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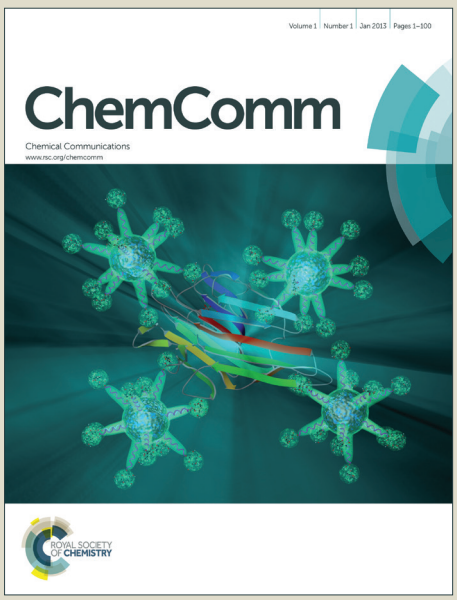

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ARTICLE TYPE

The Copper(II)-Phytate-Terpyridine Ternary System: The First Crystal Structures Showing the Interaction of Phytate with bivalent metal and ammonium cations†‡

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Phytate, an abundant molecule in eukaryotic cells, interacts strongly with inorganic cations and polyamines. This interaction is essential in determining the possible functions of this biomolecule. We present here the first solution and crystallographic study on the formation of phytate complexes in the systems Cu(II)–phytate–terpy and phytate–terpy (terpy = 2,2';6',2"–terpyridine).

Inositides, particularly inositol phosphates and phosphatidylinositol lipids, are molecules of the utmost importance in relation to cell signaling events such as ion–channel functions, vesicle trafficking, apoptosis, transcriptional regulation, motility, and cell proliferation.[1] The highly phosphorylated inositides, particularly *myo*–inositol hexakisphosphate (also called phytate, InsP_6, L^{12-} in its fully deprotonated form), play critical roles in signal transduction and cellular regulation.[2] Phytate is an ubiquitous molecule in eukaryotic cells,[3] and is the primary storage compound of phosphorous in seeds.[4]

The chemical speciation of phytate under different conditions (including those media of immediate biological relevance) has been studied in deep.[5] Chemical species of phytate at pH values near neutrality in 0.15 M NMe_4Cl and 37.0 °C are the highly charged $[H_4L]^{8-}$ and $[H_5L]^{7-}$.[5a] Accordingly, the interaction with cations is a very important aspect to be taken into account when the biological functions of this molecule are considered. Even the interaction with +1 cations, such as Na^+ and K^+, or with protonated polyamines are relevant to know the chemical speciation of this molecule. This interaction has been quantitatively determined for most cations, and the species stoichiometry is known.[5] Conversely, the structure of the species in solution is not known in detail, even though semiempirical structural optimizations show that, in the species with 1:1 molar ratio, more than one phosphate group would be bound to the metal ion, which completes its coordination sphere with water molecules.[5b]

A second aspect to be taken into account is the dominant formation of sparingly soluble solids in the presence of metal ion excess. These species may play a very important role in the chemical behavior of phytates in mammalian cells. For example, the existence of solid species like $[Mg_5(H_2L)]$ with a relatively high solubility, seems to be responsible for the solubilization of InsP_6 in the cytosol and nucleus of mammalian cells in spite of the high Mg^{2+} concentration (0.8 mM).[5c] The stoichiometry and solubility of the solid phases have been determined in some detail.[5a,b,c, 6] However, the structure of such species containing metal excess is still uncertain. Only the X–ray structure of $Na_{12}L{\cdot}38H_2O$ has been reported.[7] It contains the phytate in the form of L^{12-} which is not biologically relevant. Phytate in this structure adopts the *myo*–inositol conformation, 5 axial–1 equatorial (5a1e) (Figure S1), which is typical for the highly deprotonated species, but not at pH values near neutrality (which is 1a5e). More recently, phytate coordination in the Fe(III)–phytate compound was studied by XANES and EXAFS, suggesting a model in which each phosphate is bonded to two iron atoms and each iron is shared by two phytate anions.[8]

The knowledge of coordination modes of InsP_6 in the complexes with metal cations is of the greatest relevance to go deeper into the biological role of this molecule. However, the formation of highly insoluble complexes has been a major obstacle for the obtaining of single crystals and then for getting a full characterization. In the present manuscript, we report for the first time, four crystal structures of phytate complexes obtained in the presence of terpyridine, as an auxiliary ligand. We studied the binary and ternary systems containing phytate, and an aromatic amine (2,2';6',2"–terpyridine, terpy), in the absence and, respectively, in the presence of Cu(II) as the metal ion. The inclusion of terpy as a component of the systems is motivated by the known capability of this molecule to promote π–stacking interactions which could improve the crystallinity of the solids. In addition, it can be used as a partial blocking ligand for Cu(II) in the Cu(II)-InsP_6-terpy ternary systems. In a first stage, the systems were studied by potentiometric titrations (see the ESI) in aqueous solution (0.15 M NMe_4Cl at 37.0 °C) to get information about the stoichiometry and stability of the chemical species, and the pH ranges in which they are predominant. In a second stage, such information was used to select the experimental conditions for the preparation of single crystals of binary and ternary complexes for X-ray analysis. Crystals of $(H_2terpy)_3(H_8L)_{1.5}{\cdot}20.5H_2O$ (**1**), $(H_2terpy)_3(H_6L){\cdot}24H_2O$ (**2**), $K[Cu_4(H_3L)(terpy)_4]{\cdot}26H_2O$ (**3**), and $[Cu_2(H_8L)(terpy)_2]{\cdot}7.5H_2O$ (**4**) were successfully grown and their crystal structures were resolved (see the ESI).

The study in aqueous solution included the determination of

the protonation constants of terpy, the hydrolysis constants of Cu(II), the individual complexation of Cu(II) with terpy or InsP_6, the interaction terpy–InsP_6, and finally the stability constants of the ternary system InsP_6–terpy–Cu(II). These values, together with the protonation constants of InsP_6 under similar conditions,[5a] allow to understand this chemical system. The full set of stability constants are presented in Table S2. The aromatic amine interacts with InsP_6 forming very stable terpy:InsP_6 1:1 and 2:1 species in different protonation states. Both species exist over a wide pH

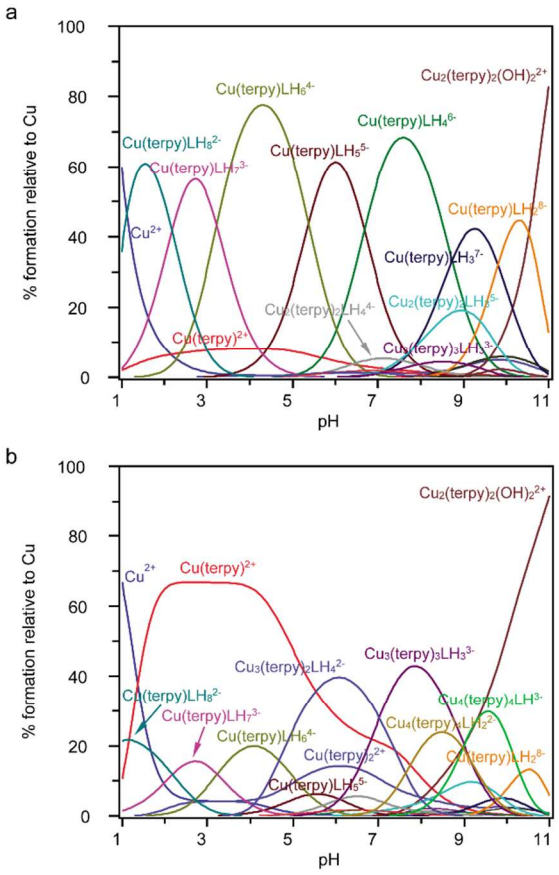


Fig. 1 Species distribution diagrams for Cu^{2+}–terpy–InsP_6 system, in 0.15 M NMe_4Cl at 37.0 °C. (a) $[Cu^{2+}]$ = [terpy] = [InsP_6] = 1 mM; (b) $[Cu^{2+}]$ = [terpy] = 4 mM, [InsP_6] = 1 mM. InsP_6 is represented as L^{12-}.

range, becoming the 2:1 species predominant as the terpy:InsP_6 molar ratio is increased. Copper also forms 1:1 species with InsP_6 in addition to the pentanuclear compound $[Cu_5(H_2L)]$ which resembles that found in the Mg(II)–InsP_6 system.[5a] In the case of systems containing the three components in equimolar amounts, variously protonated species with 1:1:1 molar ratio are among the most important complexes in solution. If the conditions are changed to lower amount of InsP_6 (molar excess of Cu(II) and terpy) the polymeric species (copper:terpy:InsP_6) 3:2:1, 3:3:1, and 4:4:1 become more abundant. This is shown in the species distribution diagrams of Figure 1.

The crystal structures of the binary terpy-InsP_6 complexes **1** and **2** show diprotonated terpyridine molecules interacting via N–H···O hydrogen bonds with partially deprotonated phytate anions, forming R1,2(10) rings (Fig 2). The terpyridine molecules interact with each other giving rise to columns of π–π stacked aromatic groups. In **2**, these columns are infinite and grow along the *a* axis, alternating terpyridine groups which point their protonated nitrogen atoms toward opposite directions. In particular, a couple of adjacent terpyridine groups point to the same side and are H–bond bridged by two phosphate groups of the same phytate anion through D2,2(10) intermolecular chains. A third terpyridine, intercalating between two of the above mentioned couples, points to the opposite direction and interacts with a different phytate (Fig 3). As a result, each phytate links two different columns by using three of its phosphate groups, the

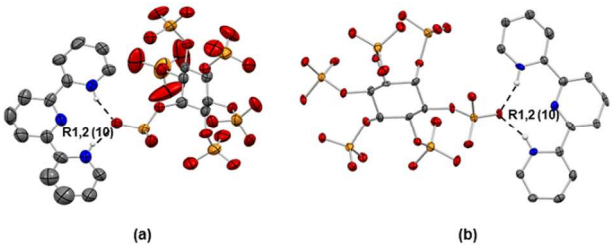


Fig. 2 R1,2(10) rings formed in **1** (a) and **2** (b) crystal structures. For clarity, only H atoms on terpy nitrogens and only one terpy group per structure are shown. Color code: N, blue; C, light grey, O, red; P, yellow.

remaining ones being involved in H–bond interactions with C–H aromatic hydrogen atoms and cocrystallized water molecules. The phytate anions display the 1a5e conformation.

Conversely, two crystallographic independent phytate anions having *myo*– (1a5e) and *scyllo*–inositol (6e) conformations (Fig S1), respectively, are present in **1**. We are not confident about the origin of this *scyllo*–InsP_6 molecule. Taking into account the natural origin of the commercial sources of InsP_6 and the improbable case of epimerization under the mild crystallization conditions, a small percentage of *scyllo* isomer in the starting material seems to be its source. The *scyllo* isomer is located around a crystallographic inversion center.

Interestingly, the two isomers behave quite differently in the crystal packing. Actually, as shown in Figure S3(a), the *myo* isomer interacts with terpyridine groups via N–H···O hydrogen bonds with a binding mode similar to that found in **2**. On the contrary, the *scyllo* isomer is placed on the apolar side of the diprotonated terpyridine groups forming weaker C–H···O and Van der Waals interactions (Figure S3(b)).

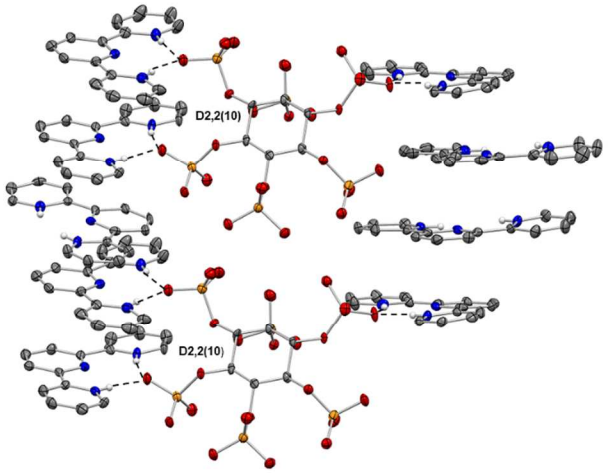


Fig. 3 NH···O interactions between phytate anions and diprotonated terpy cations and columns of stacked aromatic groups in **2**. For clarity, only H atoms on terpy nitrogens are shown. Color code: N, blue; C, light grey, O, red; P, yellow.

The crystal structures of compounds **3** and **4** were obtained for the phytate–Cu(II)–terpyridine system. They are shown, crystallization water molecules being omitted, in Figures 4 and 5, respectively, while selected bond lengths and angles for these structures are reported in Tables S4 and S5 of the ESI. In both complexes, each copper atom is coordinated by a tridentate

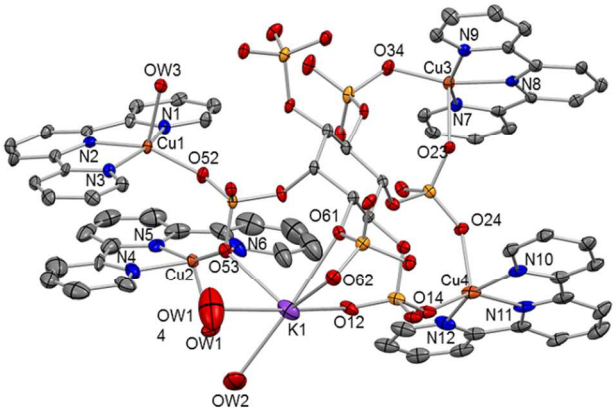
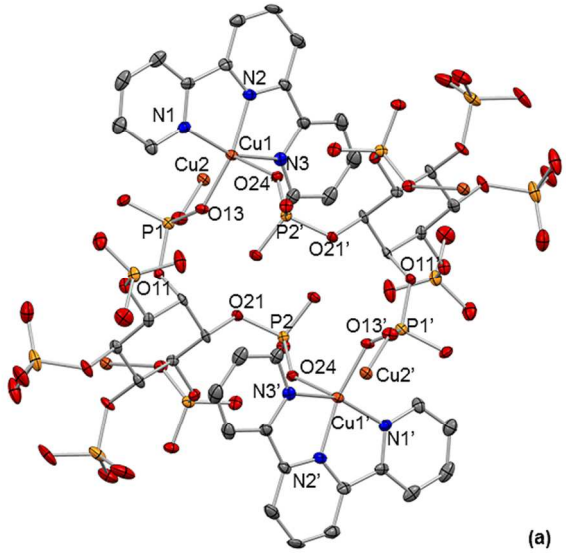


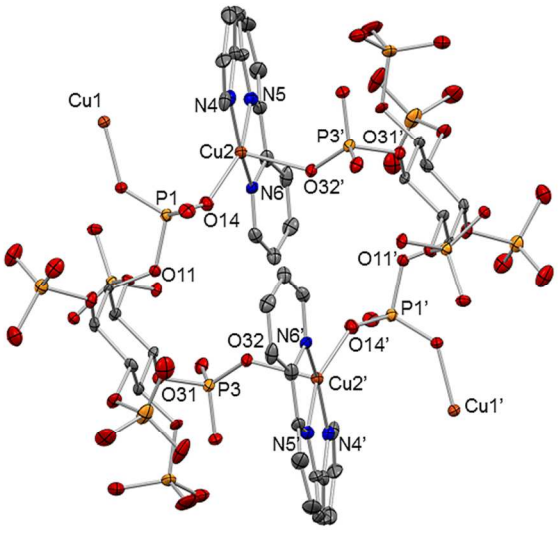
Fig. 4 Crystal structure of **3**, crystallization water molecules omitted for clarity.

terpyridine ligand, the metal coordination geometry being a distorted square pyramid, generally completed by oxygen atoms from the phytate anions. The asymmetric unit of both structures contains only one phytate, which adopts the *myo*–inositol 1a5e conformation and uses five (in **3**) and three (in **4**) phosphate groups, respectively, for metal coordination. The phytate ion is triprotonated (H_3L^{9-}) in **3** and octaprotonated (H_8L^{4-}) in **4**, according to the experimental conditions adopted for the preparation of these complexes and in line with expectations based on solution studies.

In the ternary Cu(II)-InsP_6-terpy complex **3** (Figure 4), three adjacent phosphate groups of H_3L^{9-}, in 1a5e conformation, are bridged by two $[Cu(terpy)]^{2+}$ cations (Cu3 and Cu4), while the remaining two $[Cu(terpy)]^{2+}$ cations (Cu1 and Cu2) are bridged by a different phosphate group. The fifth phosphate is involved, as a bidentate ligand, in the coordination to the potassium ion. The basal plane of the square-pyramidal coordination spheres of all Cu1-Cu4 ions is defined by three terpyridine nitrogen and one phosphate oxygen atoms, while the apical positions are occupied by water molecules, in the case of Cu1 and Cu2, and by phosphate oxygen atoms in the case of Cu3 and Cu4. The potassium ion is hexacoordinated by two water molecules and four oxygen atoms from three phosphate groups. Two of these phosphate groups are also engaged in the binding of copper ions, while the third one is of the above mentioned group behaving as a bidentate ligand.

In complex **4** (Figure 5), the asymmetric unit contains two different $[Cu(terpy)]^{2+}$ cations and one H_8L^{4-} anion, in 1a5e conformation, which involves only three adjacent phosphate groups in metal coordination, the other three remaining not coordinated. Both copper ions are five-coordinated in square-pyramidal coordination environments, with basal planes defined by the three nitrogen atoms of a terpyridine unit and the oxygen atom of a phosphate group. The two basal oxygen atoms belong to the same phosphate groups, which consequently bridges the two metal ions. The apical position of each metal is instead occupied by a phosphate oxygen of a phytate anion symmetry–related by an inversion center. As a result, the crystal packing features infinite tapes formed by alternating 18– and 20–membered rings (Figures 5a and 5b, respectively), sharing





three bonds and containing two copper ions (Figure S4).

Fig. 5 18– (a) and 20–membered rings (b) in the crystal structure of **4** (crystallization water molecules omitted for clarity).

Conclusions

The systems InsP_6–terpy and InsP_6–terpy–Cu(II) were studied in aqueous solution. They show the presence of species containing InsP_6 with high thermodynamic stability.

The isolation of such species and the X–ray diffraction analysis reveal, for the very first time, the interaction mode of InsP_6 with a protonated polyamine alone and in the presence of a bivalent cation. Two of these structures contain Cu^{2+} and show for the very first time the phytate binding mode toward a bivalent cation. It is worth mentioning that the observed conformation of the ligand is 5 equatorial–1 axial (1a5e) (Figure S1), which is known to occur in most biological relevant species of InsP_6 but it is not present in the unique crystal structure up to now available for this biomolecule. The other two structures visualize the formation of anion-complexes with a polyammonium substrate. There are plenty of ammonium groups in biological systems that can give rise to similar phytate-ammonium interactions. Also in this case, it is the very first time that the occurrence of similar interactions is ascertained and displayed by X-ray structures.

In the case of H_2terpy^{2+}, the molecular recognition is verified by three double (chelated) H–bonds which involve a single oxygen atom of a phosphate group. Using the IUPAC nomenclature for cyclitols,[9] equatorial phosphate groups attached to C1, C3, and C4, but not the axial C2 is involved in the direct H–bond interaction with the protonated amine. On the contrary, C2 plays an important role in the interaction with Cu^{2+} in compound **4**. It is the phosphate group which interacts simultaneously with different copper ions supporting the polynuclear skeleton of the structure. Phosphate groups at C3 and C4 interact with a single bivalent ion. These interaction modes could be the structural basis for the formation of the biologically relevant polynuclear species containing phytate.

Notes and references

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† Electronic Supplementary Information (ESI) available: synthesis and characterization details and CIF files. Details of the potentiometric titrations and tables of stability constants. Speciation diagram figures for the systems studied. See DOI: 10.1039/b000000x/

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