Heterometallic manganese/zinc-phytate complex as a model compound for metal storage in wheat grains

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Abstract

Myo-inositol-1,2,3,4,5,6-hexakisphosphate, also known as phytate, is a natural metal chelate present in cereals, an important feedstock worldwide. This article reports the characterization of three metal storage model complexes: the homometallic Mn(II) myo-inositol-1,2,3,4,5,6-hexakisphosphate (IP6), the heterometallic Zn(II), Mn(II) analogue Na4MnZn4(C6H6O24P6)⋅(NO3)2⋅8H2O (MnZn4IP6) and the homometallic Zn(II) metal complex Na3Zn5(C6H6O24P6)OH⋅9H2O (Zn5IP6). The techniques of high-resolution 23Na, 13C and 31P NMR, electron paramagnetic resonance (EPR) and X-ray photoelectron spectroscopy (XPS) were applied in this study. The complexation of Zn(II) and Mn(II) by phosphate groups of IP6 is demonstrated by NMR and XPS results. 13C NMR results show a conformation for IP6 consisting of five equatorial phosphate groups to one axial group showing only one chemical environment for Zn and two for Mn, when characterized by XPS and EPR, in both Mn complexes. These results support, for the first time, a probable supramacromolecular structure for phytate complexes of transition metals. Based on the similarity between the EPR spectra of wheat seeds and that of the MnZn4IP6 compound, the manganese storage centers in wheat grains can be assigned to similar heterometallic phytate complexes.

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1. Introduction

The myo-inositol-1,2,3,4,5,6-hexakisphosphate (IP6), phytate, is the major phosphorous component of plants and special cereal grains [1,2]. Phytate has long been known as an antinutritional factor since it binds nutrient mineral cations, especially from milk products, making them unavailable for nourishing the body and therefore interfering in their homeostasis [3–6]. Phytate is present in several foods and especially large values have been
found in roasted peanuts, dry roasted cashews, shredded wheat cereals and wheat flour [7]. Therefore, phytate is of concern in diets based on cereals and nuts. Interest in these inositol phosphate compounds has increased since it has been proposed that they have roles as an antioxidant [8], as a secondary messenger and DNA repair agent [9] and for treatment of calcium oxalate renal lithiasis [10].

In plants, phytic acid is responsible for 50–80% of the total phosphorous content [11] and therefore plays an important role in phosphorus storage. In cereal seeds, phytic acid is mainly located in globoid agglomerates in the aleuronic layer region [11]. The interest in studying the interaction of the manganese ion with phytate is due to the important micronutrient role of this metal for plants, and to the fact that phytate can play a role for manganese homeostasis in plants. Few authors have reported data on the molecular structure of transition metal phytate complexes. Gerard et al. [12] reported a tetrahedral structure for soluble Zn(II) phytate and cobalt phytate complexes based on potentiometric studies, $^{31}$P NMR and UV–visible spectroscopy. Pierce [13] reported enthalpic results obtained from Differential Scanning Calorimetric and thermogravimetric analysis of non-crystalline insoluble complexes of polynuclear Zn(II) phytate with water coordinated to the metal centers. The isolated Zn(II) phytate had a Zn(II):IP6 ratio of 5:1.

In this paper, we report the synthesis and characterization of the insoluble Zn(II) and Mn(II) complexes of phytate as model complexes for manganese storage in wheat seeds. To investigate the chemical structure of the MnZn$_4$IP$_6$, Mn$_5$IP$_6$ and Zn$_5$IP$_6$ model compounds, the following techniques were applied: elemental analysis, infrared absorption spectroscopy (FT-IR), multinuclear magnetic resonance spectroscopy with magic angle spinning (MAS NMR), X-ray photoelectron spectroscopy (XPS), and electron paramagnetic resonance (EPR). In addition, EPR analyses of wheat seeds and flour from different parts of the world (Brazil and Denmark) are reported to help identify possible manganese storage compounds in cereals.

2. Experimental

2.1. Materials

Dodecasodium phytate, IP$_6$, was used as supplied from Sigma (St. Louis, USA). Manganese perchlorate was supplied from Aldrich (St. Louis, USA), triethylamine was supplied by Acros (Geel, Belgium), and Zn(II) nitrate was supplied by Vetec (São Paulo, Brazil). The water used for the synthesis was de-ionized water with a resistivity of 18 MΩ. Methanol was supplied by Vetec (São Paulo, Brazil).

The Brazilian wheat seed was kindly supplied by EMBRAPA Trigo, Rio Grande do Sul. The Danish wheat seed were winter variety (Bunker for biscuits) kindly supplied by the Department of Food Science, Cereal Group of The Royal Veterinary and Agricultural University (KVL), Frederiksberg, Denmark. The wheat flour obtained from a special wheat varietal for biscuits was provided by Biska (Hiørring, Denmark).

2.2. Syntheses

2.2.1. Manganese phytate synthesis, $(\text{Na}_3\text{Mn}_5(C_6\text{H}_6\text{O}_{24}\text{P}_6)\text{OH} \cdot 9\text{H}_2\text{O}), \text{Mn}_5\text{IP}_6$

Two hundred fifty milligrams (0.25 g) of dodecasodium phytate (0.27 mmol) were diluted in 21 mL of a methanol:de-ionized water mixture (20:1, v:v). Then, 0.20 mL of triethylamine and 10.0 mL of methanol solution of Mn(ClO$_4$)$_2 \cdot 6$H$_2$O (0.27 g, 0.75 mmol) were added, under stirring and heating at $\sim$60 °C for 3 h. The solution pH during the synthesis was between 7 and 8. A dark brown precipitate was collected by vacuum filtration. The yield of Na$_3$Mn$_5$(C$_6$H$_6$O$_{24}$P$_6$)OH $\cdot$ 9H$_2$O was 63% based on manganese perchlorate.

2.2.2. Zinc phytate synthesis, Na$_3$Zn$_5$(C$_6$H$_6$O$_{24}$P$_6$)OH $\cdot$ 9H$_2$O, Zn$_5$IP$_6$

Two hundred fifty milligrams (0.25 g) of dodecasodium phytate (0.27 mmol) were diluted in 40 mL of methanol:de-ionized water (1:1, v:v). Afterwards, 0.20 mL of triethylamine and 0.27 g of Zn(NO$_3$)$_2 \cdot 6$H$_2$O (1.2 mmol) were added, under continuous stirring and heating at $\sim$60 °C for 3 h. The solution pH during the synthesis was between 7 and 8. A white powder was precipitated and was collected by vacuum filtration. The yield of Na$_3$Zn$_5$(C$_6$H$_6$O$_{24}$P$_6$)OH $\cdot$ 9H$_2$O was 70% based on zinc nitrate.

2.2.3. Heterometallic zinc/manganese phytate synthesis, Na$_4$MnZn$_4$(C$_6$H$_6$O$_{24}$P$_6$)$_6$ $\cdot$ (NO$_3$)$_2$ $\cdot$ 8H$_2$O, MnZn$_4$IP$_6$

The heterometallic compound was prepared in a similar procedure to that used for the Na$_3$Zn$_4$(C$_6$H$_6$O$_{24}$P$_6$)$_6$OH $\cdot$ 9H$_2$O by mixing 0.27 g of Zn(NO$_3$)$_2 \cdot 6$H$_2$O (1.2 mmol) and 0.0018 g (5.0 μmol) of Mn(ClO$_4$)$_2 \cdot 6$H$_2$O. The solution pH during the synthesis was between 7 and 8. The Na$_4$MnZn$_4$(C$_6$H$_6$O$_{24}$P$_6$)$_6$ $\cdot$ (NO$_3$)$_2$ $\cdot$ 8H$_2$O yield was 70% based on manganese perchlorate.

Each synthesis has been repeated and the data reported herein are the average of several measurements.

2.3. Elemental analysis

All samples were dried under vacuum, ≈626 mmHg, at 353 K for 2 h in a MA 030, MARCONI oven (Piracicaba, Brazil). They were analyzed in an EA1110 CHNS-O, CE INSTRUMENTS elemental analyzer equipment (Milan, Italy).
2.4. Magic angle spinning nuclear magnetic resonance

High-resolution solid-state $^{13}$C NMR measurements were carried out in a Varian Unity INOVA spectrometer at a field of 9.4 T, using a 7 mm magic angle spinning (MAS) probe. Samples were spun up to 7 kHz in silicon nitride rotors. Spectra were measured with a single pulse excitation and also by $^1$H-$^{13}$C cross-polarization (CP) technique. The CP experiment was carried out using a $\pi/2$ $^1$H pulse of 3.5 $\mu$s, contact time of 1 ms, recycle delay of 30 s and $^1$H decoupling of 80 kHz. In single pulse experiments a $\pi/2$ pulse of 3.5 $\mu$s was used with recycle delay of 30 s and $^1$H decoupling of 80 kHz. Also, the delayed $^1$H decoupling technique was used to suppress non-quaternary carbon signals, using a delay time of 30 $\mu$s between the end of the excitation pulse and the beginning of the decoupling irradiation [14]. $^{31}$P NMR experiments were carried out using a $\pi/2$ pulse of 4.0 $\mu$s, recycle delay of 400 s and $^1$H decoupling. $^{23}$Na NMR experiments were carried out using a $\pi/16$ pulse of 0.5 $\mu$s, recycle delay of 2 s, and no $^1$H decoupling. References for chemical shift of $^{13}$C, $^{31}$P and $^{23}$Na were TMS, 85% $\text{H}_3\text{PO}_4$ and 0.1 M NaCl, respectively.

2.5. X-ray photoelectron spectroscopy

The XPS measurements were performed in an OHMNICROM spectrometer using non-monochromatic Al K$\alpha$ radiation (1486.6 eV, power of 140 W) and a hemispherical analyser with pass energy of 44 eV. The base pressure in the chamber was in the range of $10^{-9}$ Torr. The spectra were charge-corrected using the Na1s binding energy at 1071.3 eV for Na$_3$PO$_4$ [15] as well as the Cls at 286.6 eV for myoinositol [16]. The atomic ratios were calculated using the Scofield cross-sections [17]. All XPS peaks of insulating samples were fitted according to the Leclercq and Pireaux’s method, using Gaussian–Lorentzian peaks [18].

2.6. Infrared absorption spectroscopy

The infrared absorption spectra of the samples were acquired in the transmission mode using KBr pellets of the samples at weight-to-weight ratio of 1:100. The samples were previously dried under vacuum at 353 K for 1 h. The spectra were obtained in a BOMEM MB-102 spectrophotometer (Quebec, Canada).

2.7. Electron paramagnetic resonance

The solid-state EPR spectra were recorded using previously dried samples in a quartz tube in an X-band CW ESP 300E Bruker (Karlsruhe, Germany) spectrometer. The modulation amplitude was 5.1 G for the wheat grains or 0.4 G for the model compounds, modulation frequency of 100 kHz. All spectra were run at room temperature unless otherwise noted.

3. Results

3.1. Electron paramagnetic resonance spectroscopy

Six-line Mn(II) EPR signals have not only been observed in wheat kernels [19], but also in several plant derived foods such as coconut milk [20], apples [21], green tea [22,23], raspberry [24], and coffee [25]. Similar spectra have also been observed in several types of human tissues and bodily fluids [26].

The spectra of flours from Biska (Denmark) and wheat seeds from Denmark and Brazil showed the same features, a weak free radical signal at $g = 2.00$, $\Delta H_{pp} = 6$ G, probably due to nitrogen-centered radicals in the gluten formed by peptide scission [27] and a more intense broad line centered around $g = 2$ with a sextet signal attributed to Mn(II) (Fig. 1(a) and (c)). This sextet signal is typical of Mn(II) ions, which have a sextet hyperfine splitting due to the $I = 5/2$ nuclear spin of the $^{55}$Mn nucleus. The husks of the seeds showed a higher intensity for the sextet lines than for the embryo part. Based on the literature [11], we supposed the Mn(II) compound to be a manganese-phytate complex and therefore a model complex was prepared in vitro. This manganese-phytate complex (Mn$_4$IP$_6$) showed an EPR spectrum with an inhomogeneous line broadened by magnetic dipole–dipole interactions between the Mn(II) centers, $\Delta H_{pp} = (644 \pm 80)$ G (Figure S1). We decided to synthesize a heterometallic Zn/Mn species (MnZn$_4$-IP$_6$) as phytate has been claimed to be associated
with Zn(II), calcium and manganese in cereals [11] and also because the Zn(II) would magnetically dilute the manganese, preventing dipolar interactions thus reducing the line broadening. The spectrum obtained from the heterometallic species, MnZn_{4}IP_{6}, (Fig. 1(b)), displays a Mn(II) hyperfine line pattern very similar to the signal observed for the wheat seeds and flours except for the absence of the free radical signal.

The spectrum of MnZn_{4}IP_{6} is similar to that of the two flours except for the absence of a free radical signal. The sextet portion of the three spectra is very similar but the intensity of the broad line relative to the sextet appears to be larger in the flour samples. Unless the zero field interaction is very small, the observed sextet feature is due to the central fine structure line, \( M_{S} = -1/2 \) to 1/2 transition. The four other fine structure lines constitute part or all of the broader line.

The Mn(II) is an \( ^{8}\text{S} \) ion with \( S = 5/2 \) whose EPR spectra is normally fitted to the spin Hamiltonian

\[
H = \beta_{B} [g_{s} S_{z} H_{s} + g_{s} S_{x} H_{x} + g_{s} S_{y} H_{y}] + D \left( S_{z}^{2} - \frac{35}{12} \right) + E [S_{x}^{2} - S_{y}^{2}] + [A_{xx} S_{x} I_{x} + A_{yy} S_{y} I_{y} + A_{zz} S_{z} I_{z}],
\]

where \( H \) is the magnetic field, \( S \) is the electron spin operator, \( I \) is the nuclear spin operator, and \( \beta_{B} \) is the Bohr magneton. The \( g_{s} \), \( A_{ii} \), \( D \) and \( E \) parameters are spin Hamiltonian parameters to be determined experimentally. For Mn(II), an \( ^{8}\text{S} \) state ion, \( A \) and \( g \) parameters are generally isotropic with values of 85–95 G and 2.00, respectively. By definition, the magnitude of the zero field parameter \( D \) is due to the central fine structure line, \( M_{S} = \pm 1/2 \leftrightarrow \pm 3/2 \) and \( \pm 3/2 \leftrightarrow \pm 5/2 \) transitions. We presumed these peaks are further broadened due to different Mn(II) sites on the IP_{6} ring with different values of \( D \) and \( E \). Since the center sextet is affected by the second-order term in \( D \) and \( E \) while the other fine structure lines are affected by a first-order term, a 10% change in \( D \) will only produce a 1% change in the center sextet.

We have used a simulation program obtained from Dr. Hogni Weihe (Department of Chemistry, University of Copenhagen,Universitetsparken 5, DK 2100 Copenhagen, Denmark, weih@kiku.dk), which can produce simulations using the spin Hamiltonian given in Eq. (1) with a personal computer in a DOS environment, to simulate the spectrum of MnZn_{4}IP_{6}. Indeed, if we assumed only one species of Mn(II), it was not possible to simulate the spectrum (Figure S2). The narrow sextet which is unevenly spaced and the presence of more than one line in the second, third and fourth hyperfine lines, counting from the left, in the spectrum could be reasonably simulated using the parameters of \( D = 0.014 \text{ cm}^{-1} \), \( E = 0.00467 \text{ cm}^{-1} \), \( A = 95 \text{ G} \), \( g = 2.003 \), with a line-width parameter of 42 G and a Lorentzian line shape. The line-width parameter is the width of the absorption line at half height. The success of this simulation showed us that the odd splitting pattern and the presence of more than six lines was not due to two separate species but rather to the presence of electron spin–nuclear spin double flips and the consequent distortion of intensities in the “allowed transitions”. This simulation did not, however, explain the broad tails and the extra width of the outside pair of hyperfine lines. Further, it produced narrow peaks for the Fine structure lines due to \( M_{S} = \pm 1/2 \leftrightarrow \pm 3/2 \) and \( \pm 3/2 \leftrightarrow \pm 5/2 \) transitions. We evaluated this effect by simulating three lines with \( D = 0.015, 0.014 \) and 0.013 cm\(^{-1}\) with \( E = D/3 \) and then averaged the three spectra (Figure S2A). This effectively broadened the peaks in the tail without affecting the center sextet but did not improve the simulation of the tails which really required a larger \( D \) value and larger line-width parameter. Therefore, we simulated a spectrum for \( D = 0.02 \text{ cm}^{-1} \) and \( E = 0.00667 \text{ cm}^{-1} \) with the same \( g \) and \( A \) values but with a line-width parameter of 200 G (Figure S2B). In order to estimate the relative concentration of these Mn species in the MnZn_{4}IP_{6} complex we used the area under the EPR absorption. The ratio between the two types of Mn from EPR experimental spectrum was 1:2. Then, we combined the two simulations in the ratio of one part of the averaged narrow line to 2 parts of the broad line to get the simulation shown in Fig. 2. The simulation seems good enough to conclude the spectrum is due to two main types of Mn(II).

![Fig. 2. Comparison of simulated EPR signal using parameters described in the text to that of the experimental spectrum for MnZn_{4}IP.](image-url)
3.2. Elemental analysis

The C, H and N content determined by elemental analysis of the Mn5IP6, and XPS atomic ratio from the XPS high-resolution results, Table 1, suggest that the Na3Mn5(C6H6O24P6)OH·9H2O molecular formula. The results for the Zn5IP6 and MnZn4IP6 suggest that the molecular formulas Na3Zn5(C6H6O24P6)OH·9H2O and Na4MnZn4(C6H6O24P6)·(NO3)2·8H2O, respectively.

3.3. Infrared absorption spectroscopy

The FT-IR spectra of the Mn5IP6, Zn5IP6 and MnZn4IP6 showed the δO–P–O, νP=O, νP–O and νP–O–C peaks in the same region at (541.5 ± 2.0), (1392.0 ± 4.0), (848.0 ± 4.0), (993.5 ± 4.0) and (1124.0 ± 3.0) cm⁻¹, respectively (Figure S3). The νP=O and νP–O are closer than observed in the dodecasodium phytate and the δO–P–O is shifted toward lower wave number showing the transition metals are coordinated to the phosphate group of phytic acid, R–OPO2⁻3. The decrease in the separation between the νP=O and νP–O is symptomatic of a probable bidentate coordination [30].

3.4. Solid-state NMR

Fig. 3(a) shows the 13C NMR spectrum of IP6, composed of two partially resolved lines at 74 and 67 ppm with a respective intensity ratio of 5:1. These values are compatible with the chemical shift measured for sodium aqueous solutions of IP6 at high pH [31], where the molecular conformation has 5 axial and 1 equatorial phosphate groups (5-ax/1-eq). For the Zn5IP6 compound the 13C signal has the two peaks at about 74 and 67 ppm similar to the IP6 in high pH (Fig. 3(b)). Thus, 13C NMR confirms the presence of the IP6 skeleton in both compounds with a 5-ax/1-eq conformation. The line at 67 ppm can be attributed to the carbon corresponding to the axial phosphate group. On the other hand, a 13C signal was not observed for MnZn4IP6 compound, most probably due to the paramagnetism of Mn(II) ions.

Fig. 4 shows the 31P NMR spectra of the three compounds. The 31P spectrum of IP6 has one symmetric peak at 7.2 ppm with line-width of 5 ppm. According to the solution NMR results, the separation between the six 31P resonances is less than 1 ppm, which precludes the resolution of these sites in the solid state [31]. On the other hand, the 31P MAS NMR spectrum of Zn5IP6 has three peaks at 6.1, 2.6 and −1.3 ppm with line-widths of about 1.5 ppm and respective intensity ratios of 1:3:2. These values for multiplicity and intensity ratio of the NMR lines indicate the presence of three non-equivalent P-sites per molecule. The values of chemical shift of these lines are more shielded than in IP6, which is an expected behavior when ions with stronger cationic potential are brought to the second coordination sphere of P(V) [32]. This is precisely the case for Zn(II) substituting Na. Also, this kind of substitution has consequences on the nuclear magnetic dipole–dipole interaction of P with neighboring nuclei. In the solid state, 23Na can establish dipolar interaction with 31P, because both species have non-zero nuclear spin. The 23Na isotope is 100% abundant, has I = 3/2 spin and a moderate gyromagnetic ratio γ. This heteronuclear dipolar coupling between 31P and 23Na produces some residual broadening of the NMR lines.

On the other hand, the only isotope of Zn(II) with non-zero spin is 67Zn, which is only 4% abundant and has a low γ, about a quarter of that for 23Na. For these reasons, the dipolar coupling with 31P is negligible.
Therefore, the substitution of Na by Zn(II) in the neighborhood of P will reduce the line-width of the NMR lines, as was observed in Fig. 4(b). Therefore, from these results we can infer the existence of a close coordination of Zn(II) ions around the IP6 molecule, causing the non-equivalence of P-sites through modifications in the molecular conformation, the shifting of the resonances due to electronic effects and the line narrowing by reducing the heteronuclear dipolar interaction. In contrast, the $^{31}$P NMR spectrum of MnZn$_4$IP6 consists of a single and very broad asymmetric peak at 4 ppm with line-width of 21 ppm, resulting from paramagnetic effects (Fig. 4(c)). Not much information can be drawn from this broad line, except that Mn(II) species should be rather close to the phytate molecule.

The $^{23}$Na NMR spectra of IP6 and Zn$_5$IP6 compounds are shown in Fig. 5. No NMR signal could be recorded for MnZn$_4$IP6, due to paramagnetic effects from Mn ions. The IP6 MAS and static spectra has a broad signal centered at $-8.3$ ppm with line-width of 14 ppm (Fig. 5(a) and (b)). The asymmetric line-shape may be associated to the overlapping of several site resonances and/or to the effect of second-order electric quadrupole interaction effects, which can also be dynamically modulated by diffusion-like motion of Na ions. The $^{23}$Na spectrum of the Zn$_4$IP6 compound has two signals at $-2.2$ and $-6.9$ ppm, with respective line-widths of 12 and 0.8 ppm (Fig. 5(c)). The relative intensity of the narrower line is 3%. The extremely small line-width value, equivalent to $83$ Hz, indicates the absence of second-order quadrupolar broadening. Therefore, these Na ions occupy crystal sites of cubic symmetry, which has a zero electric field gradient, or alternatively undergo fast diffusion causing motional narrowing of the quadrupolar broadening. We believe that the former hypothesis is more probable, as will be discussed later. A static NMR measurement in Zn$_5$IP6 (Fig. 5(d)) showed a featureless resonance with line-width of 16 ppm, very similar to the value of 12 ppm measured for the line centered at $-2.2$ ppm line in the MAS spectrum (Fig. 5(d)). This result indicates that Na ions contributing to the $-2.2$ ppm line are undergoing rapid motions, causing an efficient averaging of the anisotropic parts of the chemical shift, quadrupolar and dipolar homonuclear interactions. The MAS averaging reduces the residual anisotropy only by 25%. On the other hand, the line of minor intensity is not resolved in the static spectrum, pointing out that there exists some anisotropic broadening which is substantially
averaged out by MAS. This observation led us to propose that this narrow resonance corresponds to Na sites with low electric field gradient, giving negligible second-order quadrupolar broadening.

The anisotropic effects shown by the static experiment are associated with chemical shift interactions. Therefore, the peak position at −6.9 ppm should be attributed essentially to the isotropic chemical shift and could be assigned to Na species in cubic symmetry. These Na ions would be loosely bonded to IP6, probably in the second sphere of coordination as hydrated counter ions. The other peak would be related to Na ions more tightly bonded to phosphate groups resulting in lower symmetry and a broader line-width. Potentiometric studies in aqueous solution of phytic acid with different alkaline metals have shown a strong influence on the pKₐ of this species, for instance, by formation of relatively strong complexes with phytic acid [33].

3.5. X-ray photoelectron spectroscopy

XP-spectrum of the Zn2p₃/₂ region reveals for Zn₅IP₆ and MnZn₄IP₆ a symmetric peak with binding energy at 1024.2 eV. Fig. 6 shows a representative spectrum of Zn₅IP₆. The symmetric peak is characteristic of only one chemical environment for Zn(II) in both complexes. The binding energy is typical of Zn(II) bound to a phosphate group [24].

The Mn2p XP-spectra of Mn₅IP₆ and MnZn₄IP₆ species show asymmetric doublets that have been fitted with two Gaussian–Lorentzian doublet functions and one singlet function, only the Mn₅IP₆ spectrum is shown in Fig. 7, for illustration.

The presence of two doublets confirms the EPR hypothesis of two different chemical environments for Mn in Mn₅IP₆ and MnZn₄IP₆. Unfortunately, the Mn2p₃/₂ binding energy value for these compounds, 641.4 and 643.5 eV, as well as the spin–orbit splitting in the Mn2p photoelectric peaks are not helpful in spectral assignment, since different Mn valence states have approximately the same parameters in similar chemical environments [16]. However, the presence of the shake-up satellite at 646.0 eV is an evidence of one Mn(II) species, at least, since the same is not observed for Mn(III) or Mn(IV). Petruska and Talham [35] reported Mn2p₃/₂ binding energies at 643.1 eV for the manganese phosphate Mn[O₃POC₃₀H₄₇ON₂], where manganese is linked to the phosphate group in a bidentate mode. Therefore, we can assign the second doublet also to a Mn(II) in a similar chemical environment as in the Mn[O₃POC₃₀H₄₇ON₂]. The other Mn(II) species must have a slightly different environment. Based on the lower binding energy value, 641.4 eV, observed, we assign the third doublet to a species where only one phosphate group is present and the coordination sphere is completed with water or hydroxide ions as suggested in the molecular formula. Indeed, in the Mn(OH)O compound the Mn2p₃/₂ binding energy was found at 641.7 eV [16]. The coordination of water or hydroxide would result in a shift of the binding energy toward lower values as compared to species where Mn(II) is bound to two phosphate groups.

The XP-spectra of the complexes Mn₅IP₆, Zn₅IP₆ and MnZn₄IP₆ in the P2p region showed an asymmetric peak that was fitted as two doublets with doublet separation for 2p₃/₂ and 2p₁/₂ peaks of 0.84 eV (Fig. 8). The binding energy value for the 2p₃/₂ components are: (a) Mn₅IP₆: 133.1 and 134.7 eV; (b) Zn₅IP₆ and...
MnZn₄IP₆: 136.9 and 136.2 eV, respectively. Similar values were reported for Zr[O₃POC₁₀H₂₁S] and Mn[O₃POC₃₀H₄₇N₂] [35,36].

The XP-O1s photoelectric lines for all compounds showed asymmetric line-shapes that were fitted with three Gaussian–Lorentzian functions with binding energy values of (530.2 ± 0.3), (532.1 ± 0.3) and (534.2 ± 0.3) eV assigned to P–O–C, P–O–Zn(II) or P–O–Mn(II) and P=O, and possibly P–O–P or shake-up satellite, respectively (Fig. 9) [34,37]. Water molecules coordinated to transition metal complexes have a typical value for O1s binding energy of 532 eV, and therefore, could not be distinguished from oxygen in the P–O-Metal bond [15,16]. The P–O–P bonding could be formed by dehydration of two phytic acids in a similar way to phosphates, however, no other photoelectric line or spectroscopic result supports this assignment, therefore, we believe the peak is due to a shake-up satellite.

4. Discussion

Elemental analysis and XPS both point to the same molecular formula where the metal-to-phytate ratio is ca. of 5:1 in agreement with previously reported data [12,13]. The phytate molecule in the metal phytates discussed in this article is in the 5 axial/1 equatorial conformation according to the ³¹P results. This is in agreement with the reported results in solution which shows 5 axial/1 equatorial conformation to be the more stable one in basic pH. The vibrational spectroscopic data and the XP-P2p results indicate a bidentate coordination mode; however, XP-P2p and XP-O1s results point to two different phosphate groups in the complexes probably resulting from the presence of P–O–Na groups. ³¹P MAS NMR analysis of the line-widths suggest that Na⁺ anions are more loosely bound to phosphate in transition metal phytates than in dodecasodium phytate, occupying probably the third coordination sphere of P(V).

Based on the XP-Zn2p results only one Zn coordination environment is observed in Zn₄IP₆ and MnZn₄IP₆, however Mn has two different Zn environments in Mn₅IP₆. The presence of P–O–P groups is ruled out by NMR data, so the higher binding energy mode is probably due to a shake-up satellite of the phosphate group. The simulation of EPR spectra for the MnZn₄IP₆ results in two sets of Mn(II)-phytate environments, the first one with a larger zero field interaction and magnetic dipolar broadening and the second one with a smaller zero field and dipolar broadening.

Using van Vleck’s equation [38] for second moments to estimate line broadening, we can estimate an average distance between Mn(II) to be 8 Å for the first environment and 13 Å for the second environment. It was inferred that the first environment has two Mn(II) in the same phytate ring while the second environment has only one Mn(II) per ring.

Based on these results and the low solubility of these compounds in polar solvents like water, acetone, dimethylformamide and dimethylsulfoxide, we propose a supramacromolecular structure for the compounds as represented in Fig. 10. The solubility for all compounds in water at 353 K is ca. of 83 μM.

Monomeric species of manganese or Zn(II) phytate have been studied in aqueous solution by potentiometry, since these species are soluble. The presence of bridging Mn as shown in Fig. 10 would result in a large increase in the molecular weight and a decrease of total net charge resulting in a loss of solubility. Also, the presence of bridging and non-bridging Mn(II) species would explain the presence of two components in the XP-Mn2p
spectrum. The same is not observed in Zn(II) compounds probably because the 2p binding energy of this metal is not as sensitive as Mn2p. The proposed structure is in agreement with the EPR results and the broad line would be due to vicinal Mn(II).

5. Conclusion

The manganese complex in wheat seeds from Brazil and Denmark contain a heterometallic diamagnetic transition metal manganese phytate complex, probably containing Zn(II). A model compound of Zn(II) and manganese phytate was synthesized and spectroscopically characterized for the first time as Na₄MnZn(II)₄(C₆H₅O₂₃P₆)·(NO₃)₂·8H₂O. In addition, other model Zn(II) phytate and manganese phytate complexes have been synthesized as Na₃Mn₅(C₆H₅O₂₃P₆)OH·9H₂O and Na₃Zn(II)₅(C₆H₅O₂₃P₆)OH·9H₂O. In the manganese compounds, it was possible to identify two different chemical environments for the Mn(II) species, the first one coordinated to more than one phosphate and the other to only one phosphate group of phytate, IP₆. These different chemical environments can lead to different chemical reactivity of the Mn in Mn₅IP₆ and metal bioavailability. Further studies on the redox reactivity and structure of Mn₅IP₆ complexes could also show their role as anti-oxidant natural compounds related to the presence of two different redox centers.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jinorgbio.2005.06.014.

References


