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Significance And Types of Phytate Degrading Enzymes; Phytase- A Review

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Abstract:
Phosphorus and inositol are stored in seeds of cereals, legumes and oil seeds in the form of phytate. Phytate is an insoluble and chelates ions as well as proteins and thus make them unavailable. They have negative effect on agriculture, nutrition and biological. Phytate degrading enzymes are very important as they hydrolyze phytic acid and make phosphorus available. Till now four types of phytase has been classified that are HAPs, BPPs, PAP and protein tyrosine phosphatases. Present review discusses the significance of phytic acid and phytase as well as the types of phytase known and their role in phytate degradation.

Keywords: Phytic acid, Phytase, Phosphorus

INTRODUCTION

The phosphorus and inositol are contained inside plant seeds in the form of phytic acid. Generally, it comprises about 3-5% of total dry weight predominantly in grains and cereals (Zhang *et al.*, 2010). The total phosphorus in most food of plant origin contains approximately 50-80% as phytate (Harland and Morris, 1995). Phytic acid has the ability to chelates positive divalent cation like calcium, magnesium, iron and zinc. These cations are indispensable in the nourishment of both humans and animals but due to binding with phytic acid the bioavailability of these cations decrease. Therefore, phytic acid has anti-nutritional properties and it reduces intake of nutrient from food in animals and humans (Urbano *et al.*, 2000; Lei *et al.*, 1993). In 1903 Posternak defined phytic acid for the first time. Peffer discovered phytic acid in early 1872. Phytic acid occurs as salts of phytic acid called phytate. Phytases are enzymes capable of hydrolyzing phytic acid to myo-inositol and inorganic phosphorus. Phytases have applicability as animal feed additive, as they improve the dietaryvalue of plant material in feed by elevating the bioavailability of minerals for monogastric animals. In addition, animal feeds lacking phytase can results in massive release of undigested phytate into the surrounding environment. Due to the excess release of phosphorus excreted in the environment the phosphorus assimilating microorganisms grows in large number resulting in eutrophication (Schroder *et al.*, 1996). Phytases are known to occur in plants, animals and microorganisms. However, microbial phytases are most promising for biotechnological application as per various studies (Angelis *et al.*, 2003). In this review we focused our attention on significance and types of phytases.

PHYTIC ACID

Phytic acid is the chief formin which phosphorus and inositol is stored in seeds of cereals, legumes and oilseeds. Phytic acid plays a vital role in seed tissue metabolism as the phosphorus, minerals and myo-inositol is stored and recovered during germination and growth. It was first recognised as calcium/magnesium salt of organic phosphate by Pfeffer in 1872 in the subcellular particles of wheat endosperm.It constitutes up to 60-80% of the total phosphorus in grains. In forage, one-third phosphorus is present as digestible inorganic phosphorus and two–third phosphorus is present as organic phosphorus (phytic acid).

A. *Distribution, content and Occurrence of phytic acid:*

Phytic acid occurs essentiallyin the form of salt of mono and divalent cations (ex.- mg salt in rice and Ca-mg-k salt in soybean) in distinctareas of cereals, legume and grains. It is formed from the esterification of phosphate groups to each of the six hydroxyl groups in myo-inositol molecule. Usually it represents 60-85% of entire phosphorus present in plant seeds. Salts of phytic acids (phytic acid forms insoluble salts with

mono-divalent cations) are called phytate. During the maturation period phytates are accrued in plant seeds and are distributed homogeneously in the cotyledons and embryonic axis in peas, pulses, beans etc. (Reddy and Salukhe, 1982 and Loewus and Murthy, 2000)

B. Chemical structure of phytic acid:

In 1914 **A**derson anticipated structure of phytic acid, which was confirmed on NMR spectroscopy by Johnson and Tate in 1969. They derived conformational structure of phytic acid from ^{31}P-NMR analysis which proposed that the phosphate at 2-position is in an axial position and other phosphate are in an equatorial position. Conversely, Blank et al (1971) resolved that the phosphate group at the 1-, 3-, 4-, 5- and 6- positions are axial and at the 2- position it is equatorial based on study from x-ray analysis. Costello and co-workers (1976) however, supported the conformation suggested by Johnson and Tate. Using^{31}P-NMR as well as pH titration methods pKa value was determined for dissociating groups of phytic acid.Theconclusion drawnthatsixgroupslies between pK_a1.1to2.1,onelies at pK_a5.70 (weak acid),twowithpK_a6.80to7.60, andthreein between pK_a10.0to12.0 (very weak acid range). These studies revealthatphyticacidhasa strongprobabilityforcomplexingmultivalentcationsandpositivelychargedproteins,asit is a stronglynegativelychargedmoleculeoverawidepHrange.

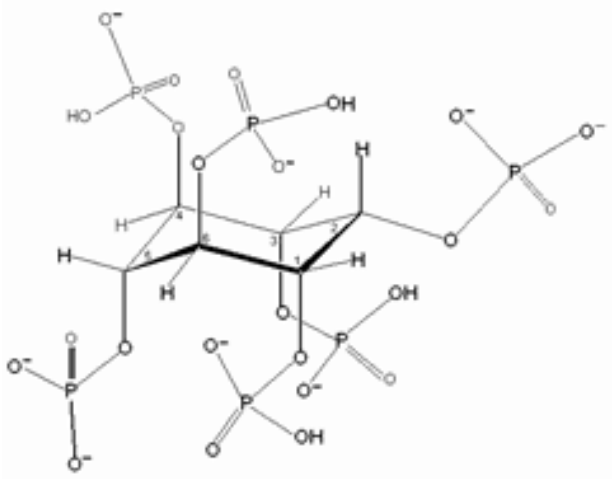

Figure 1. Conformation of phytic acid (D-configuration)(Costello*et al.*1976)

Thus, phytic acid in D-configuration with phosphate at 2-postion in axial position is the most stable conformation.

C. Significance of phytic acid

1. ***Agricultural significance:*** The amount of phosphorus in plants is present in optimal amount that would meet the requirement of optimal growth of animal if all the phytate bound phosphorus were available to animals. The ruminant animals are able to digest phytate because of the phytate hydrolysing enzyme produced by rumen micro-organisms. However, the monogastric animals such as fish, swine, poultry, and humans could not utilise phytate bound phosphorus due to the absence of phytate hydrolysing enzyme in their guts (Singh and Satyanarayana, 2010). Monogastric animals thus excrete the undigested phytic acid in their faeces, which on degradation by soil micro-organisms, release phosphorous in the soil. Therefore, to meet the P requirement additional inorganic phosphorus that is very expensive and non-renewable is added to the animal feed that ultimately increases the feed cost. The undigested phosphorus causes the pollution when it enters the water bodies via soil runoff. Inorganic phosphorus is usually the limiting factor in most fresh waters, therefore its excess in water bodies cause eutrophication (Schroder *et al.*, 1996).
2. ***Nutritional significance:*** Due to the presence of six reactive phosphate groups the phytic acid can chelates with most of the positive molecules such as cations, proteins, starch and lipids. This in turn reduces the digestibility of these molecules.Thus, phytic acid act as an anti-nutritional factor (Urbano *et al.*, 2000 and Lei *et al.*, 2003).

3. ***Effect on bioavailability of minerals:*** **T**here is an inverse relationship between the absorption of minerals and phytic acid. The interaction of minerals with phytic acid reduces the bioavailability of minerals. The interaction of phytic acid with minerals is dependent on pH.(Nolan *et al.*, 1987). Phytic acid is a highly negatively charged molecule and remains highly reactive at a wide pH range. So, if it is a part of diet of animals it reduces the bioavailability of minerals and multivalent ions. The complexes formed are more soluble at acidic pH in comparison to alkaline pH (Fedlund *et al.*, 2006; Lonnerdal *et al.*, 2000; Torre *et al.*, 1991). Another important aspect to be considered is the degree of phosphorylation of inositol, when it is high the absorption of cations is significantly inhibited. The inositol phosphate to mineral ratio plays an important role in solubility of the complexes (Graf and Eton, 1986).
4. ***Effect on bioavailability of proteins:*** The degree of interaction of phytic acid withprotein depends on various factors such as conformation, net charge and interaction with mineral at any given pH.Below the isoelectric point of proteins, at low pH the phytic acid phosphate esters bind to the cationic cluster of basic amino acids, such as, arginine, histidine, lysine, and may form phytate-protein complexes. The interaction occurs through the formation of complexes with divalent cations such as Ca^{++}, or Mg^{++}at a pH above the isoelectric point of proteins. The formation of phytate-protein complex affects the conformation of proteins which may reduce the enzyme activity and also decreases function, solubility, absorption and digestibility of proteins (Kumar *et al.*, 2010).Principally the capability to constrain proteolytic enzymessuch as trypsin, chymotrypsin, amylolytic (amylase) and lipolytic (lipase) is accountable of their anti-nutritional properties (Cheryan and Rackis, 1980).
5. ***Biological significance:*** Recent studies show numerousbiologicalrolesof phytic acid in plant grains and seeds. Phytic acid play several functions such as

a) aphosphorussources
b) anenergysources
c) acation sources
d) asasourceof *myo*-inositol(acellwallprecursor)
e) initiationofdormancy.

Reddy*et al.,* 1989 showed that phyticacidpossiblyaidsseveralotherunfamiliarfunctionsinseeds.Graf *et al.* (1987) suggested thefunctionofphyticacidasanaturalantioxidantinseedsduringdormancy. It is assumed that the antioxidant property of phytic acid is based on the fact that phyticacideffectivelyblocksiron-drivenhydroxylradicalformation.Some studies showed that thepresenceofundigestedphyticacidinthecolonmayprovide protectionagainstthedevelopmentof coloniccarcinoma(Dvorakova,1998) and hence might exertanantineoplasticeffectinanimalmodelsofbothcolonandbreastcarcinomas. Several studies haveconfirmedtheroleofinositolphosphate intermediatesinthetransportofmaterialintothecell. The role of phytic acid in form of intermediates such asinositol triphosphates,insignaltransductionandregulationofcellfunctionsinplantandanimalcells isaveryvigorouslyresearched area(WodzinskiandUllah,1996. A variety of physiological events triggers by the increase of cytosolic free Ca^{2+}. Anantagonist-stimulatedsurge ininositol(1,4,5) triphosphateandinositol(1,3,4,5) tetraphosphateisfrequentlyrelatedwith anincreaseincytosolicfree.

PHYTASE

Phytases (myo-inositol hexakisphosphatephosphohydrolase) are phytate degrading enzymes that hydrolyze phytic acid to *myo*-inositol and inorganic phosphates through a series of *myo*-inositol phosphate intermediates, and eradicate its anti-nutritional characteristics. Suzuki *et al.* in 1907 discovered phytase four years later the discovery of phytic acid in a study done on rice bran hydrolysis in which he observed that the phosphatidylinositol exhibiting varying degree of phosphorylation were generated as intermediates or in some case the end product.

A. Classification of Phytase:

Phytases can be classified on the basis of their catalytic mechanisms, the order in which phosphate groups are liberated or pH optima.

1. ***Based on pH optima:*** Acidic Phytase include enzymes belonging to HAP, PAP and PTP where as alkaline phytase include BPP from bacillus.
2. ***Based on carbon at intialdephoshphorylation in the myo-inositol ring:*** According to the International Union of Pure and Applied Chemistry and The International Union of Biochemistry (IUPAC-IUB), depending on the site where the hydrolysis is initiated in phytate molecule, phytases can be of three types. Firstly **the 3-phytase** (myo-inositolhexakisphosphate-3-phosphohydrolase, E.C. 3.1.3.8) It favourablyreleases the phosphate moiety at C3 position and includes all microbial phytases except *S. Ruminantium*, Second are **6-phytase** (myo-inositolhexakisphosphate-6-phosphohydrolase E.C. 3.1.3.26) It favourablyreleases the phosphate moiety at C6 position and includes mostly plant phytases. Whereas third one are **5-phytase** (myo-inositolhexakisphosphate-5-phosphohydrolase E.C. 3.1.3.72) Itfavourablyreleases the phosphate moiety at C5 position and includes phytases from *S. ruminantium*.
3. ***Based on catalytic mechanism:*** They include four types of phytases that are discussed in the following text.

Histidine acid phosphatasesare heterogenous group of enzymes that at acidic pH catalyse the transfer of a phosphoryl group from phosphomonoesters to water using histidine at an active site. Studies shows that acid phosphatase have two conserved regions of similar sequence, each centred about a conserved histidine residue, that aids in catalytic mechanism. HAPs can hydrolyse different phosphate containing substrate. Not all but Some HAPs can hydrolyze phytate. Both the mouse and fruit fly myo inositol hexakis phosphatase represent a kind of HAPs that do not degrade phytate. Phytate degrading/ hydrolyzing HAPs constitutes a major class of phytases that works under acidic pH. These contains active sites with N-terminal motif RHGXRXP and the C-terminal motif HD (Wodzinski and Ullah, 1996).At acidic pH the active site of HAPs is positively charged at in both prokaryotes and eukaryotes thus can efficiently bind to phytate and hydrolyse it. The hydrolysis of phytic acid completes in a dual-step process. First step involves a nucleophilic attack on phosphorous atom by the histidine in active site, this is followed by hydrolysis of resulting phosphor-histidine intermediate (Vincent *et al.*, 1992).

The crystal structure of HAPs consists of an alpha helical domain and a conserved alpha helical/beta sheet domain, with two helices on each side of the seven stranded sheet. The active site is located at the interface between the two domains. Two important factors determining the enzymes activity/ catalysis are glycosylation and disulphide bridges.

Beta propeller phytase are alkaline phytases that remain active at neutral to alkaline pH with pH optimum (7-8). The molecular structure of this enzyme chiefly comprises of Beta sheets and resembles a hexa-blade propeller; hence named beta propeller phytase. These enzymes need ca^{2+} for their catalytic activity and have specific substrate specificity. The chelation of Ca^{2+}-inositol phosphate mediates the substrate specificity of beta propeller phytase. The function of ca^{2+} ion is activation of water molecule, coordination of the negative charge that build ups in the transition state.The catalytic mechanism involves a catalytic site, an affinity site and a central electronegative solvent-accessible channel that binds seven calcium ions. The structure of a novel thermostable Beta propeller phytase for the first time was determined from Bacillus amyloliquefacience strain. The crystal structure revealed a six bladded beta propeller in which each blade consists of 4-5 stranded anti parallel beta sheet. Catalytic activity is Ca^{2+} dependent and the fully active form contain six ca^{+2} ions. The ca^{2+} phosphate in phytate is axially bonded. The BPP is a thermostable enzyme and its thermostability is dependent on calcium binding to high affinity calcium binding site. Thermostable beta propeller phytase are different from others in having the ability to cleave the phosphate group at C-2 position (Wyss *et al.*, 1999). As these enzymes are dynamic from neutral to alkaline pH and exhibit higher thermostability they could find application in feed industry. Thus, it can be concluded that the enzyme prefers the hydrolysis of every second phosphate and alternatively remove phosphate group with the end product being myoinositol triphosphate.

The bioinformatics studies on microbial genomes and environmental metagenomes advised that the BPP phytase are dispersed more widely and may play some role in bioremediation as phytate phosphorus cycling in soil and aquatic environment.

Purple acid phosphataseis discovered from plants. They have recently been isolated from the cotyledons of germinating soyabeans. PAP are metalloenzymes that require metal ions for their activity. These enzymes have been well studied and searched. Although, genomic database reveals the PAP are present in plants, mammals, fungi and bacteria but only PAP isolated from soybeans has been shown to have some noteworthy phytase activity.The catalysis mechanism and three-dimensional structure for PAP have also been determined.

Protein tyrosine phosphatases are a quiet a recently discovered class of phytase. PTP have been isolated from bacteria inhabiting the gut of ruminant animals (Nakashima *et al.*, 2007). The active site sequence contains motif (His-Cys-(X)5-Arg). The dephosphorylation mechanism consists of a two-step, acid-base mechanism with activity towards phosphorylated tyrosine residues (Zhang, 2004). The exact biological substrates and roles of bacterial PTP-like phytases is yet to be identified and not properly understood.Fascinatingly, sequence and structural homologyof the characterized PTP-like phytases from ruminal bacteria shown similarity with the mammalian PTP-like phosphoinositide/-inositol phosphatase PTEN (Puhl*et al.*, 2007).

COMMERCIAL DEVELOPMENT OF PHYTASE

The very first attempt to commercialize phytase was taken by IMC (International Mineral and Chemicals) in 1962. The attempt was not successful but provided an important fungal isolate *Aspergillusniger* NRRL 3135 that produced highest yield of phytase with two pH optima 5.5 and 2.5 in 1968. The phytase was purified and characterized from *A. niger*, at ARS, southern research centre. In 1991, the researchers at Gist-Brocades cloned, sequenced and over expressed *phy*A from *A. niger* NRRL 3135 which resulted in 52-fold improvement of phytase yield. They also cloned the enzyme along with amyloglucosidase promoter and *A. niger* NRRL 3135 leader sequence into *A. niger*CBS 51388 which resulted in 1400-fold improvement of phytase yield in one of the yield-type non-producer.

Table 1. Commercially available microbial phytases (modified from Cao *et al.* 2007)

Trademark	Company	Country	Phytase source	Production strain
Allzymephytase	Alltech	USA	*A. niger*	*A. niger*
AMAFERM	Biozyme	USA	*A. oryzae*	*A. oryzae*
Avizyme	Fin feeds International	Finland	*A. awamori*	*T. reesei*
Bio-Feed phytase	DSM	USA	*P. lycii*	*A. oryzae*
Finase	AB Enzymes	Germany	*Aspergillus awamori*	*Trichodermareesei*
Finase	Roal	Finland	*A. awamori*	*T. reesei*
Natuphos	BASF	Germany	*A. niger*	*A. niger*
Phyzyme	Fermic	Mexico	*A. oryzae*	*A. oryzae*
Ronozyme® Roxazyme®	Novozyme	Denmark	*Peniophoralycii*	*A. oryzae*
ROVABIO	Genencor International	USA	*P. simplicissimum*	*Penicilliumfuniculosum*
SP, TP and SF	Alko Biotechnology	Finland	*A. oryzae*	*A. oryzae*

The bioengineered strain secreted 7.9g/l of purified phytase with specific activity of 2100 nkat/mg protein. The Gist-Brocades tested the bioengineered enzyme extensively in swine and poultry and after receiving approval from several countries and FDA as GRAs for use in food, the first phytase product was sold by BASF under the trade name Natuphos (Haefner*et al.*, 2005). Several organisms have been screened for the production of phytase with higher catalytic properties. Table 1. list the name of companies that produces phytase at commercial level.

REFERENCES

1. Angelis MD, Gallo G, Corbo MR, Mc Sweeney PLH, Faccia M, Giovine M, and Gobbetti M (2003). Phytase activity in sourdough lactic acid bacteria: purification and characterization of a phytase from Lactobacillus sanfranciscensis CB1. Int. J. Food Microbiol., 87: 259-270.
2. Cao L, Wang W, Yang C, Yang Y, Diana J, Yakupitiyage A, Luo Z and Li D (2007). Application of microbial phytase in fish feed. Enz. Microb. Technol., 40: 497-507.
3. Cheryan M (1980). Phytic acid interactions in food systems. Crit. Rev. Food Sci. Nutr., 13: 297-335.
4. Costello AJR, Glonek T and Myers TC (1976). Phosphorus-31 nuclear magnetic resonance-pH titration of hexaphosphate (phytic acid). Carbohydr. Res., 46: 156-171.
5. DvorakovaJ(1998).Phytase:Sources,PreparationandExploitation.FoliaMicrobiol.,43: 323-338.
6. Fredlund, K., Isaksson, M., Rossander-Hulthén, L., Almgren, A., & Sandberg, A. S. (2006). Absorption of zinc and retention of calcium: dose-dependent inhibition by phytate. Journal of Trace elements in Medicine and Biology, 20(1), 49-57.
7. Harland BF and Morris ER (1995). Phytate: A good or a bad food component. Nutr. Res., 15: 733-754.
8. Kumar, V., Sinha, A. K., Makkar, H. P., & Becker, K. (2010). Dietary roles of phytate and phytase in human nutrition: A review. Food chemistry, 120(4), 945-959.
9. Lei XG, Ku PK, Miller ER, Yokoyama MT andUllrey DE (1993b). Supplementing corn-soybean meal diets with microbial phytase maximizes phytate phosphorus utilization by weanling pigs. J. Anim. Sci., 71: 3368-3375.
10. Loewus, F. A., & Murthy, P. P. (2000). myo-Inositol metabolism in plants. Plant science, 150(1), 1-19.
11. Loewus, F. A., & Murthy, P. P. (2000). myo-Inositol metabolism in plants. Plant science, 150(1), 1-19.
12. Lonnerdal B (2000). Dietary factors influencing zinc absorption. J. Nutr., 130 (5S Suppl): 1378S-1383S.
13. Nakashima, B. A., McAllister, T. A., Sharma, R., & Selinger, L. B. (2007). Diversity of phytases in the rumen. Microbial ecology, 53, 82-88.
14. Puhl, A. A., Gruninger, R. J., Greiner, R., Janzen, T. W., Mosimann, S. C., & Selinger, L. B. (2007). Kinetic and structural analysis of a bacterial protein tyrosine phosphatase-like myo-inositol polyphosphatase. Protein science, 16(7), 1368-1378.
15. Reddy NR, Pierson MD, Sathe SK and Salunkhe DK (1989). Phytates is cereals and legumes. CRC Press, Inc. Boca Raton Fla., 7: 57-70.
16. Reddy NR, Sathe SK andSalunkhe DK (1982). Phytases in legumes and cereals. Adv. Food Res., 82: 1-92.
17. Singh B and Satyanarayana T (2010). Plant growth promotion by an extracellular HAP-phytase of a thermophilic mold Sporotrichum thermophile. Appl. Biochem. Biotechnol., 160 (5): 1267-1276.
18. Suzuki U, Yoshimura K and Takaishi M (1907). About the enzyme “phytase”, which splits “anhydro-oxy-methylene diphosphoric acid” Bulletin of the College of Agriculture, Tokyo Imperial University., 7: 503-512.
19. Urbano, G., Lopez-Jurado, M., Aranda, P., Vidal-Valverde, C., Tenorio, E., &Porres, J. (2000). The role of phytic acid in legumes: antinutrient or beneficial function?. Journal of physiology and biochemistry, 56(3), 283-294.
20. Vincent, J. B., Crowder, M. W., & Averill, B. A. (1992). Hydrolysis of phosphate monoesters: a biological problem with multiple chemical solutions. Trends in biochemical sciences, 17(3), 105-110.
21. Wodzinski RJ and Ullah AHJ (1996). Phytase. Adv. Appl. Microbiol., 42: 263-301.
22. Wyss M, Brugger R, Kronenberger A, Remy R, Fimbel R and Oesterhelt O (1999b). Biochemical characterization of fungal phytases (myoinositolhexakisphosphate-phosphohydrolases): Catalytic properties. Appl., Environ. Microbiol., 65: 367-373.
23. Wyss M, Pasamontes L, Friedlein A, Remy R, Tessier M and Kronenberger A (1999a). Biophysical characteri
24. zation of fungal phytases (myoinositolhexakisphosphate - phosphohydrolases): Molecular size, glycosylation pattern and engineering of proteolytic resistance. Appl. Environ. Microbiol., 65: 359-366.
25. Zhang L, Lijia A, Xiaorong G and Wang Y (2004). Properties of A. FicuumAS3.324 phytase expressed in tobacco. Process Biochem., 40: 213-216.