Binding of Cu^{2+}, Zn^{2+}, and Cd^{2+} to Inositol Tri-, Tetra-, Penta-, and Hexaphosphates

Hans Persson

Physical Chemistry I, Center for Chemistry and Chemical Engineering, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden

Maria Türk*

Department of Food Science, Chalmers University of Technology, P.O. Box 5401, SE-402 29 Göteborg, Sweden

Margareta Nyman

Applied Nutrition and Food Chemistry, Center for Chemistry and Chemical Engineering, Lund Institute of Technology, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden

Ann-Sofie Sandberg

Department of Food Science, Chalmers University of Technology, P.O. Box 5401, SE-402 29 Göteborg, Sweden

myo-Inositol hexaphosphate, the salt of *myo*-inositol hexaphosphoric acid (IP6), is a common constituent of many plant foods, such as cereals and legumes. IP6 interacts with mineral elements, influencing their bioavailability. Processed foods contain a mixture of different inositol phosphates, i.e., IP6 and its degradation products with five or less phosphate groups (IP5−IP1). The interaction of the lower inositol phosphates with mineral elements is not well-known. In this study, the interaction between metal ions (Cu^{2+}, Zn^{2+}, and Cd^{2+}) and isolated fractions of inositol phosphates with 6, 5, 4 and 3 phosphate groups (IP6−IP3) was investigated by using a potentiometric technique. The study was performed at pH 3−7, which is the pH range in the upper part of the duodenum, where mineral absorption takes place. The inositol phosphate fractions studied had a pronounced binding capacity between pH 5 and 7. Thus, mineral complex formation with lower inositol phosphates is likely to occur in the duodenum, which would be important from a nutritional point of view. The mineral binding capacity as calculated per phosphate group was similar for IP6, IP5, IP4, and IP3, but the binding strength was lower for the lower inositol phosphates (IP4 and IP3). At increasing pH, within the range (pH 3−7), the metal complex formation generally began in the order copper, zinc, cadmium for all inositol phosphates indicating the same order of binding strength, i.e., Cu > Zn > Cd. For IP6 the difference was small between Cu and Zn.

Keywords: *Inositol phosphates; mineral ions; complex formation; binding strength*

INTRODUCTION

The ability of inositol phosphates to interact with mineral elements has attracted a great deal of interest in human nutrition (Kelsay, 1982; Lönnerdal et al., 1989; Sandberg et al., 1993).

Many plant foods, e.g., cereals and legumes, contain *myo*-inositol hexaphosphate, the salt of *myo*-inositol hexaphosphoric acid (IP6). It is the major storage form of phosphorus in the plant and possesses tremendous potential for chelating minerals. IP6 forms phytate complexes with divalent metal ions such as Fe^{2+}, Zn^{2+}, Mg^{2+}, and Ca^{2+}. These complexes have a low solubility at physiological pH, and thus the bioavailability of essential elements can be reduced, but for potentially toxic mineral elements the interaction could be considered positive if the metal ions bound to inositol phosphates are excreted in feces and not absorbed (Pallauf and Rimbach, 1997; Wise and Gilburt, 1981). Besides being an antinutrient, dietary phytate may have benefical effects. IP6 and its lower phosphorylated forms are present in most mammalian cells, where they are important in regulating vital cell functions. For example the 1,4,5-IP3 acts as a "second messenger", causing the release of stored calcium inside the cell. Recent observations have been made on dietary phytate as an anticarcinogen, protecting against colon cancer (Shamsuddin, 1995; Graf and Eaton, 1985; Harland and Morris, 1995). The mechanism of this action is not fully understood, but phytate may supply an important antioxidant function by complexing iron and thereby reducing the formation of hydroxyl radicals in the colon. Several carcinogenic substances are generated through

* Address correspondence to this author at the Department of Food Science, Chalmers University of Technology, c/o SIK, P.O. Box 5401, SE-402 29 Göteborg, Sweden (telephone +46 31 335 13 35; fax +46 31 83 37 82; e-mail mt@fsc.chalmers.se).

S0021-8561(97)01055-8 CCC: $15.00
Published on Web 07/21/1998

iron-catalyzed lipid peroxidation. It is not yet known whether lower inositol phosphates derived by hydrolysis of phytate have anticancer action. However, *myo*-inositols phosphorylated in the 1, 2, and 3 positions have been demonstrated to inhibit iron-catalyzed hydroxyl radical formation (Phillipphy and Graf, 1997).

Numerous studies have been made on the protonation constants of IP6, but the results are often conflicting. This conflict could be due to the fact that the protonation constants of IP6 to a large extent are dependent on the ionic strength of the medium (Bieth and Spiess, 1986).

According to Erdman (1979), in the phytic acid molecule, 6 of the 12 replaceable protons are strongly ($pK_a < 3.5$) and six are weakly ($pK_a = 4.6-10$) dissociated. Therefore, IP6 is strongly negative charged over a wide pH range and easily interacts with divalent cations. By binding to one phosphate group or bridging two phosphate groups, various cations could form chelate complexes with phosphate groups within an inositol phosphate molecule as well as with phosphate groups in different molecules.

Several studies demonstrate the importance of conditions such as pH and the phytate-to-mineral molar ratio for the formation of phytate complexes (Wise and Gilburt, 1981; Evans and Pierce, 1982; Graf, 1983; Grynspan and Cheryan, 1983; Cheryan et al., 1983; Martin and Evans, 1986; Wise, 1995; Pallauf and Rimbauch, 1997; Nolan et al., 1987). Another important factor is the synergistic effect of "secondary cations", among which Ca^{2+} has been most prominently mentioned. Two cations may, when present simultaneously, act together to increase the quantity of phytate precipitation (Oberleas, 1973; Simpson and Wise, 1990).

Studying the solubility and relative stability of various IP6−metal complexes by potentiometric titration, Vohra et al. (1965) found the order of stability at pH 7.4 to be $Cu^{2+} > Zn^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Fe^{3+} > Ca^{2+}$, whereas Maddaiah et al. (1964), using a different titration method, indicated the order of stability at this pH as $Zn^{2+} > Cu^{2+} > Co^{2+} > Mn^{2+} > Ca^{2+}$. However, in both studies it was concluded that Cu and Zn appeared to have a high affinity for phytate and that the metal binding to phytate as well as the solubility of the complex was pH-dependent.

During food processing, e.g., baking (Phillippy et al., 1988; Sandberg, 1996; Türk and Sandberg, 1992; Türk et al., 1996) and passage through the digestive tract, phytate may be hydrolyzed to a mixture of inositol penta- to monophosphates (IP5−IP1). A large proportion of lower inositol phosphates has been detected in ileal contents and feces of man (Sandberg et al., 1987; Skoglund et al., 1997).

Knowledge about the binding strength of lower inositol phosphates to different metal ions is limited. Kaufman and Kleinberg (1971) studied Ca^{2+} binding of IP6, IP5, IP4, and IP3 over a pH range of 2.0−12.0. With increasing degree of inositol phosphate hydrolysis, the pH at which calcium phytate first precipitated increased, indicating that the mineral binding strength was becoming progressively lower. Persson et al. (1991) demonstrated that the soluble fiber fraction of cereals bound Cu^{2+}, Zn^{2+}, and Cd^{2+} in the order $Cu^{2+} > Zn^{2+} \approx Cd^{2+}$. Phytase treatment reduced the binding capacity considerably but not totally. The remaining binding capacity was ascribed to lower inositol phytates formed during the phytase treatment or to other ligands.

Results are conflicting as to whether lower inositol phosphates impair mineral absorption. Studies in suckling rats suggest that, when added in a molar ratio of phytate to mineral ion 4:1, isolated inositol hexa- and pentaphosphates (IP6 and IP5) inhibit zinc and calcium absorption, while inositol tetra- and triphosphates (IP4 and IP3) have no effect (Lönnerdal et al., 1989). Zinc absorption in humans was also depressed when IP6 or IP5 was added to a meal with white wheat rolls, while no effect of IP4 was found (Sandström and Sandberg, 1992). IP6 and IP5 had a strong negative effect on iron solubility at simulated physiological conditions (Sandberg et al., 1989) and iron absorption (Sandberg et al., 1993), but no such effect was found for IP4 or IP3. However, in studying iron absorption from bread meals (Brune et al., 1992) a strong negative correlation was found between the sum of IP6−IP3 and iron absorption, suggesting that IP4 and IP3 also contributed to the negative effect on iron absorption.

The aim of this work was to study the solubility of some metal ions under pH conditions that may be encountered in the duodenum. Early in the digestion of a meal, the doudenal contents has an almost neutral pH, and during the next 1.5 h the pH falls succesively to 5, 4, and 3. Duodenal neutralization is very rapid and effective, so that only a short segment of the duodenum is acidified for a brief time. In normal subjects the average pH of contents of the middle of the second part of the duodenum lies between 5.4 and 7.8 (Davenport, 1982). However, at pH above 7, hydroxo complexes dominate for copper and measurements with amalgam electrodes are less reproducible. Therefore, the interaction between single metal ions (Cu^{2+}, Zn^{2+}, and Cd^{2+}) and isolated fractions of inositol phosphates with 6, 5, 4, and 3 phosphate groups (IP6−IP3) was investigated over a pH range of 3−7.

The ionic strength of the solutions was 100 mM, which appeared to be within plausible physiological range for conditions in the duodenum. The mean electrolyte composition of the duodenal secretion is Na^+ 145 mM, K^+ 6.3 mM, Cl^- 136 mM, and HCO_3^- 17 mM (Davenport, 1982). To get an excess of mineral ions to binding sites and thereby to achieve saturation, a molar ratio of 1:13 (IP*x* to mineral) was used. Inositol phosphate fractions were prepared by nonenzymatic hydrolysis of sodium phytate. The binding to metal ions was studied by using a potentiometric technique (Persson, 1970; Norberg and Persson, 1984).

MATERIALS AND METHODS

Preparation of Inositol Tri-, Tetra-, and Pentaphosphates. Sodium phytate was used in the IP6-samples. IP5, IP4 and IP3 were prepared according to Sandberg et al. (1989). Sodium phytate (1.5 g, BDH Chemicals, Poole, England) was hydrolyzed with 100 mL of 0.5 M HCl for 7 h. The hydrolysate was evaporated to dryness at reduced pressure and a temperature of 40 °C and dissolved in 10 mL of deionized water. The inositol phosphates formed were separated by anion-exchange chromatography. Glass columns (1 × 70 cm) containing 10 mL of resin (AG 1 × 8, 200−400 mesh) were used. Fractions of 30 mL were eluted by a linear gradient from 0.05 to 0.5 M HCl. The initial concentration of 0.05 M HCl (2 × 30 mL) was used to separate inositol mono- and diphosphates from the hydrolysate. IP3 started to eluate at a concentration above 0.05 M. Three portions of the following concentrations (0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, and 0.50 M) were used for elution in the first step. The columns were then washed with 2.0 M HCl. A sample of each eluted fraction was evaporated to dryness, dissolved in the mobile phase, and

analyzed by high-performance liquid chromatography (HPLC) to determine the content of IP5, IP4, and IP3. The pure fractions were collected and the fractions containing a mixture of inositol phosphates were passed through the anion-exchange resin again, using a similar procedure until pure fractions were obtained. The pure fractions were quantified by HPLC (Sandberg et al., 1987; Sandberg and Ahderinne, 1986).

Composition of Inositol Phosphate Isomers. The inositol phosphate fractions thus prepared by nonenzymatic hydrolysis of IP6 and used in the experiment contained a mixture of isomers. Isomers of inositol phosphates were determined by the method of Skoglund et al. (1997), with improvements according to Skoglund et al. (1998). A determination of IP5−IP3 with good separation of isomers was obtained. The method includes purifying of samples, ion chromatography, acidic gradient eluation, postcolumn reaction with $Fe(NO_3)_3$, and UV detection.

The IP5 fraction contained DL-Ins(1,3,4,5,6)P_5 (28%), DL-Ins(1,2,4,5,6)P_5 (31%), DL-Ins(1,2,3,4,5)P_5 (30%), and Ins(1,2,3,4,6)P_5 (11%); the IP4 fraction contained DL-Ins(1,4,5,6)P_4 (11%), Ins(2,4,5,6)P_4 (4%), DL-Ins(1,2,5,6)P_4 (20%), DL-Ins(1,3,4,5)P_4 (16%), DL-Ins(1,2,4,6)P_4 (9%), Ins(1,3,4,6)P_4 (6%), DL-Ins(1,2,4,5)P_4 (10%), DL-Ins(1,2,3,4)P_4 (20%), and Ins(1,2,3,5)P_4 (3%); and the IP3 fraction contained DL-Ins(1,5,6)P_3 (18%), DL-Ins(1,4,5)P_3 (14%), DL-Ins(1,2,6)P_3 (8%), Ins(1,2,3)P_3 (24%), DL-Ins(1,2,4)P_3 and DL-Ins(1,3,4)P_3 (together 24%), and 12% of an unidentified IP3 isomer.

Complex Formation. The interaction between the inositol phosphate fractions Cu^{2+}, Zn^{2+}, and Cd^{2+} ions, respectively, was studied using a modification of the potentiometric technique elaborated by Persson (1970) and described previously (Norberg and Persson, 1984). The emf (E) of galvanic cells of the following composition was measured:

$$M(Hg)|S + T_1 + T_2 + T_3||10.0\ mM\ NaCl,\ 90\ mM\ Na\ ClO_4|Ag(s), AgCl(s)$$

where M is Cu, Zn, or Cd.

To 20.0 mL of a solution (S) containing $M(ClO_4)_2$ of the concentration 3.22 mM (Cu), 3.52 mM (Zn), and 3.33 mM (Cd), respectively, and 0.100 M $NaClO_4$ as ionic medium, 0.500 mL of a solution T_3 containing 10.0 mM IPx (x = 3, 4, 5, or 6) was added. By addition of a solution T_1 (30.0 mM $HClO_4$ and 70 mM $NaClO_4$), pH was then adjusted to such a low value that the interaction between metal ions and inositol phosphates could be neglected (i.e., pH 2.5−3.0). The resulting solution was titrated with a solution T_2 (6.00 M NaOH and 94 mM $NaClO_4$) to pH 7. By emf measurements at 25 °C with amalgam electrodes in solutions containing known total concentrations of metal ions (C_M) and a certain inositol phosphate, the concentration of free metal ion $[M^{2+}]$ was determined from

$$E = E° - 29.58 \log [M^{2+}] \quad (1)$$

This relation was proven to be valid within ± 0.2 mV for all metals at C_M = 0.5−5 mM. Thus, the fraction of metal bound to inositol phosphate or complexed otherwise, $(C_M - [M^{2+}])/C_M$, could be calculated. The interaction was studied as a function of pH.

The formation of hydroxo complexes was determined from a separate measurement series performed without addition of inositol phosphate. From the total amount of bound metal at a certain pH, the experimentally determined fraction existing as hydroxo complexes (C_{MOH}) was subtracted, and the net metal binding to IPx obtained. Every titration series was repeated at least once. The protolytic properties of IP3−IP6 were studied by titrating the different inositol phosphates with sodium hydroxide solution, without metal ions present. Before the titrations, pH was about 3 in the solutions containing IP3, IP4, and IP5, while perchloric acid had to be added to the IP6 solution to obtain the corresponding pH value. In all solutions the pH was adjusted below 3 by adding perchloric acid.

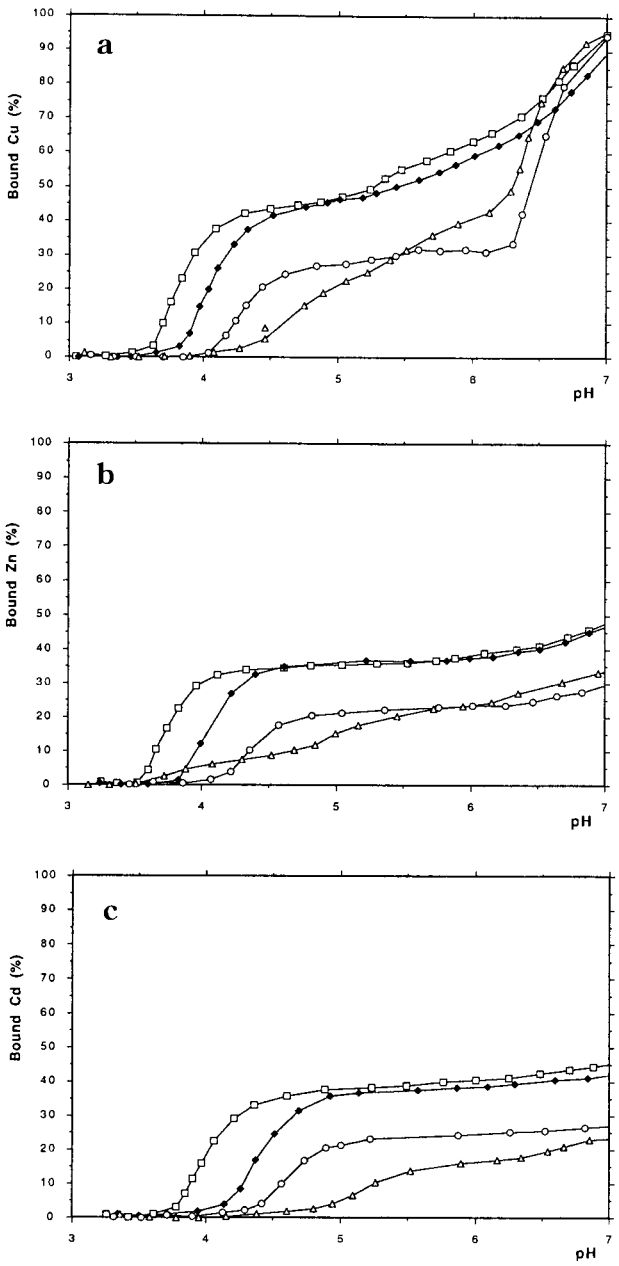

Figure 1. Bound metal $(C_M - [M^{2+}])/C_M$ as a function of pH at addition of different inositol phosphates. (a) Copper; (b) zinc; (c) cadmium. (□) IP6; (◆) IP5; (○) IP4; (△) IP3. The starting concentrations were: inositol phosphates 0.24 mM, copper (II) 3.14 mM, zinc (II) 3.43 mM and cadmium (II) 3.25 mM.

RESULTS

Complex Formation. The total amounts of copper, zinc, and cadmium bound to the different inositol phosphates, IP6−IP3, or existing as hydroxo complexes are shown as functions of pH in Figure 1. A considerable binding between the metal ions studied and all inositol phosphates was observed. For IP6 and IP5 the complex formation started at about pH 3.5 and for lower inositol phosphates at about pH 4. The values in the measurement series, of the percentage of metal ion bound at a certain pH, were generally reproduced within ±2 units.

The maximum amount of metal ion bound by the various inositol phosphates was approximately the same for IP6 and IP5 but decreased at further dephosphory-

lation, i.e., at decreasing number of phosphate groups on the inositol molecule.

To compare the net binding of different metals to a certain inositol phosphate molecule, the hydroxo complexes were subtracted. In Figure 2 a comparison of the binding of Cu, Zn, and Cd is shown for IP6−IP3. At increasing pH, in the interval 3.5−7, the metal complex formation generally began in the order Cu, Zn, Cd for all inositol phosphates, indicating the same order of binding strength, i.e., $Cu^{2+} > Zn^{2+} > Cd^{2+}$. For IP6, however, the difference between Cu and Zn was small.

The total concentration of presumptive ligand groups, i.e., the phosphate groups on the inositol molecule, was less than the concentration of metal ions. At pH 5−6 the complex formation curve flattens out, indicating saturation of the available binding sites on the inositol phosphates. The drop-off in the curves for Cu at still higher pH values is due to preferential formation of hydroxo complexes.

In the pH range studied, the titration curves in Figure 3 show two steps indicating two different groups of protons. From the pH value halfway to the first jump was obtained an average value $pK'_a = 3.2 \pm 0.2$. From the pH values just between the two pH jumps on the titration curves, an average of $pK''_a = 6.2 \pm 0.2$ was obtained. According to Erdman (1979), 6 of the 12 replaceable protons on phytic acid have low pK_a values ($pK_a < 3.5$) and 6 have higher ($pK_a = 4.6-10$). However, Barré et al. (1954) found three groups of acidic hydrogens with different average pK_a values: six with $pK_a = 1.84$, two with $pK_a = 6.3$, and four with $pK_a = 9.7$.

By use of the concentrations of different inositol phosphates (C_{IPx}), the total concentration of metal (C_M), and the concentrations of free metal ions ($[M^{2+}]$) and of hydroxo complexes (C_{MOH}), the number of metal ions (n_M) bound per inositol phosphate molecule can be calculated as

$$n_M = ((C_M - [M^{2+}]) - (C_{MOH}))/C_{IPx} \qquad (2)$$

At pH ≈ 5 the complex formation curves (Figure 2) flattened out, indicating saturation of the available binding sites on the inositol phosphates. Table 1 contains values of n_M calculated at these plateaus.

Table 1 generally indicates, within the error limits, that every phosphate group on the inositol phosphate molecule can bind at most one metal ion.

DISCUSSION

Inositol phosphates interact with minerals in vivo. Although the in vivo system is much more complex, in vitro studies of mineral binding to inositol phosphates can be used to explain the mechanism behind this. During food processing and passage through the digestive tract, phytate may be hydrolyzed to a mixture of inositol penta- to monophosphates (Skoglund et al., 1997). It is therefore important, from a nutritional point of view, that the mineral binding studies are not concentrated solely on the hexaphosphate but also include its degradation products. The aim of this work was to study the solubility of metal ions under pH conditions that may be encountered in the duodenum.

Our results indicate that IP6 and its derivatives IP5, IP4, and IP3 bind metal ions (Cu^{2+}, Zn^{2+}, and Cd^{2+}) in vitro at pH values similar to that in the duodenum.

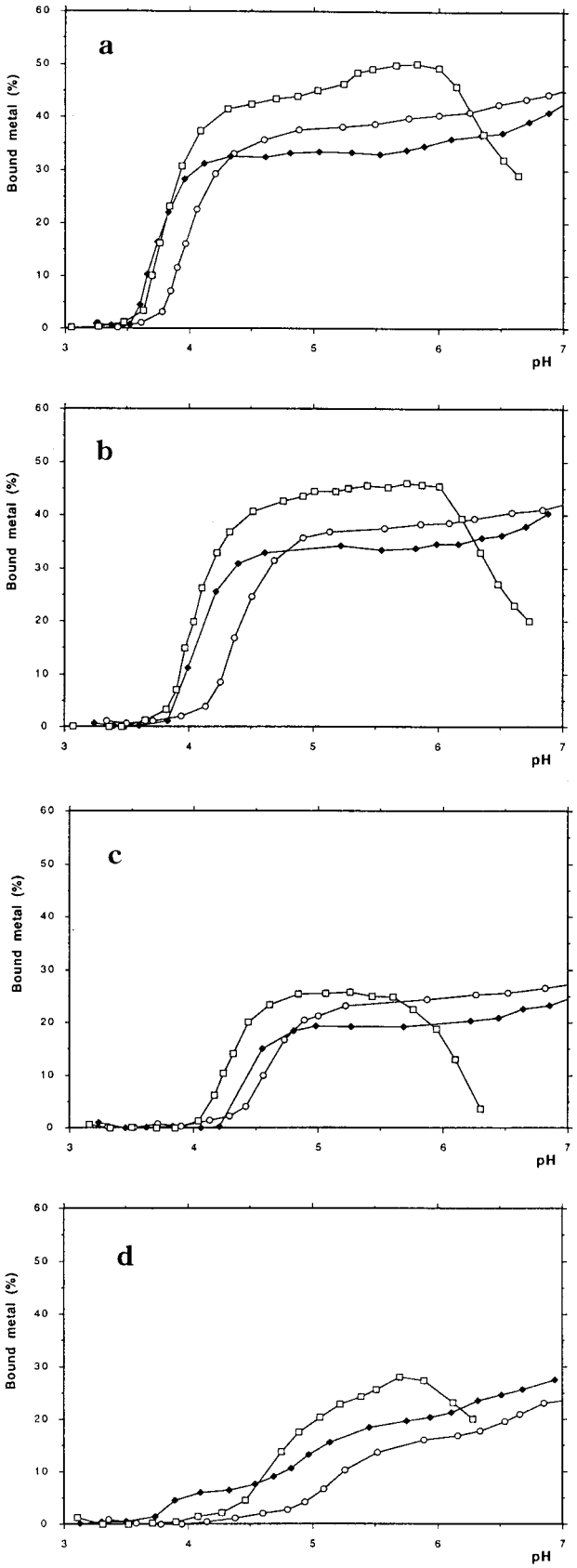

Figure 2. Metal bound to inositol phosphates ($C_M - [M^{2+}]$)/C_M as a function of pH. Inositol hexa- (a), penta- (b), tetra- (c), and tri- (d) phosphates are shown. (□) Cu; (◆) Zn; (○) Cd. The starting concentrations were inositol phosphates, 0.24 mM; copper(II), 3.14 mM; zinc(II), 3.43 mM; and cadmium(II), 3.25 mM. Corrections for formation of metal-hydroxo complexes have been made.

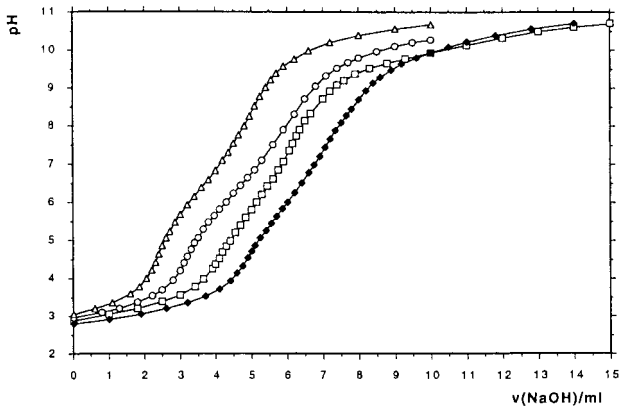

Figure 3. Titration of different inositol phosphates with sodium hydroxide. pH is shown as a function of the added volume of 6.00 mM sodium hydroxide. (□) IP6; (◆) IP5; (○) IP4; (△) IP3. The starting concentration of inositol phosphate was 0.24 mM.

Table 1. Number of Metal Ions Bound per Inositol Molecule at pH ≈5−6

IP*x*	$n_{Cu^{2+}}$	$n_{Zn^{2+}}$	$n_{Cd^{2+}}$
IP6	5.8	4.9	5.3
IP5	5.7	4.8	5.1
IP4	3.3	3.0	3.3
IP3	3.1	3.0	2.4

The strength of the complexes was found to be in the order $Cu^{2+} > Zn^{2+} > Cd^{2+}$ for all inositol phosphates. For IP6 the difference was small between Cu and Zn. The capacity of a certain inositol phosphate to bind metal ions was found to be a function of the number of phosphate groups on the molecule.

In the present study, the inositol phosphate fractions had a pronounced binding capacity at pH between 5 and 7, and thus mineral complex formation can be important from a nutritional point of view. Lower inositol phosphates (IP4 and IP3) bound less metal than higher inositol phosphates (IP6 and IP5) in all cases and the complex formation for IP4 and IP3 also started at a higher pH value, indicating weaker complexes. However, the mineral binding capacity, at pH above 5, per number of phosphate groups was similar for all inositol fractions. Generally one phosphate group could bind at most one metal ion (Table 1).

The phytate:mineral ratios in Table 1 are nonstoichiometric, which is in agreement with the work of Evans and Pierce (1982). This could mean that some inositol phosphates are further deprotonated than others with the same degree of phosphorylation and therefore able to bind more cations. According to the study of Bieth and Spiess (1986) on protonation of IP6, a mixture of protonated species is present at each pH value.

Copper and zinc appear to have a high affinity for phytate. In the present study, the ability of metal ions to form complexes was found to be in the order $Cu^{2+} > Zn^{2+} > Cd^{2+}$ for all inositol phosphates, which is in agreement with the work of Persson et al. (1991) on soluble fiber fractions from cereals and also with the work of Vohra et al. (1965), who found the order of stability of phytate−metal complexes at pH 7.4 to be $Cu^{2+} > Zn^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Fe^{2+} > Ca^{2+}$. In contrast to this Maddiah et al. (1964) found Zn^{2+} to form more stable complexes with IP6 than Cu^{2+}. However, in our study, for IP6 the difference was small between Cu and Zn.

Complex binding to inositol phosphates could faciliate the elimination of potentially toxic heavy metals from the organism. The interaction of IP6 with Pb^{2+} and Be^{2+} was studied by calorimetry (Evans and Martin, 1992), and strong affinity for IP6 was found for both cations. Furthermore, the complex binding properties of D-1,2,6-IP3 with Cd^{2+}, Pb^{2+}, and Hg^{2+} were investigated by Lapp and Spiess (1991). The complexes with the studied metals were quite stable in a wide pH range. In the present study, Cd^{2+} bound to all inositol phosphate fractions studied.

The metal−inositol phosphate complexes seem to become more soluble as the number of phosphate groups decreases (Jackman and Black, 1951). There is also some evidence for weaker complexes when phosphate groups are removed from IP6 (Kaufman and Kleinberg, 1971). Thus, the fact that lower inositol phosphates in the diet seem to interact less with mineral bioavailability than higher inositol phosphates can be due both to increased solubility of the complexes and to weaker metal complexes.

Hydrolysis of IP6 results in the formation of a large number of isomers of IP5 to IP1, and a further possibility is that these different isomers have different mineral binding capacities (Phillipy and Graf, 1997; Mernissi-Arifi et al., 1994). However, the results of the present study generally indicate that for these groupings of positional isomers the mineral binding capacity was a function of the number of phosphate groups rather then their position.

Further, IP6 exists in two configurations: 5eq/1ax (five phosphates in the equatorial position and one phosphate in the axial position) and 1eq/5ax (one phosphate in the equatorial position and five phosphates in the axial position) (Reddy et al., 1989). Barrientos and Murthy (1996) describe a ^{1}H NMR spectroscopic investigation of the conformations of IP6−IP1. According to their results, IP6−IP1 would all be in the 5eq/1ax form in the pH range 3−7, i.e., the pH values in the present study. However, Champagne and Fischer (1990) found that, at pH 7, IP6 existed in both forms. According to their study, at molar ratios of phytate to metal 1:10, 5 or 6 Cu^{2+} ions bound to phytate compared to 4 Zn^{2+}. The Cu−phytate complexes stayed in solution while the Zn−phytate complexes slowly precipitated. They proposed that Zn^{2+} preferentially bound the 5ax/1eq phytate, which they found to be present in a small amount in equlibrium with the predominant 5eq/1ax conformer, while Cu^{2+} preferentially bound the 5eq/1ax phytate. Thus, if inositol phosphates can exist in both forms at physiological pH, one of these configurations may be favorable for binding metal ions.

An understanding of how inositol phosphates bind metal ions is of importance to improve the mineral bioavailability of processed food and also to prevent absorption of potentially toxic metals.

ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; IP3, inositol triphosphate; IP4, inositol tetraphosphate; IP5, inositol pentaphosphate; IP6, inositol hexaphosphate; Ins, accepted NC−IUB abbreviation for *myo*-inositol, with the numbering of the D configuration (numbered counterclockwise) unless the prefix L (numbered clockwise) is explicitly added.

LITERATURE CITED

Barré, R, Courtois, J. E.; Wormser, G. Étude de la structure de l'acide phytique au moyen de ses courbes de titration et de la conductivité de ses solutions (Studies of the structure of phytic acid by means of its titration curves and the conductivity of its solutions). *Bull. Sté. Chim. Biol.* **1954**, *36* (1−2), 455−474.

Barrientos, L. G.; Murthy, P. P. N. Conformational studies of *myo*-inositol phosphate. *Carbohydr. Res.* **1996**, *296*, 39−54.

Bieth, H.; Spiess, B. A comparative study of the protonation of myo-inositol hexakis(phosphate). *J. Chem. Soc., Faraday Trans. 1* **1986**, *82*, 1935−1943.

Brune, M.; Rossander-Hultén, L.; Hallberg, L.; Gleerup, A.; Sandberg, A.-S. Iron absorption from bread in humans: Inhibiting effects of cereal fiber, phytate and inositol phosphates with different numbers of phosphate groups. *J. Nutr.* **1992**, *122*, 442−449.

Champagne, E. T.; Fischer, M. S. Binding differences of Zn-(II) and Cu(II) ions with phytate. *J. Inorg. Biochem.* **1990**, *38*, 217−223.

Cheryan, M.; Andersson, F. W.; Grynspan, F. Magnesium−phytate complexes: Effects of pH and molar ratio on solubility characteristics. *Cereal Chem.* **1983**, *60*, 235.

Davenport, H. W. In *Physiology of the digestive tract*; Year Book Medical Publishers Inc.: Chicago, 1982; Chapters 4 and 12.

Erdman, J. W., Jr. Oilseed phytates: Nutritional implications. *J. Am. Oil Chem. Soc.* **1979**, *56*, 736−741.

Evans, W. J.; Pierce, A. G., Jr. Interaction of phytic acid with the metal ions, Copper (II), Cobalt (II), Iron (III), Magnesium (II), and Manganese (II). *J. Food Sci.* **1982**, *47*, 1014−1015.

Evans, W. J.; Martin, C. J. Interactions of inositol hexaphosphate with Pb(II) and Be(II). A calorimetric study. XVIII. *J. Inorg. Biochem.* **1992**, *45*, 105−113.

Graf, E. Calcium binding to phytic acid. *J. Agric. Food Chem.* **1983**, *31*, 851.

Graf, E.; Eaton, J. W. E. Dietary suppression of colonic cancer. *Cancer* **1985**, *56*, 717−718.

Grynspan, F.; Cheryan, M. Calcium phytate: effect of pH and molar ratio on *in vitro* solubility. *J. Am. Oil Chem. Soc.* **1983**, *60*, 1761.

Harland, B. F.; Morris, E. R. Phytate: A good or bad food component? *Nutr. Res.* **1995**, *15*, 733−754.

Jackman, R. H.; Black, C. A. Solubility of iron, aluminium, calcium and magnesium inositol phosphates at different pH values. *Soil Sci.* **1951**, *72*, 179.

Kaufman, H. W.; Kleinberg, I. Effect of pH on calcium binding by phytic acid and its inositol phosphoric acid derivatives and on the solubility of their calcium salts. *Arch. Oral Biol.* **1971**, *16*, 445−460.

Kelsay, J. Effects of fiber on mineral and vitamin bioavailability. In *Dietary Fiber in Health and Disease*; Vahouny G. V., Kritchevsky, D., Eds.; Plenum Press: New York, 1982.

Lapp, C.; Spiess, B. Complexation studies on inositol-phosphates III. Cd^{2+}, Pb^{2+} and Hg^{2+} complexes of D-*myo*-inositol 1,2,6-trisphosphate. *J. Inorg. Biochem.* **1991**, *42*, 257−266.

Lönnerdal, B.; Sandberg, A.-S.; Sandström, B.; Kunz, C. Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. *J. Nutr.* **1989**, *119*, 211−214.

Maddaiah, V. T.; Kurnick, A. A.; Reid, B. L. Phytic acid studies. *Proc. Soc. Exp. Biol. Med.* **1964**, *115*, 391.

Martin, C. J.; Evans, W. J. Phytic acid−metal ion interactions. II. The effect of pH on Ca(II) binding. *J. Inorg. Biochem.* **1986**, *27*, 17−30.

Mernissi-Arifi, K.; Wehrer, C.; Schlewer, G.; Spiess, B. Complexation studies on inositol-phosphates. V. Cu^{2+}, Zn^{2+}, Fe^{2+}, and Fe^{3+} complexes of some *myo*-inositol triphosphates. *J. Inorg. Biochem.* **1994**, *55*, 263−277.

Nolan, K. B.; Duffin, P. A. Effects of phytate on mineral bioavailability. In vitro studies on Mg^{2+}, Ca^{2+}, Fe^{3+}, Cu^{2+} and Zn^{2+} (also Cd^{2+}). Solubilities in the presence of phytate. *J. Sci. Food Agric.* **1987**, *40*, 79−85.

Norberg, A.; Persson, H. Accumulation of heavy metal ions by *Zoogloea ramigera*. *Biotechnol. Bioeng.* **1984**, *26*, 239−246.

Oberleas, D. Phytates. In *Toxicants occurring naturally in foods*; National Academy of Sciences: Washington, DC, 1973; pp 363−371.

Pallauf, J.; Rimbach, G. Nutritional significance of phytic acid and phytase. *Arch. Anim. Nutr.* **1997**, 301−319.

Persson, H. The complex formation in the zinc thiosulfate and the cadmium thiosulfate systems. *Acta Chem. Scand.* **1970**, *24*, 3739−3750.

Persson, H.; Nyman, M.; Liljeberg, H.;Önning, G.; Frölich, W. Binding of Mineral Elements by Dietary Fibre Components in Cereals-*In vitro* (III). *Food Chem.* **1991**, *40*, 169−183.

Phillippy, B. Q.; Graf, E. Antioxidant functions of inositol 1,2,3-trisphosphate and inositol 1,2,3,6-tetrakisphosphate. *Free Radical Biol. Med.* **1997**, *22* (6), 939−946.

Phillippy, B. Q.; Johnston, M. R.; Tao, S.-H.; Fox, M. R. S. Inositol phosphates in processed foods. *J. Food Sci.* **1988**, *53* (2), 496−499.

Reddy, N. R.; Pierson, M. D.; Sathe, S. K.; Salunkhe, D. K. In *Phytates in cereals and legumes*; Reddy, N. R., Ed.; CRC Press Inc.: Boca Raton, FL, 1989.

Sandberg, A.-S.; Brune, M.; Carlsson, N.-G.; Hallberg, L.; Rossander-Hultén, L.; Sandström, B. The effects of various inositol phosphates on iron and zinc absorption in humans. In *Bioavailability '93: Nutritional, Chemical and Food Processing Implications of Nutrient Availability; Proceedings, Part 2, Symposium, Ettlingen, Germany, May 9−12, 1993; Schlemmer, U., Ed.; Bundesforschungsanstalt für Ernährung: Karlsruhe, Germany, 1993.*

Sandberg, A.-S. Food processing influencing iron bioavailability. In *Iron nutrition in health and disease*; Proceedings of the Swedish Nutrition Foundation's 20th International Symposium and the Swedish society of Medicine Berzelius Symposium XXXI, Saltsjöbaden, Stockholm, Sweden, August 24−27, 1996; Hallberg, L., Asp, N.-G., Eds.; Libbey: London, 1996; Chapter 35.

Sandberg, A.-S.; Ahderinne, R. HPLC method for determination of inositol tri-, tetra-, penta- and hexaphosphates in foods and intestinal contents. *J. Food Sci.* **1986**, *51*, 547−550.

Sandberg, A.-S.; Andersson, H.; Carlsson, N.-G.; Sandström, B. Degradation products of bran phytate formed during digestion in the human small intestine: Effect of extrusion cooking on digestability. *J. Nutr.* **1987**, *117*, 2061−2065.

Sandberg, A.-S.; Carlsson, N.-G.; Svanberg, U. Effects of inositol tri-, tetra-, penta- and hexaphosphates on in vitro estimation of iron availability. *J. Food Sci.* **1989**, *54*, 159−161.

Sandström, B.; Sandberg, A.-S. Inhibitory effects of isolated inositol phosphates on zinc absorption in humans. *J. Trace Elem. Electrolytes Health Disease* **1992**, *6*, 99−103.

Shamsuddin, A. M. Inositol phosphates have novel anticancer function. *J. Nutr.* **1995**, *125*, 725S−732S.

Simpson, C. J.; Wise, A. Binding of zinc and calcium to inositol phosphates (phytate) in vitro. *Br. J. Nutr.* **1990**, *64*, 225−232.

Skoglund, E.; Carlsson, N.-G.; Sandberg, A.-S. Determination of isomers of inositol mono- to hexaphosphates in selected foods and intestinal contents using high-performance ion chromatography. *J. Agric. Food Chem.* **1997**, *45*, 431−436.

Skoglund, E.; Carlsson, N.-G.; Sandberg, A.-S. High-performance chromatographic separation of inositol phosphate isomers on strong anion exchange columns. *J. Agric. Food Chem.* **1998**, *46*, 1877−1882.

Türk, M.; Sandberg, A.-S. Phytate reduction during breadmaking: effect of phytase addition. *J. Cereal Sci.* **1992**, *15*, 281−294.

Türk, M.; Carlsson, N.-G.; Sandberg, A.-S. Reduction in the levels of phytate during wholemeal bread making; effect of

yeast and wheat phytases. *J. Cereal Sci.* **1996**, *23*, 257−264.
Vohra, P.; Gray, A.; Kratzer, F. H. Phytic acid−metal complexes. *Proc. Soc. Exp. Biol. Med.* **1965**, *120*, 447.
Wise, A. Phytate and zinc bioavailability. *Int. J. Food Sci. Nutr.* **1995**, *46*, 53−63.
Wise, A.; Gilburt, D. J. Binding of cadmium and lead to the calcium−phytate complex in vitro. *Toxicol. Lett.* **1981**, *9*, 45−50.

Received for review December 12, 1997. Revised manuscript received May 26, 1998. Accepted June 2, 1998.

JF971055W