THE STRUCTURE OF MYO-INOSITOL HEXAPHOSPHATE DODECASODIUM SALT OCTATRIACONTAHYDRATE: A SINGLE CRYSTAL X-RAY ANALYSIS

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SUMMARY

Sodium phytate·$38H_2O$ crystals from water belonging to space group Cc gave cell dimensions a = 23.082, b = 12.203, c = 22.885 Å, β = 108.30°, D_c = 1.7457 gm/cm^3 with four molecules per unit cell. Phytate has the hexaorthophosphate ester structure. The phosphates at positions C-1, 3, 4, 5 and 6 are axially disposed with that at C-2 equatorial rather than the reverse stereochemistry reported by Johnson and Tate. The twelve sodium atoms are coordinated with phosphate and water oxygens in 2-hexa, 8-octa and 2-decahedral arrangements.

INTRODUCTION

Phytic acid (myo-inositol hexaphosphate) is widely distributed in plants and is the major component of the acid stable organic phosphorous fraction of some seeds (1). Metabolic roles suggested for phytate include a storage form of phosphate in seeds (2), a phosphagen capable of phosphate transfer to certain diphosphonucleotides (3), and an agent that may slow metabolism prior to dormancy through its chelating properties for divalent cations (4). Phytate also exhibits cofactor characteristics for hemoglobin analogous to 2, 3-diphosphoglycerate (DPG) (5).

This communication presents structural evidence which defines the stereochemistry of phytate. Certain properties of phytate are discussed in light of new structural considerations.

MATERIALS AND METHODS

Sodium phytate (Sigma) crystallizes from water as a hydrate (38 H_2O) in space group Cc (6) with cell dimensions a = 23.082, b = 12.203, c = 22.885 Å, β = 108.30°, D_o = 1.7459 gm/cm^3 (by flotation in chloroform: dibromomethane), and D_c = 1.7457 gm/cm^3 for four molecules per unit cell. Intensity data were collected on a Picker automatic diffractometer using CuK_α radiation. The structure was solved by the symbolic addition procedure for non-centrosymmetric space groups with centered cells (7). Anisotropic least squares refinement (8) of all non-hydrogen atoms in the structure was carried out over 5030 observed reflections for $\sin\theta \leq 0.906$ to a residual $R = \Sigma \left| |F_o| - |F_c| \right| / \Sigma |F_o|$ of 0.077.

RESULTS AND DISCUSSION

This crystal structure is a realistic model for phytate in the solution state since it is highly hydrated and reflects the ionic character (9) of phytic acid predictable under physiological conditions. The molecular configuration and conformation of phytate is shown in Fig. 1 without its accompanying solvation components. The hexaorthophosphate structure of phytate concurs with that proposed originally by Anderson (10). However, Johnson and Tate's interpretation of the NMR spectra of phytate (11, 12) is inconsistent with its actual conformation. We find phosphates at C-1, 3, 4, 5, and 6 axially oriented and the phosphate on C-2 in the equatorial position. Although sterically equivalent groups normally prefer that conformation with the maximum number of equatorial positions, the dipolar and coulombic forces of the phosphate ion can be best minimized by vicinal, trans phosphates being diaxial. The supposition is made that nearest neighbor phosphate interactions play a dominant role in governing the conformational stereochemistry. Results from preliminary optical

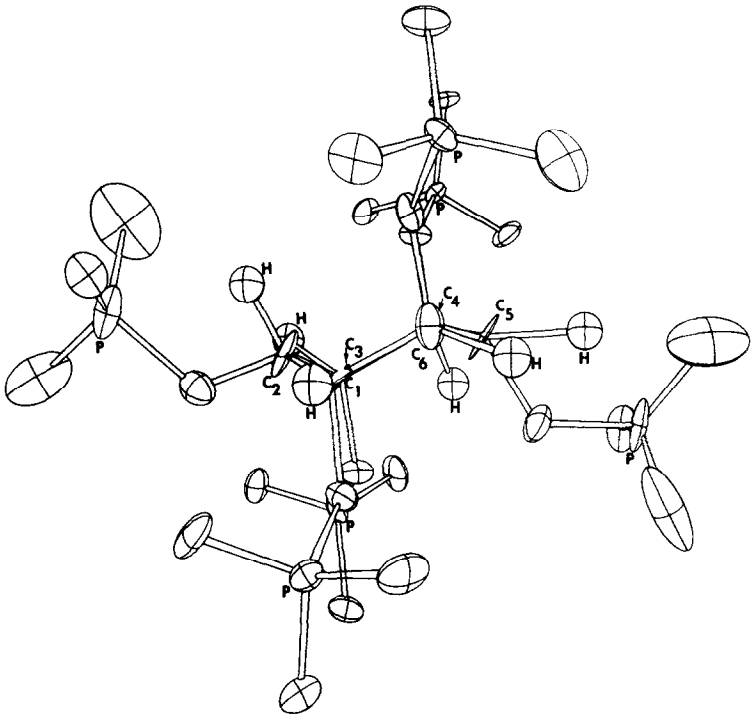


Fig. 1. The structure of phytate, a projection along the vector from C_6 to C_4.

rotation studies by Brown (13) suggest similar conformational properties for (+)-trans-cyclohexane-1, 2-diphosphate and a glycerol myo-inositol triphosphate. In an analogous manner electrostatic forces govern the spatial disposition of the halogen atoms in substituted cyclohexanes (14-16).

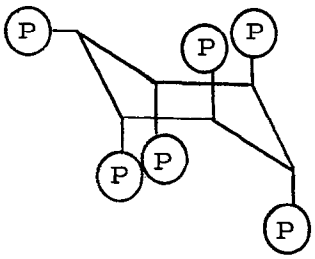
While sequential hydrolytic cleavage of the inositol hexaphosphates by phytases, meso-inositol-hexaphosphate phosphohydrolase (E. C. 3. 1. 3. 8), has been well documented for both the wheat bran (17-21) and bacterial enzymes (12, 21-23), conclusions from these studies are based on the incorrect conformation for phytate. The initial hydrolysis of phytate by the enzyme of either wheat bran (18, 24) or Aerobacter (22) yields L-myo-inositol 1, 2, 3, 4, 5-pentaphosphate*, while D-myo-inositol 1, 2, 4, 5, 6-

*The absolute configuration is denoted in accordance with the IUPAC-IUB Tentative Cyclitol Nomenclature Rules (European J. Biochem. 5, 1 (1968)).

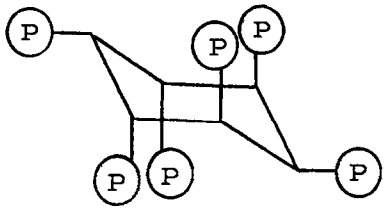
pentaphosphate is produced by phytases from Pseudomomas (21, 23, 25) and Neurospora crassa (12) (see Table I). The enzymes then generally remove

Table I

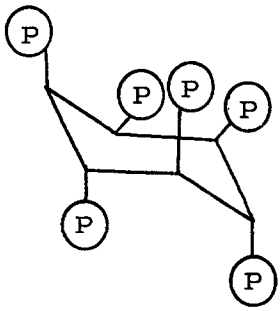
Proposed Conformations of Some Inositol Polyphosphates*



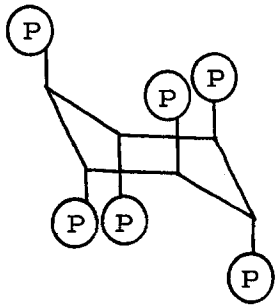
myo-



neo-

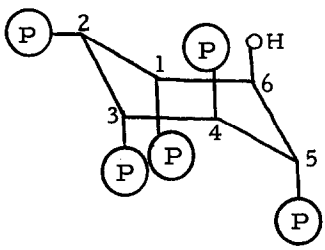


chiro-

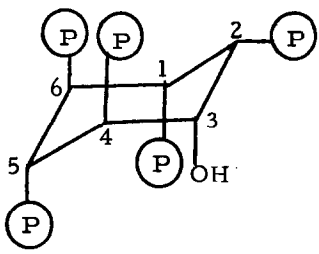


scyllo-

Inositol Hexaphosphates



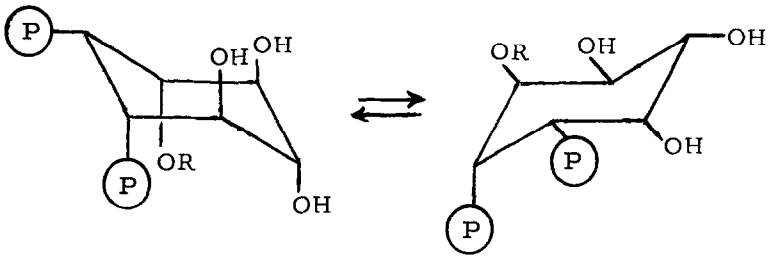
Pentaphosphate from Wheat Bran



Pentaphosphate from Bacteria (Pseudo)

*(P) = $PO_4^{\equiv}$

phosphate adjacent to free hydroxyl groups yielding the penultimate product inositol-2-phosphate, with the possible exception noted by Tomlinson and Ballou (18). Revised conformations of isomeric substrates and some enzymatic hydrolysis products are depicted in Table I based on the structural considerations presented here. Both myo-inositol 1,2-diphosphate (R = H) and myo-inositol 1, 2, 3-triphosphate ($R = PO_3^{=}$) would be expected to prefer conformation (II) of the two chair forms. In (II) the phosphates to be removed are equatorial rather than axial. Since other inositol hexaphosphate isomers also serve as substrates (26), the enzyme's substrate binding requirements are not clearly apparent.



(I) (II)

The minimization of the electrostatic repulsions by the axial orientation of the negatively charged phosphates in phytate compensates for the unfavorable 1,3-steric interactions involving the ester oxygen atoms at carbons 1, 3, 4, 5 and 6. To relieve these interactions the cyclohexane ring undergoes considerable distortion in torsion angles causing ring flattening and angular distortion particularly at C_5. The accuracy of the present refinement does not allow a critical quantitative evaluation of these structural features at this time; for this reason more accurate data are being collected.

The solvation sphere of sodium phytate consists of eight octa-, two hexa-, and two decahedrally coordinated sodium atoms. Although all of the

phosphates are coordinated to sodium, the phosphates at positions C_2 and C_5 show the fewest of these associations. Four molecules of water are not coordinated to sodium but are involved in hydrogen bonds to phosphate oxygens and other water oxygens.

Phytate possesses the ability to bind to hemoglobin and alter hemoglobin's affinity for oxygen in a fashion similar to DPG (5). The blood of birds contains inositol polyphosphates, predominantly 1, 3, 4, 5, 6-inositol pentaphosphate (11), rather than DPG. This similarity in biochemical activity may understandably reflect a resemblance of the conformation of the phosphates on carbons two and three of DPG with the diaxially oriented vicinal, trans phosphates of these inositol esters. A similar conformation has been proposed for DPG based on stereochemical considerations of a model of deoxyhaemoglobin (27).

ACKNOWLEDGEMENTS

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