, the groups involving with the high p*K*a in polyamines and IP are preferable for the adduct formation. Spm, whose p*K*a is higher than that of 3,3,3-tet, showed to shift the log K_e – n plot curve in (IP)H_n(Spm) to small n (Figure 9). In this point the natural polyamine, Put, Spd, Spm are excellent the base properties and showed wide range of the adduct species.

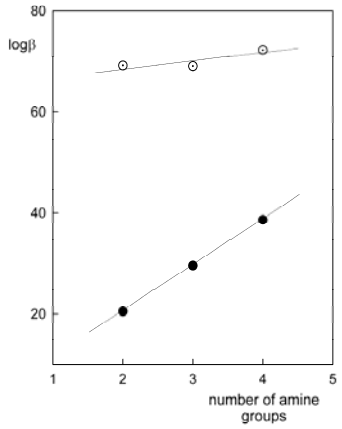


Figure 6 Overall protonation constants log β_{10n} ● of Put, Spd and Spm, and overall stability constants log β_{116} ○ of (IP)H_6(Put), (IP)H_6(Spd) and (IP)H_6(Spm) adducts *vs.* number of amine groups in polyamine n

The above discussed results suggest that the interactions are of the ion-H^+-ion type with positive centers at protonated – NH_x^+ groups of polyamines and negative centers at deprotonated phosphate groups from phytate. High lability of the first protons dissociating from the phosphate groups results in information of complexes at low pH values. The model proposed is strongly confirmed by the fact that the decomposition of all adducts occurs at pH of amine deprotonation. This observation is in agreement with results obtained for recently studied polyamine/nucleotide (or nucleoside) systems for which the effectiveness of the reaction, except for charge, also depends on the amine structure [22,23,33,43]. In the systems, the pH ranges of adduct formation correspond clearly to those of amine protonation and nucleotide (or nucleoside) deprotonation, which confirms the proposed model of the ion-ion type interaction. For example, pH range of uridine adduct formation (protonation constants for uridine (log K^H=9.22) is shifted

(relative to that of cytidine, log K^{H}=4.49) towards higher pH values, which is a result of changes in the degree of the polyamine protonation [45]. Formation of negatively charged reaction sites at the phosphate groups causes that the nucleotide complexes occur at pH values much lower than those of the occurrence of nucleoside complexes [22], as it is in the case of the systems with IP.

3.2. 2 ^{31}P NMR study of phytic acid/polyamine system

Plots of four ^{31}P signals *vs*. pH (supplemental Figure 3 and Table 1) showed most of ^{31}P signals moved to highfield Δδ (-0.2 - 2.5ppm) in the presence of Spm. The signal movement should be due to the IP-Spm adduct formation and may be explained by two steps : (1) interaction of Spm NH---H^+---O^-P, (2) H^+ rearrangement from interacted PO_3H to non-interacted PO_3 due to the competition with ligand donor. At pH 11.5 Δδ (ppm) = 0.1(P1,3), 0.0(P2), 1.9(P4,6), 0.0(P5) indicated Spm binding at P1,2,3,5 in 5ax1eq IP. Δδ (ppm) = 0.2(P1,3), 0.2(P2), 0.6(P4,6), 0.3(P5) at pH 5 showed major P1,2,3 binding in 5eq1ax IP. P2 may be a good target for Spm binding because P2 has a unique steric configuration and elongated P2-P1,3 distance makes Spm NH/NH2 interaction preferable. This steric requirement also adapts Mg coordination at P2-P1,3 (Figure 7, *vide infra*). The peak broadings due to 5ax1eq ⟸ 1ax5eq conformational exchange were observed at pH 8 for IP, and pH 11 for IP-Spm (supplemental Figure 4). From the IP broading at pH 8 and the species distribution of H_nIP (Figure 2) preferable conformations of IP are 5ax1eq of $H_{2,3}IP$ and 5eq1ax of $H_{5\text{-}7}IP$. This may be explained by $–OPO_3^{2-}$---H^+---$^{2-}O_3PO$- interaction in 5ax1eq (P1-P3, P4-P6) and in 5eq1ax (P3-P4-P5-P6-P1) (supplemental Figure 5). In the presence of Spm the pH shift from 8 to 11 showed Spm preferably bound 5eq1ax IP below pH 11 (Figure 7) and it is in accordnace with the fact that the maximum log K_e were observed in $IPH_{5\text{-}7}$(Spm) presented in pH 7 – 10.5 (Table 2).

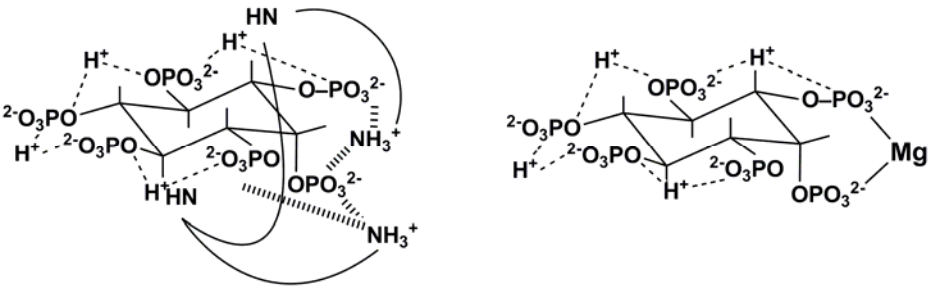


Figure 7 Possible interaction modes in $IP(H_6)Spm$ and $Mg(IP)H_4$

3.3. Equilibrium study of complex formation in polyamine/metal ion systems

Nucleic acid, a big anion, should be associated with cation such as polyamines and metal ions. Crystal structure of phe t-RNA revealed both spermine (Spm) and Mg incorporated in RNA formed high ordered structure [47]. The Mg ions belong to hard acids and show a high affinity to hard bases, hence its great effectiveness in formation of complexes with oxygen donors from IP molecules. Therefore metal ions should be regarded as potential competitors to polyamines in their reactions with IP and related compounds such as DNA and phosphorylated protein in living organisms. Since there is no study of the binding difference between polyamine and Mg in solution, we studied the complex formation of Mg-IP system.

Stability constants of complexes log β_{pqr}, determined from computer-aided analysis of potentiometric data of systems studied, Mg/IP ($Mg_p(IP)_qH_r$ complexes), are shown in Table 3. Log K_e values were evaluated by replacement of polyamine A with M. Not only 1:1 Mg:IP but also n:1 Mg:IP (n=2-4.5) study showed the presence of polynuclear Mgp(IP)Hr (p=2, 3) complexes. Phytate tends to form multinuclear complexes $M_p(IP)H_r$, where p=2 and 3, which is undoubtedly associated with large number of donor atoms in the ligand molecule.

Table 3. Stability constants log β_{pqr}* for $Mg_p(IP)_qH_r$ complexes

pqr	log β_{pqr}	pqr	log β_{pqr}	log K_e
012	23.029(3)	115	56.19 (2)	4.33
013	34.409(2)	114	49.94 (1)	5.56
014	44.382(2)	113	42.43 (2)	8.02
015	51.859(2)	112	33.04 (3)	10.01
016	57.701(2)	213	46.49 (3)	
017	60.948(2)	212	38.10 (4)	
		311	33.50 (3)	
		310	23.50 (6)	

* values in the parentheses refer to standard deviations σ given by SUPERQUAD

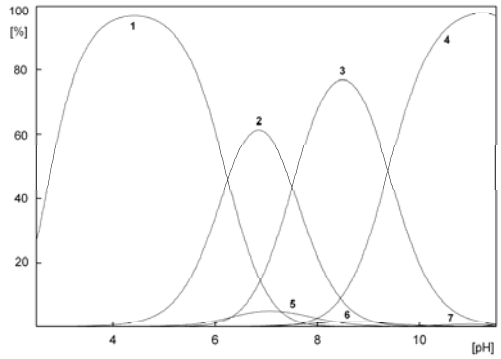


Figure 8 Distribution diagram for Mg/IP complexes.
1–$Mg(IP)H_5$, 2–$Mg(IP)H_4$, 3–$Mg(IP)H_3$, 4–$Mg(IP)H_2$, 5–$Mg_2(IP)H_3$, 6–$Mg_2(IP)H_2$, 7–$Mg_3(IP)H$;
[IP]=0.001 M, [Mg]=0.0005 M, Percentage refers to total metal.

Calculated distribution diagrams of complexes formed was shown in Figure 8. Low values of IP protonation constants cause that complexes begin to form at lower pH < 3 and no free Mg was observed in pH >5. In the 1:1 Mg/IP system the dominant complexes in neutral pH is $Mg(IP)H_4$, and its relative fraction is about 60%, which is analogous to that in the reaction between H_4IP and polyamine. Since log K_1 = 1.57 in monodentate CH_3OPO_3-Mg complex [41], large value of log K_1 (=log β_{114}-log β_{014}) = 5.56 in IP-Mg complex indicated that Mg is above bidentate, however there is no report of bidentate mode in Mg-phosphate ester coordination, probably the phosphate bridged coordination $-OPO_3^{2-}$-Mg^{2+}-$^{2-}O_3PO-$ at P-2 and P-1(P-3) is preferable because $4H^+$ in H_4IP bind other phosphates (P-3(1), 4, 5, 6) and form the hydrogen bond $-OPO_3^{2-}$---H^+---$^{2-}O_3PO-$ in 5eq1ax which stabilizes the $Mg(IP)H_4$ complex (Figures 8).

The charge of phosphate mono-anion in $Mg(IP)H_4$ complex is similar to that of phosphodiester moiety in nucleic acid. We may look the property difference of the binding between Mg and polyamine in nucleic acid by comparing the stability constants in IP system. The values of log K_e for the adducts $IP(H_{4-8})$Spm (6.0-7.4(H_6)) were larger than log K_e value of $Mg(IP)H_4$ complexes (5.6) (Figure 9). On the other hand the value for $IP(H_{4-8})$Spd (3.9-5.5(H_7)) was similar or below that of the Mg complex, that is, the stabilization order of phosphate anion : Spm (4N) > Mg ≥ Spd (3N). Thus the non-covalent interaction involving 4N of Spm was over Mg coordination. however, that of 3N Spd was not over Mg coordination. These selectivity in phosphate binding indicated a vital role of stabilizing nucleic acid structure by polyamine.

$Mg(IP)H_4$

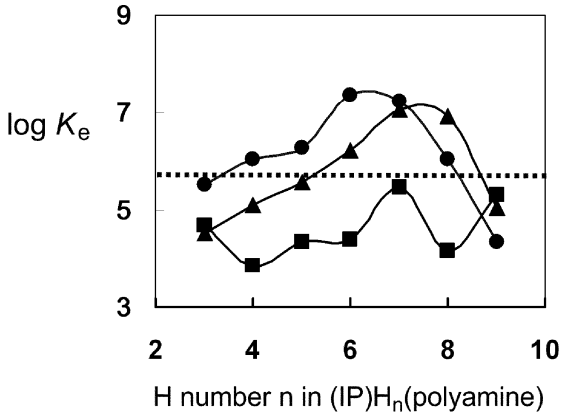


Figure 9 Comparison of equilibrium constants for IP/polyamine adducts with those for Mg(IP)H_4 complexes.
● - IP/Spm system, ■ - IP/Spm system, ▲ - IP/3,3,3-tet system, --- Mg(IP)H4 complex

4. Conclusions

By comparing equilibrium constants of formation of IP/polyamine adducts with those for metal/IP complexes and by taking into consideration the character of noncovalent bonds, one can suggest that the interaction between IP and polyamine is of polydentate type. Such polydentate effect as well as the chelate effect makes the interaction to be strong and the interaction of big polyamine such as 4N Spm is over Mg coordination. However, from log K_e-structure of many polyamines relation the polydentate effect did not show the large dependence of the fused ring number which is typical in the coordinated chelate ring. Large dependence of the proton number n on adduct formation constant log K_e in (IP)H_n(A) complex indicated the importance of the positive charge, that is, the polyamine NH_3^+ showed the strong interaction but the NH_2 state did not show the typical interaction. Such difference of the interaction is also shown in polyamine dependence of log K_e values. Since the actual structure of IP is involved with proton and polyamine in equilibria, the difference of the interaction between the NH_3^+ and the H^+ may be small. In conclusion the effectiveness of the polyamine on the phosphate-amine interaction is based on non-selective polydentate character.

Acknowledgements

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References

1 F. Grases, B.M. Simonet, I. Vucenik, R.M. Prieto, A. Costa-Bauza, J.G. March and A.M. Shamsuddin, BioFactors, 2001, **15**, 53-61;

2 P. Ekholm, L. Virkki, M. Ylonen and L. Johansson, Food Chemistry, 2003, **80**, 165-170;

3 Qing-Chuan Chen and B.W. Li, J. Chromatogr. A, 2003, **1018**, 41-52;

4 L. Oatway, T. Vasanthan and J.H. Helm, Food Rev. Int., 2001, **17**, 419-431;

5 A. Bebot-Brigout, C. Dange, N. Fauconnier and C. Gerrard, J. Inorg. Biochem., 1999, **75**, 71-78;

6 R.T. Hawkins, D.R. Poyner, T.R. Jackson, A.J. Letcher, D.A. Lander and R.F. Irvine, Biochem. J., 1993, **294**, 929-934;

7 B.F. Harland and G. Narula, Nutr. Res., 1999, **19**, 947-961;

8 G. Urbano, M. Lopez-Jurado, P. Aranda, C. Vidal-Valverde, E. Tenorio and J. Porres, J. Physiol. Biochem., 2000, **56**, 283-294;

9 K. Midorikawa, M. Murata, S. Oikawa, Y. Hiraku, and S. Kawanishi, Bioch. Biophys. Res. Comm., 2001, **288**, 552-557;

10 R.M. Burger, S.B. Horwitz and J.Peisach, Biochemistry, 1985, **24**, 3623-3629;

11 P.K. Gupta, J. Singh and R. Bharathi, Nutrition and Cancer, 2003, **46**, 66-72;

12 L.A. Hanakahi and S.C. West, EMBO J., 2002, **21**, 2038-2044;

13 M. R. Mecbeth, H. L. Schubert, A. P. VanDemark, A. T. Lingam, C. P. Hill and B. L.Bass, Science, 2005, **309**, 1534-1539.

14 A. Saiardi, R. Bhandari, A. C. Resnick, A. M. Snowman and S. H. Snyder, SCIENCE, 2004, ***306***, 2101-2105.

15 V. Chukkapalli, I. B. Hogue, V. Boyko, W.S. Hu and A. Ono, J. Virol., 2008, **82**, 2405-2417;

16 C.J. Martin and W.J. Evans, J. Inorg. Biochem., 1986, **26**, 169-183;

17 C.J. Martin and W.J. Evans, J. Inorg. Biochem., 1986, **28**, 39-55;

18 C. Brigando, J.C. Mossoyan, F. Favier and D. Benlian, J. Chem. Soc. Dalton Trans. 1995, 575-578;

19 C.W. Tabor and H. Tabor, Ann. Rev. Biochem., 1984, **53**, 749-790;

20 Progress in Polyamine Research, V. Zappia, A.E. Pegg (Eds), Plenum Press, NY, 1988;

21 L. Lomozik, in Handbook of Metal-Ligand Interactions in Biological Fluids, G. Berthon (Ed), Marcel Dekker, NY, vol. I, p. 686;

22 L. Lomozik, A. Gasowska and R. Bregier-Jarzebowska, J. Inorg. Biochem., 2004, **98**, 1319-1330;

23 L. Lomozik, A. Gasowska and L. Bolewski, J. Chem. Soc. Perkin Trans. 2, 1997, 1161-1165;

24 M. Felemez and B. Spiess, J. Inorg. Biochem., 2001, **84**, 107-111;

25 M.H. Irving, M.G. Miles and L.D. Pettit, Anal. Chim. Acta, 1967, **38**, 475-488;

26 T. Yajima, R. Takamido, Y. Shimazaki, A. Odani, Y. Nakabayashi, O. Yamauchi, Dalton, **2007**, 299-307.

27 P. Gans, A. Sabatini and V. Vacca, J. Chem. Soc. Dalton Trans., 1985, 1195-1200;

28 N. Ingri, W. Kakolowicz, L.G. Sillen and B. Warqvist, Talanta, 1967, **14**, 1261-1286;

29 L. Lomozik, M. Jaskolski and A. Wojciechowska, Polish J. Chem., 1991, **65**, 1797-1807;

30 P.K. Glasoe and F.A. Long, J. Phys. Chem., 1960, **64**, 188-190;

31G.R. Hedwig and H.K.J. Powell, J. Chem. Soc. Dalton Trans., 1973, 793-797;

32 K. Mernnisi-Arifi, I. Imbs, G. Schlewe and B. Spiess, Biochim. Biophys. Acta, 1996, **1289**, 404-410;

33 L. Lomozik and A. Gasowska, J. Inorg. Biochem., 1998, **72**, 37-47;

34 N. Li, O. Wahlberg, I. Puigdomenech and L.O. Ohman, Acta Chem. Scan., 1989, **43**, 331-339;

35 C. De Stefano, D. Milea and S. Sammartano, J. Chem. Eng. Data, 2003, **48**, 114-119;

36 H. Bieth and B. Spiess, J. Chem. Soc. Faraday Trans. 1, 1986, **82**, 1935-1943;

37 W.J. Evans, E.J. McCourtney and R.I.Shrager, J. Am. Oil Chem. Soc., 1982, **59**, 189-191;

38 C. Brigando, J.C. Mossoyan, F. Favier and D. Benlian, J. Chem. Soc. Dalton Trans., 1995, 575-578;

39 C. De Stefano, D. Milea, A. Pettignano and S. Sammartano, Anal. Bioanal. Chem., 2003, **376**, 1030-1040;

40 C. De Stefano, O. Giuffre, D. Milea, C. Rigano and S. Sammartano, Chem. Spec. Bioavail., 2002, **15**, 29-36;

41 A. Saha, N. Saha, L.-n Ji, J. Zhao, F. Gregan, S. Ali A. Sajadi, B. Song and H. Sigel, J. Biol. Inorg. Chem., 1996, **1**, 231-238;

42 A.J.R. Costello, T. Glonek and A.C. Myers, Carbohydr. Res., 1976, **46**, 159-171;

43 L. Lomozik, A. Gasowska and L. Bolewski, J. Inorg. Biochem., 1996, **63**, 191-206;

44 Ma Yunmei and M.R. Lieber, J. Biol. Chem., 2002, **277**, 10756-10759;

45 L. Lomozik and R. Jastrzab, J. Inorg. Biochem., 2003, **93**, 132-140;

46 G.E.Blank, J.Pletcher and M.Sax, Acta Crystallogra., 1975, **B31**, 2584-2592.

47 L. Jovine, S. Djordjevic and D. Rhodes, J. Mol. Biol., 2000, **301**, 401-414.

Figure Captions

Figure 1. Formulae of the ligands

Figure 2 Distribution curves of particular species of IP/H^+. [IP]=0.001M

Figure 3 Titration curves of IP and IP/Spd systems. $c_{IP}=c_{Spd}=0.001$M
—— experimental titration curve of IP
- - - experimental titration curve of the IP/Spd system

Figure 4 Calculated distribution diagram for IP/Spd system.
Percentage refers to total IP; [IP]=0.001M, [Spd]=0.001M

Figure 5 Average values of log K_e for IP/polyamine adducts *vs.* number of protons in the adducts.

a) diamines b) triamines c) tetramines

Figure 6 Overall protonation constants log β_{10n} [●] of Put, Spd and Spm, and overall stability constants log β_{116} [○] of $(IP)H_6$(Put), $(IP)H_6$(Spd) and $(IP)H_6$(Spm) adducts *vs.* number of amine groups in polyamine n

Figure 7 Possible interaction modes in $IP(H_6)$Spm and $Mg(IP)H_4$

Figure 8 Distribution diagram for Mg/IP complexes.

1–$Mg(IP)H_5$, 2–$Mg(IP)H_4$, 3–$Mg(IP)H_3$, 4–$Mg(IP)H_2$, 5–$Mg_2(IP)H_3$, 6–$Mg_2(IP)H_2$, 7–$Mg_3(IP)H$;
[IP]=0.001 M, [Mg]=0.0005 M, Percentage refers to total metal.

Figure 9 Comparison of equilibrium constants for IP/polyamine adducts with those for $Mg(IP)H_4$ complexes.

● - IP/Spm system, ■ - IP/Spm system, ▲ - IP/3,3,3-tet system, --- Mg(IP)H4 complex

Table 1. Overall protonation constant* log β_{10n} of polyamines A, log β_{01n} of IP and successive protonation constant log K_n

compound	log β_{101}**	log β_{102}	log K_2	log β_{103}	log K_3	log β_{104}	log K_4
en	10.15 (2)	17.45 (2)	7.30				
tn	10.70 (2)	19.64 (2)	8.96				
Put	10.83 (1)	20.51 (2)	9.68				
dien	9.94 (2)	19.04 (2)	9.10	23.34 (3)	4.30		
2,3-tri	10.47 (1)	19.90 (1)	9.43	26.16 (1)	6.26		
3,3-tri	10.48 (2)	20.22 (2)	9.74	28.03 (3)	7.81		
Spd	10.97 (1)	21.03 (1)	10.06	29.61 (2)	8.58		
3,3,3-tet	10.36 (2)	20.38 (3)	10.02	29.01 (3)	8.63	36.39 (4)	7.38
Spm	10.91 (2)	21.28 (2)	10.37	30.39 (3)	9.11	38.67 (3)	8.28
IP	log β_{012}	log β_{013}	log β_{014}	log β_{015}	log β_{016}	log β_{017}	
	23.73 (1)	34.30 (3)	43.64 (2)	50.83 (2)	56.72 (1)	59.34 (1)	
	log K_1 log K_2	log K_3	log K_4	log K_5	log K_6	log K_7	log $K_{8\text{-}12}$
	~12	10.57	9.34	7.18	5.89	2.63	<2

*values in the parentheses refer to standard deviations σ given by SUPERQUAD
** log β_{101} = log K_1

Table 2. The overall stability constants* (log β_{pqr}) and equilibrium constants (log K_e) for molecular complexes formed in the $A_p(IP)_qH_r$ systems

			log β_{pqr}								
pqr			en	tn	Put	dien	2,3-tri	3,3-tri	Spd	3,3,3-tet	Spm
112										27.58 (4)	28.70 (6)
113			38.88 (8)	39.28 (5)	39.42 (9)				39.39 (4)	38.61 (4)	40.17 (2)
114			48.99 (6)	50.16 (7)	50.63 (6)		50.32 (4)		49.14 (3)	49.77 (6)	51.25 (2)
115			58.50 (8)	60.44 (7)	60.89 (5)	58.60 (3)	59.87 (5)	60.06 (7)	59.72 (3)	60.25 (6)	61.85 (2)
116			66.93 (6)	68.90 (7)	69.23 (8)	66.76 (8)	69.42 (5)	70.03 (6)	69.11 (4)	70.25 (4)	72.29 (2)
117			73.45 (9)	74.96 (8)	75.77 (7)	73.62 (9)	77.51 (6)	78.10 (5)	78.72 (4)	79.69 (3)	81.27 (1)
118			79.08 (6)	79.83 (9)	80.62 (9)	79.94 (8)	83.02 (8)	83.60 (6)	84.62 (5)	86.96 (2)	88.34 (2)
119							87.31 (8)		91.64 (8)	92.27 (1)	93.84 (2)
			log K_e								
X(IP)	Y(A)	r	en	tn	Put	dien	2,3-tri	3,3-tri	Spd	3,3,3-tet	Spm
2	1	3	5.00	4.85	4.86				4.69	4.52	5.53
3	1	4	4.54	5.16	5.50		5.54		3.87	5.11	6.04
3	2	5			6.08				4.36		
4	1	5	4.71	6.10		5.02	5.76	5.94		6.25	7.30
4	2	6	5.84	5.62	5.08	4.08	5.88	6.17	4.41	6.22	7.37
4	3	7				6.64	7.71	6.43	5.47	7.04	7.24
5	2	7	5.17	4.49	4.43						
4	4	8								6.93	6.03
5	3	8				5.77	6.03	4.93	4.18		
6	2	8	4.90	3.47	3.39						
5	4	9								5.05	4.34
6	3	9					4.43		5.31		

*values in the parentheses refer to standard deviations σ given by SUPERQUAD

Table 3. Stability constants log β_{pqr}* for $Mg_p(IP)_qH_r$ complexes

pqr	log β_{pqr}	pqr	log β_{pqr}	log K_e
012	23.029(3)	115	56.19 (2)	4.33
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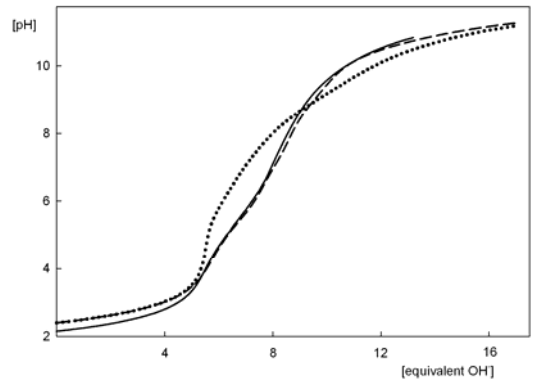
015	51.859(2)	112	33.04 (3)	10.01
016	57.701(2)	213	46.49 (3)	
017	60.948(2)	212	38.10 (4)	
		311	33.50 (3)	
		310	23.50 (6)	

* values in the parentheses refer to standard deviations σ given by SUPERQUAD

Supplement

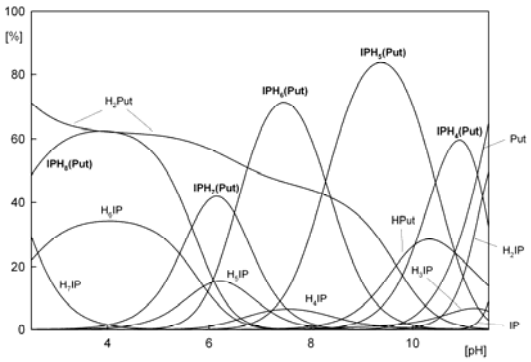


Supplemental Figure 1 Experimental and simulated titration curves for IP/Spm system. $c_{IP}=c_{Spm}=0.001M$

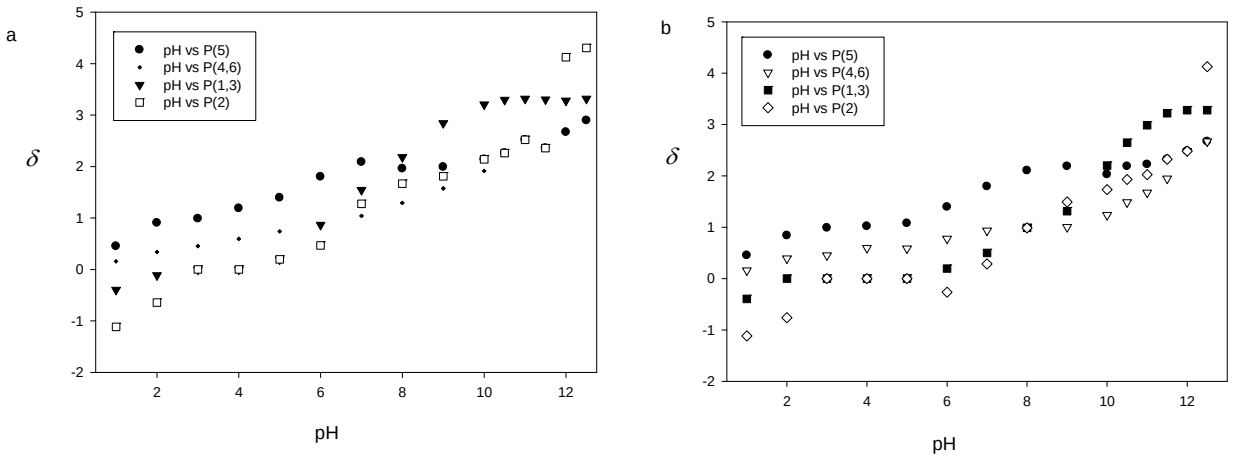
——— experimental titration curve for IP/Spm system

····· simulated titration curve for IP/Spm system; adduct formation was not taken into account

– – – – simulated titration curve for IP/Spm system; adduct formation was taken into account

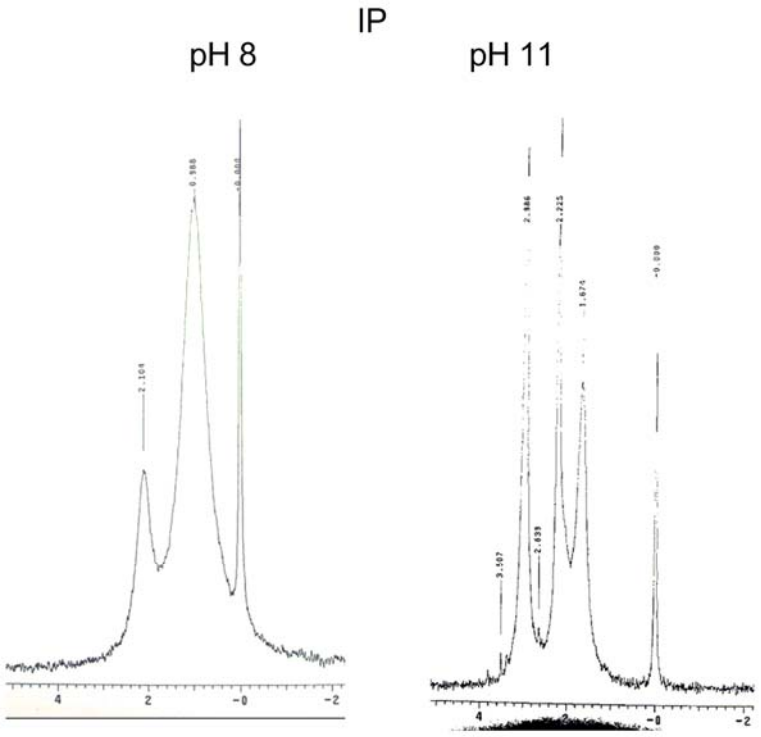


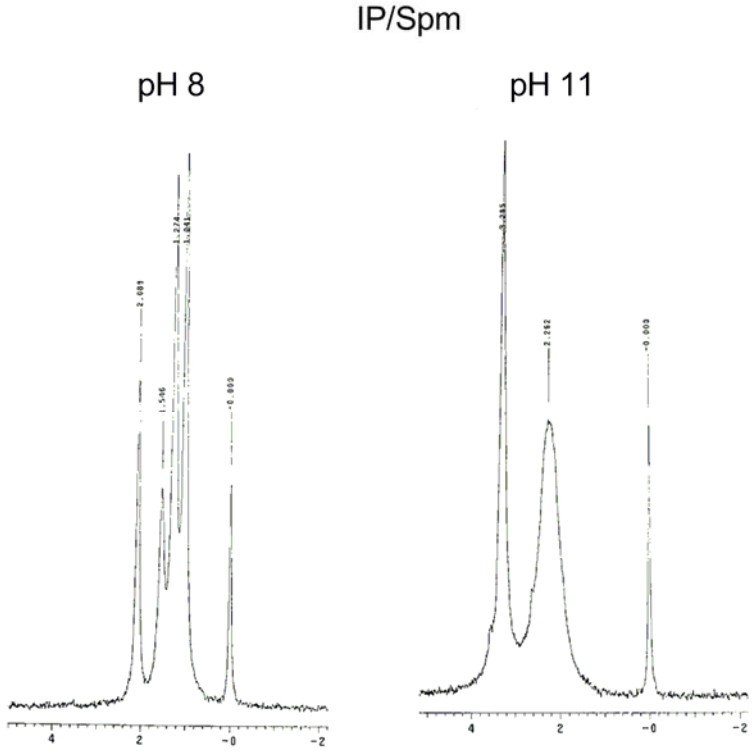
Supplemental Figure 2 Distribution diagram for IP/Put system. Percentage refers to total IP; $c_{IP}=0.001M$, $c_{Put}=0.001M$

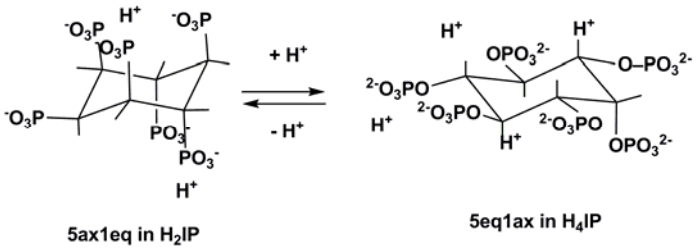


Supplemental Figure 3 Plot δ in ^{31}P-NMR vs. pH for a) H_n(IP) and (IP)H_n(Spm) systems

Supplemental Figure 4 ^{31}P NMR spectra for IP and IP/Spm systems







Supplemental Figure 5 Possible preffered conformations of 5ax1eq in H_2IP and 5eq1ax in H_4IP

Supplemental Table 1 ^{31}NMR shifts for IP and IP/Spm systems

IP system

pH	P(5)	P(4,6)	P(1,3)	P(2)
1	0.596	0.287	-0.256	-0.95
3	--	0.437	0	0
4	--	0.596	0	0
5	1.395	0.739	0.196	0.196
6	1.802	0.867	0.468	0.468
7	2.089	1.041	1.546	1.274
8	1.961	1.292	2.184	1.667
9	1.991	1.576	2.843	1.81
10	2.142	1.915	3.205	2.142
10.5	2.262	2.262	3.295	2.262
11	2.519	2.519	3.318	2.519
11.5	2.36	2.36	3.303	2.36
12	2.67	2.67	3.28	4.125
12.5	2.896	2.896	3.318	4.306

IP/Spm

pH	P(5)spm	P(4,6)spm	P(1,3)spm	P(2)spm
1	0.452	0.158	-0.395	-1.116
3	0.988	0.452	0	0
4	--	--	0	0
5	1.078	0.588	0	0
6	1.395	0.777	0.196	-0.264
7	1.795	0.935	0.498	0.287
8	2.104	0.988	0.988	0.988
9	2.187	1.003	1.312	1.493
10	2.029	1.237	2.202	1.734
10.5	2.187	1.486	2.647	1.931
11	2.225	1.674	2.986	2.025
11.5	2.323	1.946	3.22	2.323
12	2.481	2.481	3.28	2.481
12.5	2.67	2.67	3.28	4.125

Difference(IP-IP/Spm)

pH	P(5)	P(4,6)	P(1,3)	P(2)
1	0.144	0.158	0.139	0.166
3		0.452	0	0
4		--	0	0
5	0.317	0.588	0.196	0.196
6	0.407	0.777	0.272	0.732
7	0.294	0.935	1.048	0.987
8	-0.143	0.988	1.196	0.679
9	-0.196	1.003	1.531	0.317
10	0.113	1.237	1.003	0.408
10.5	0.075	1.486	0.648	0.331
11	0.294	1.674	0.332	0.494
11.5	0.037	1.946	0.083	0.037
12	0.189	2.481	0	1.644
12.5	0.226	2.67	0.038	0.181

Supplemental Table 2 IP and IP/Spm experimental condition for ^{31}P NMR Measurment

number	V_{sample} [cm^3]	$V_{[(Et)4N]OH}$ [cm^3]	mol of IP	mol of $[(Et)_4N]OH$	pH
IP					
1.	21	---	0.00066990	---	0.86
2.	20.1	0.930	0.00064119	0.0022599	1.98
3.	19.1	1.251	0.00060929	0.0030399	2.99
4.	18.1	1.414	0.00057739	0.0034360	4.33
5.	17.1	1.468	0.00054549	0.0035672	4.99
6.	16.1	1.600	0.00051359	0.0038880	5.87
7.	15.1	1.780	0.00048169	0.0043254	6.93
8.	14.1	1.920	0.00044979	0.0046656	7.85
9.	13.1	2.030	0.00041789	0.0049329	8.92
10.	12.1	2.140	0.00038599	0.0052002	9.82
11.	11.1	2.235	0.00035409	0.0054310	10.47
12.	10.1	2.325	0.00032219	0.0056498	10.96
13.	9.1	2.445	0.00029029	0.0059414	11.51
14.	8.1	2.545	0.00025839	0.0061844	11.98
15.	7.1	2.645	0.00022649	0.0064274	12.50
IP/Spm					
16.	21	---	0.0006699	---	0.96
17.	20	0.771	0.0006380	0.0018735	1.99
18.	19	0.970	0.0006061	0.0023571	2.97
19.	18	1.115	0.0005742	0.0027094	4.29
20.	17	1.220	0.0005423	0.0029646	4.97
21.	16	1.375	0.0005104	0.0033412	5.86
22.	15	1.526	0.0004785	0.0037082	6.92
23.	14	1.630	0.0004466	0.0039609	7.86
24.	13	1.770	0.0004147	0.0043011	8.94
25.	12	1.920	0.0003828	0.0046656	9.86
26.	11	2.070	0.0003509	0.0050301	10.51
27.	10	2.181	0.0003190	0.0052998	10.95
28.	9	2.330	0.0002871	0.0056619	11.47
29.	8	2.480	0.0002552	0.0060264	11.99
30.	7	2.615	0.0002233	0.0063544	12.49

the content with a illustration.

Polyamines bind myo-inositoltetrakisphosphate through polyamine-H^+-phosphate interaction. Stability constant for Spermine interaction being higher than Mg coordination indicates polyamine biological significance.