The Interaction of the Vanadyl(IV) Cation with Phytic Acid

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ABSTRACT

The interactions of VO^{2+} with phytate to form both soluble and insoluble complexes, have been studied by electronic absorption spectroscopy. A soluble $1:1$ VO^{2+}: phytate complex is formed at pH < 1. At higher pH-values insoluble complexes are produced. Two different solid complexes, obtained respectively at pH $= 2$ and 4, were isolated and characterized. The maximal bonding ratio of VO^{2+}:phytate was found to be 4, on the basis of a pH binding profile.

Index Entries: Vanadyl(IV)-phytate complexes; effect of the pH value; soluble and insoluble complexes; spectroscopic characterization.

INTRODUCTION

The nutritional importance of phytic acid (mio-inositol hexaphosphate) stems in part on its tremendous chelating potential, which produces the formation of insoluble complexes with polyvalent metal cations and thereby affects its bioavailability. There is an extensive, and often conflicting, literature on this subject (for example (1–4) and references therein).

Phytic acid (Fig. 1) is a dodecaprotic acid, with six pK_a values around 1.5 and the other six ranging from 2 to 12 (5).

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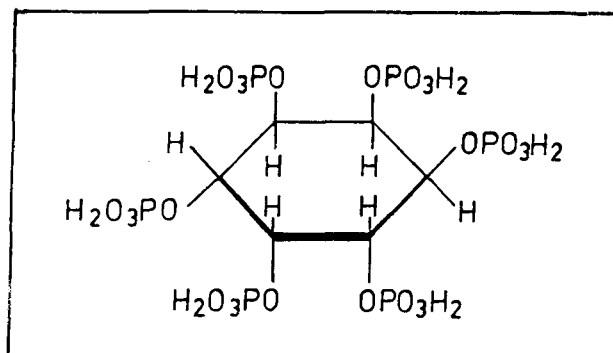$$H_2O_3PO \qquad OPO_3H_2$$

Fig. 1.   Schematic representation of the phytic acid molecule.

In the last years, a number of studies on the interaction of phytic acid with various essential metal ions have been undertaken (2,3,6–13).

As part of our current research on different aspects of the inorganic biochemistry of vanadium, we have now investigated the interaction of phytic acid with the vanadyl(IV) cation VO^{2+}, which is one of the most relevant vanadium species present in biological systems (14–16). This investigation also extends previous studies on the interaction of the same cation with other phosphate containing molecules of biological interest (17–22).

MATERIAL AND METHODS

The dodecasodium salt of phytic acid (Sigma) and $VOSO_4{\cdot}5H_2O$ (Merck) were used as supplied.

Electronic absorption spectra were obtained with a Shimadzu UV-300 spectrophotometer using 1 cm-quartz cells. Diffuse reflectance spectra of the solid complexes were registered with the same instrument, using MgO as a standard. Infrared spectra were obtained with a Perkin Elmer 580 B spectrophotometer using the KBr pellet technique.

The solid complexes were obtained by mixing aqueous solutions of VO^{2+} and phytate (phy) in a 6:1 molar ratio in buffered solutions (KCl/HCl, pH-2 and sodium acetate/acetic acid, pH = 4). The light blue solids were collected on a sintered glass funnel, washed with cold water, and dried in a vacuum desiccator over $CaCl_2$.

RESULTS AND DISCUSSION

VO^{2+} Phytate Interactions in Aqueous Solutions

Electronic Spectra

At pH > 1 a mixture of VO^{2+} and phytate in a 6:1 molar ratio began to precipitate. Solution studies were, therefore, performed at pH = 1.

The visible electronic spectra of VO^{2+} and VO^{2-}/phy solutions are shown in Fig. 2. The shift of the electronic bands to higher wavenumbers (from 625/763 to 650/810 nm) is consistent with the coordination of VO^{2+} through phosphate groups (*17,21*).

The crystal field parameter 10Dq, which can be obtained directly from the higher energy band (*23*) has a value of 15385 cm^{-1}, which is slightly lower than that observed in the VO^{2+}/thiamine diphosphate system (*20*).

Stoichiometry of the Complex at pH = 1

In order to determine the stoichiometry of the generated complex, we have used the continuous variation's method (Job's method) (*24*). A series of solutions with the mole fraction of the ligand, X, ranging from X = 0 to X = 1, were prepared and absorbance measurements performed at 800 nm.

As both, the metal ion and the complex, present great absorption at this wavelength but the ligand does not, we introduce a new quantity, $A_{calc.}$, defined as: $A_{calc.} = A_{obs.} - A_{theor.}$ (with $A_{theor.}$: the theoretical absorbance owing to the metal alone, at the selected wavelength). The results are presented in Table 1 and a plot of $A_{calc.}$ against the mole fraction of the ligand X, is shown as an inset in Fig. 2. As can be seen, the maximum of this plot is obtained for X = 0.5, indicating the formation of a 1:1 complex between VO^{2+} and phytate at pH = 1.

Interaction of VO²⁺ and Phytate at Different pH Values

At pH > 1 insoluble complexes were formed with a decrease of the absorbance of the remaining solution (A_M). In the absence of ligand, no turbidity appeared up to pH = 4 and the absorbance (A'_M) remains constant.

According to Martin and Evans (*6,8,9*) the mols of VO^{2+} bound per mole of phytate can be determined from the relation:

$$\text{mols } VO^{2+} \text{ bound} = 6 \left[1 - \frac{A_M}{A'_M} \right]$$

where, as indicated, A_M is the molar absorptivity of the VO^{2+}/phytate species (after centrifugation and separation of the precipitate) and A'_M refers to the metal solution without phytate. The details of the analysis are given in Table 2 and the binding profile is shown in Fig. 3. For comparative purposes in this figure, similar plots for the respective calcium, copper and cobalt complexes, adapted from (*9*), are also shown.

An apparent saturation is observed at pH > 2 with four VO^{2+} cations bound to phytate. Martin et al. (*6,8,9,12,25*) found that the mols of cations bound to one mole of ligand are five for Ca(II) and Mn(II), four for Fe(III) and Zn(II), and six for Ni(II), Co(II) and Cu(II).

The conformation of phytic acid is pH dependent (*6,26*). At pH below 5, phytate exists in the so-called "equatorial form" (cyclohexane
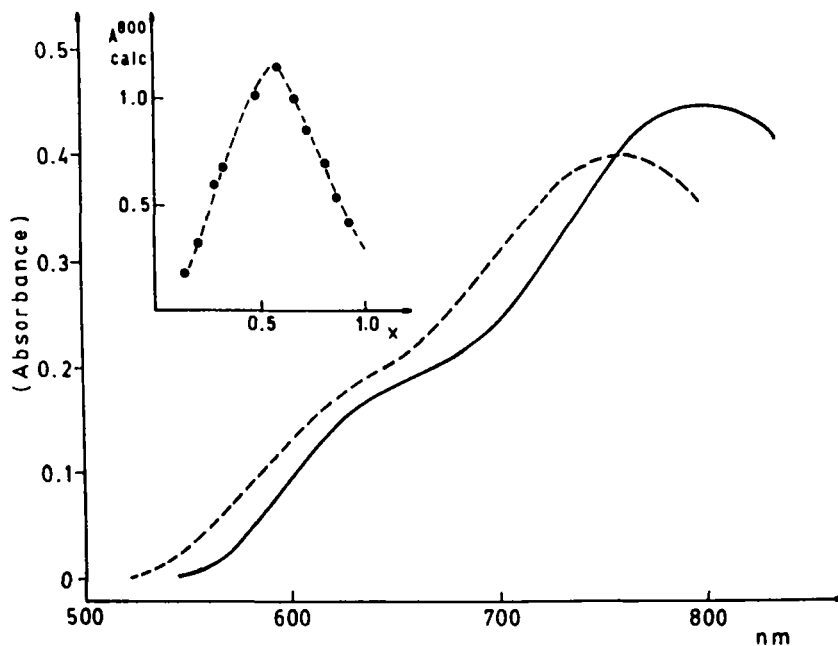
Fig. 2.   Electronic spectra of an aqueous VO^{2+} solution (-----)
and a 1:1 VO^{2+}/phy solution at pH $= 1$ (———); $[VO^{2+}] = 2.5 \times$
$10^{-2}M$. (Inset: Job's plot at pH $= 1$; the ordinate is $A_{calc} \times 10$ (cf. Table
1)).

Table 1
Analysis of the VO^{2+}/Phytate System
by Job's Method

X	A_{calc}
0.13	0.018
0.20	0.033
0.27	0.061
0.33	0.067
0.50	0.105
0.60	0.116
0.67	0.100
0.73	0.086
0.83	0.071
0.87	0.053
0.93	0.042

$[VO^{2+}] = [phy] = 0.05M$; pH $= 1$; $\lambda = 800$ nm

ring in the chair form, five phosphate groups in equatorial position and
the remainder one, in axial position), leading to an open structure that
allows the formation of soluble complexes at low pH-values.

The analogy of the VO^{2+} binding profile with those of Cu(II) and
Co(II), suggests that all these cations bind to this "equatorial form" (cf.
also (7,9)).

Table 2
Calculation of VO^{2+} mols Bound Per Mol Phytate

pH	A_M	A_M	$6[1 - A_M/A_M']$
1.30	0.720	0.973	1.56
1.65	0.580	0.995	2.50
2.00	0.460	0.997	3.23
2.40	0.408	0.997	3.55
3.00	0.432	0.997	3.40
3.50	0.429	0.991	3.40
4.00	0.393	1.009	3.68
4.50	0.362	1.078	3.99

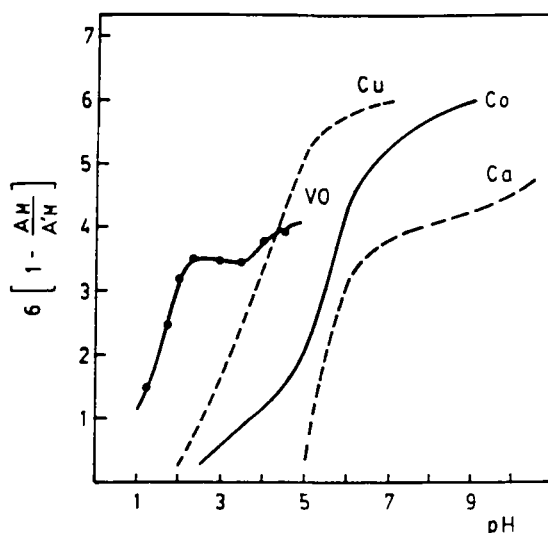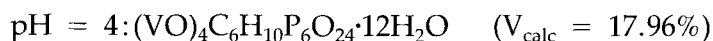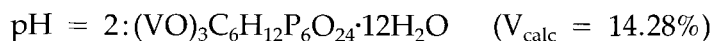$[VO^{2+}] = 60$ mM; [phy] $= 10$ mM

Fig. 3. Binding of VO^{2+} and other divalent metal cations as a function of pH.

Studies on Solid VO^{2+}-Phytates

Synthesis and Characterization

Two different solid complexes were obtained when solutions of phytate and VO^{2+} (1:6 molar ratio) were mixed at pH = 2 and 4, respectively. The elemental analysis of these solids show only poor reproducibility, probably owing to the formation of quite stable VC phases during the combustion. But, on the other hand, vanadium determinations using a spectrophotometric technique (formation of tungstophosphovanadic acid and absorbance measurements at 400 nm) (27) were highly reproducible. The following results were obtained: at pH = 2, V = 14.43% and at pH = 4, V = 17.82%. The stoichiometry of the solid compounds which agree with these results were as follows:

$$pH = 2 : (VO)_3C_6H_{12}P_6O_{24} \cdot 12H_2O \quad (V_{calc} = 14.28\%)$$

$$pH = 4 : (VO)_4C_6H_{10}P_6O_{24} \cdot 12H_2O \quad (V_{calc} = 17.96\%)$$

As can be seen, the proposed stoichiometry agree with those proposed above on the bases of the binding isotherms.

Electronic Spectra

The diffuse reflectance spectra for the two solid complexes showed also a shift of the bands to higher wavenumbers, in comparison to values of $VOSO_4 \cdot 5H_2O$. They present a good defined band at 825 nm and a shoulder at ca. 650 nm indicating, again, the coordination of the VO^{2+} cation to the phosphate groups of the ligand.

Infrared Spectra

Figure 4 shows the infrared spectra of the free ligand and the two solid complexes in the spectral range between 1400–700 cm^{-1}. The broad band of phytate located at ca. 1125 cm^{-1} can be assigned to the antisymmetric PO_3-stretching mode. The other strong, and better defined band, at 980 cm^{-1} may be the corresponding symmetric vibration (*18,28*), which can probably be coupled with skeletal modes of the cyclohexane ring (*29*). Lower intensity bands, at 845 and 785 cm^{-1} can also be assigned to ring vibrations (*29*).

After formation of the complexes a strong broadening in the PO_3-stretching region, as well as a displacement of the $\nu_s (PO_3)$ band to 1000 cm^{-1} can be observed. This behavior is characteristic for metal-phosphate interactions (*18,28,30*). Also the two lower energy bands are slightly displaced to higher frequencies, showing that the complex formation affects the ring vibrations in some way.

Unfortunately, it is not possible to localize the $\nu (V = O)$ stretching band in these two complexes. This band appears usually between 970–1020 cm^{-1} (*31*), i.e., in the same region in which the strong PO_3-bands are found. This is probably another reason for the important broadening in the spectral region around 1000 cm^{-1}. It should be remarked that in the case of VO^{2+} complexes with nucleotides this stretching could also never be observed (cf. (*18,22*)).

CONCLUSIONS

This study has clearly demonstrated the existence of specific interaction between the VO^{2+} cation and phytate and allowed the characterization of both, soluble and insoluble vanadyl(IV)/phytate complexes. On the other hand, the analysis of the pH binding profile suggests that VO^{2+} interacts with the so-called "equatorial form" of phytate and showed that the maximal bonding ratio of VO^{2+} to phytate is four.
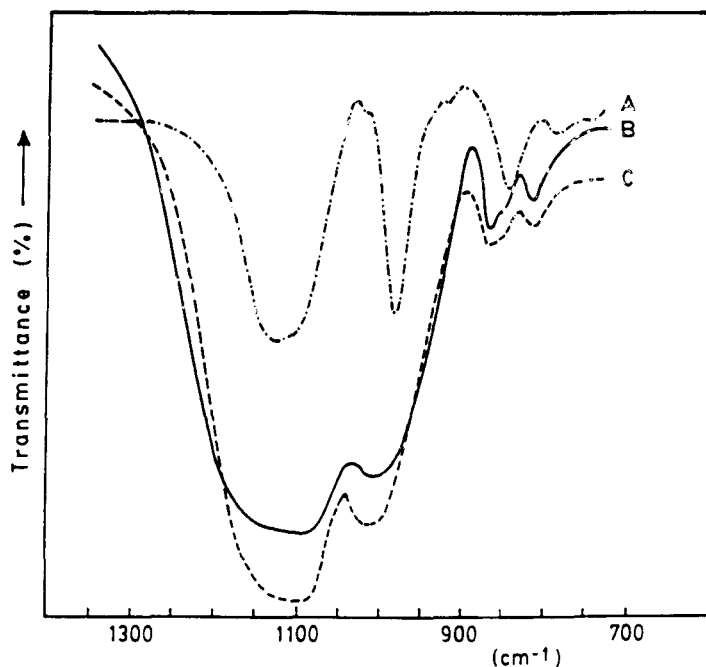
Fig. 4. Infrared spectra of sodium-phytate (A) and of the solid vanadyl(IV) phytates obtained at pH = 2 (B) and pH = 4 (C).

ACKNOWLEDGMENT

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