Characteristics of Phytase Enzyme and its Role in Animal Nutrition

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A B S T R A C T

Cereals, legumes and oilseed crops are grown over 90% of the world’s harvested area. Together they serve as a major source of nutrients for the animal kingdom. An important constituent of seeds of these crops is phytic acid (myo-inositol 1, 2, 3, 4, 5, 6-hexakisphosphate; Ins P$_6$). The salt form, phytate, is an anhydrous storage form of phosphate accounting for more than 80% of the total phosphorus in cereals and legumes. In contrast to other organo-phosphate molecules, phytate contains a high phosphate content, which results in a high negative charge over a wide pH range. Under normal physiological conditions phytic acid chelates essential minerals such as calcium, magnesium, iron and zinc. Phytic acid also binds to amino acids and proteins and inhibits digestive enzymes. Thus, phytic acid is an anti-nutritive component in plant-derived food and feed, and therefore, the enzymatic hydrolysis of phytic acid is desirable. The enzyme phytase is able to release the bioavailable phosphorus from phytic acid, consequently improving the phosphorus bioavailability and uptake of minerals.

Keywords
Phytase, Phytate, phosphorus, Hydrolysis, Nutrition

Introduction

Phosphorus is one of the most limiting nutrients for animals because most of the phosphorus in the plant seeds including the feeding plants is in the form of phytic acid, most of which cannot be digested by monogastric animals and acts as an antiinutritional factor hindering the uptake of a range of minerals. In the current poultry industry, phytase is commonly added to poultry diets to improve P utilization, which leads to a decrease in feed cost and P excretion in the environment (Dersjant et al., 2015). A better understanding of in vivo phytase activity is important so as to use phytase more economically and efficiently.

myo-Inositol hexakisphosphate

myo-inositol hexakisphosphate is the most abundant phosphorylated derivative of myo-inositol found in nature and has been more commonly referred to as phytic acid (Figure 1). Phytic acid is chemically described as myo-inositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate or Ins P$_6$ (IUPAC-IUBMB, 1992).

Occurrence and Distribution

The seeds of cereal grains and legumes show the highest content of phytate among plants (Reddy et al., 1989). Ins P$_6$ has also been found in pollen, spores (DeMaggio and Stetler, 1985), and vegetative tissues, such as...
roots, stems and leaves (Campbell et al., 1991). Until recently, Ins P₆ was thought to be restricted to plants, but later studies have revealed that higher inositol polyphosphates are widespread and perhaps ubiquitous among eukaryotes (Sasakawa et al., 1995). Non-ruminants are unable to metabolize phytate, making a majority of phosphorus in seed unavailable to these animals. The addition of phytase to the diet of monogastric livestock has been examined as a way to reduce phosphorus pollution resulting from intensive livestock operations (Reddy et al., 1989).

**Phytic acid hydrolysis**

The principle end products of phytase action are phosphoric acid and myo-inositol, but the phosphatidylinositols representing various degrees of dephosphorylation from inositol hexakisposphate to inositol are generated as intermediates, or in some cases, as end products (Figure 2) (Wodzinski and Ullah, 1996).

Phytate also chelates trace elements of iron and zinc between phosphate groups within a single phytate molecule or between two phytate molecules. Phytase is the only known enzyme that can initiate the phosphate hydrolysis at carbon 1, 3 or 6 in the inositol ring of phytate. The removal of phosphate group by phytase results in releasing of calcium, iron, zinc, and other metals. Most characterized phytases hydrolyze Ins P₆ in a stepwise manner, yielding myo-inositol pentakis-, tetrakis-, tris-, bis- and monophosphate products (Konietzny and Greiner, 2002).

**Sources of phytases**

**Microbial sources**

Phytate degrading enzymes have been most commonly found in fungi, particularly from the Aspergillus species (Konietzny and Greiner, 2002). The phytase from A. ficuum was the first studied for use as a commercial product. Phytate degrading enzymes have also commonly been found in many bacteria.

**Plant sources**

Phytase activity has been found in many plants, such as maize (Laboure et al., 1993), barley, rye (Greiner et al., 2000), spelt (Konietzny et al., 1995), canola seed (Houde et al., 1990) and lily pollen (Scott, 1991).
Animal sources

Phytate-degrading enzymes have been isolated from the intestinal mucosae of some monogastric animals (Chi et al., 1999). A multiple inositol polyphosphate phosphatase (MIPP) displaying phytate degrading activity was also identified in rat hepatic tissue, localized in the ER lumen. A phytate-degrading enzyme has also been purified and characterized from the protozoan Paramecium (Freund et al., 1992).

The physiological nature of phytate

Phytate can exist in a metal-free form or in metal–phytate complex, depending on the pH of the solution and the concentration of metal cations. At acidic pH, protonation of the phosphate groups of phytate generates the metal-free form. At neutral pH, in contrast, deprotonation of the phosphate groups of phytate enhances the affinity for divalent metal cations and thus phytate forms metal–phytate complexes with divalent metal cations, mostly Mg$^{2+}$ and Ca$^{2+}$ (Maenz et al., 1999). During the germination of seed, phytate is degraded by phytases, providing phosphate and minerals for the growing seedlings. In addition to its role in phosphate storage, phytate acts as a strong chelator for divalent metal cations and exists as a stable metal–phytate complex with divalent metal cations (mostly K$^+$, Mg$^{2+}$, Ca$^{2+}$, or Zn$^{2+}$) in plants (Reddy et al., 1989).

Storage of phytic acid

Phytic acid and its co-precipitated cations are stored in electron dense spherical particles named globoids. The globoids are localised predominantly in the aleurone layer (wheat and barley) or in the embryo (maize) (O’Dell et al., 1972). They are compartmentalised inside protein storage vacuoles in the seeds. The phosphorus fraction stored as phytate range from 30% in roots up to 80% in seeds and cereals. The highest amount of phytate among cereals is found in maize (0.83-2.22%) and among legumes in dolique beans (5.92-9.15%) (Reddy et al., 1989).

Dephytinisation and nutrition

The chelating properties of PA not only result in the binding of cations in seeds. Iron and zinc uptake have both been shown to be inhibited when the phytic acid: metal ratio increases above 10:1 (Gharib et al., 2006). Milling of cereals removes the phytic acid, but this treatment also removes the major parts of the minerals and dietary fibres and cannot therefore be a nutritional solution to the problem. Similarly, soaking or extracting in aqueous solutions can remove up to two thirds of the PA by the action of endogenous phytase activity, but loss of minerals, water-extractable proteins and vitamins also occurs (Hurrell, 2004).

Avoiding PA formation in the first place or catalysing its degradation by the use of PA hydrolysing enzymes would therefore be more beneficial approaches to dephytinisation. Reducing phytate content through lpa mutants have been attempted through knock-out of genes involved in PA biosynthesis. Furthermore, the yield or germination ability is affected if PA content is reduced more than 50%, thereby making this approach unattractive from an economic perspective.

Instead of blocking its biosynthesis, an attempt to reduce PA in wheat products has been performed by introducing the Aspergillus niger phytase gene phyA into a wheat variety by particle bombardments of immature wheat embryos (Holm et al., 2002). Wheat, barley and rye all have high phytase activity in the grain, whereas maize, millet and sorghum have low initial phytase activity that increase rapidly after germination (Egli et al., 2002).
The biochemical properties of phytases

Most phytases belong to either the acid phytases or the alkaline phytases, depending on their optimal pH for catalytic activity. Histidine acid phosphatases (HAPs) from *E. coli*, *K. terrigena* (Greiner et al., 1997), *Aspergillus niger*, *Aspergillus fumigatus* (Ullah et al., 2000), canola seeds (Houde et al., 1990), and spelt (Konietzny et al., 1995) have an optimal pH range of 4.5–5.5. In contrast, alkaline phytases from *Bacillus* (Idriss et al., 2002) and some plant seeds, such as *Typha latifolia* L. pollen and *Lilium longiflorum* pollen (Scott, 1991), have an optimal pH range of 6.5–8.0. All phytases are monomeric proteins, except for phytase B from *A. niger*, which is a tetramer. The molecular masses of the enzymes are quite variable, within the range 38–100 kDa. Most phytases have an optimal temperature of 44–60°C. In contrast, phytases from *Aspergillus fumigatus* and *Bacillus amyloliquefaciens* have an optimum temperature of about 70°C. To use phytases as animal feed additives, thermostability of the enzyme is a highly desirable property during the animal feed-pelleting process (80–100°C). Alkaline phytases from *Bacillus* are quite stable at the high temperature range of 80–95°C, while other phytases are rapidly inactivated above 60°C (Tomschy et al., 2002). The phytase from *Bacillus amyloliquefaciens* is an extremely thermostable enzyme, based on Tm values (80°C, the denaturation temperature) determined by differential scanning calorimetry (Ha et al., 2000).

Molecular and biophysical characteristics of phytases

They are classified according to their pH optimum as acid or alkaline phytases, according to the position of their initial hydrolysis of phytate as 3-phytases, 6-phytases or 5-phytases, and according to their catalytic mechanisms as belonging to the histidine acid phosphatases (HAPs), purple acid phosphatases (PAPs), cysteine phosphatases (CPs) or β-propeller phytases.
One of the major classes is the family of HAPs sharing a highly conserved RHGXRXP motif (Van Etten et al., 1991). The HAP class can be further subdivided into three 368 different groups (PhyA–PhyC), based on amino acid sequence homology and biochemical properties, such as optimal pH and the position-specificity of phytate hydrolysis. Group I, PhyA, consists of enzymes with 465–469 amino acids and includes extracellular HAPs from Aspergillus niger, Aspergillus niger (awamori) (Piddington et al., 1993), Aspergillus fumigatus, Aspergillus terrus, Emericella nidulans, Talaromyces thermophilus (Pasamontes et al., 1997). These phytases have two optimal pH values (2.5, 5.5) and an optimal temperature at 55–60°C (Wyss et al., 1999). The molecular mass of the unglycosylated phytases is predicted to be 48–50 kDa.

Group II, PhyB, contains phytases that are extracellular HAPs from Aspergillus niger (Ethrlich et al., 1993), Saccharomyces cerevisiae and Shizosaccharomyces pombe (Elliott et al., 1986). They are composed of 453–479 amino acids and the molecular mass is ~48–50 kDa. The apparent molecular mass of the glycosylated proteins is 65 kDa by SDS-PAGE and about 270 kDa by analytical centrifugation (Kostrewa et al., 1999). These phytases have a single optimal pH of 2.5, lack any activity at pH 5.0 or higher, and have an optimal temperature of 55–60°C. PhyB hydrolyzes the same position of metal-free phytates and is classified as a 3-phytase.

Group III, PhyC, contains acid phytases from E. coli (Dossa et al., 1990), lysosomal (Geier et al., 1991), and prostatic acid phosphatases (Van Etten et al., 1991) from rat and human. These phytases are intracellular proteins composed of 354–439 amino acids, with a molecular mass of ~42–45 kDa; and these monomeric proteins are nonglycosylated enzymes. PhyC have a single optimal pH (~5.0–6.0) and exhibit an optimal temperature at 40–60°C. Group III phytases are also capable of hydrolyzing not only metal free phytate but also various other phosphate esters, like other HAPs (Wyss et al., 1999). However, these phytases are considered as a 6-phytase, since they hydrolyze phytate preferentially at the D-6 (1-4) position (Lei et al., 2013).

Another major class (Class II) contains alkaline phytases, which differ from HAPs in many aspects, including optimal pH, molecular mass, tertiary structure, substrate specificity, and calcium ion requirement for enzymatic catalysis. Based on these biochemical differences and phylogenetic data, alkaline phytases from Bacillus and some plant seeds can be classified as another group: PhyD. Group IV, PhyD, consists of the phytate-specific enzymes from Bacillus and some plants, such as T. lattifolia pollen (Hara et al., 1985), L. longiflorum pollen (Barrientos et al., 1994), and some legume seeds (Scott, 1991). Comparison of amino acid sequences among these enzymes is impossible, since no amino acid sequence data is available for the plant alkaline phytases. However, these plant enzymes have biochemical characteristics very similar to those of the phytases from Bacillus (Oh et al., 2001). They are composed of 383 amino acids and encode an extracellular monomeric protein. The molecular mass is ~42 kDa; and SDS-PAGE gives an apparent molecular mass of ~38–44 kDa. A phytase that works over a wide range of pH and is active up to the stomach and upper intestine would be the ideal phytase for animal feed (Dersjant et al., 2015).

Crystal structure

The crystal structure of the A. niger and E. coli enzyme closely resembles the overall fold
of other histidine acid phosphatases. These structures contain a conserved α/β-domain and a variable α-domain. The active site is located at the interface between the two domains. Differences in substrate binding have been attributed to differences in the α-domain. The proposed structures also provide information about substrate binding and the catalytic mechanism on the molecular level (Lim et al., 2000).

The ruminants digest phytic acid through the action of phytases produced by the anaerobic gut fungi and bacteria present in their rumenal microflora. However, monogastric animals such as pig, poultry and fish utilize phytate phosphorus poorly because they are deficient in gastrointestinal tract phytases. Therefore, supplemental inorganic phosphate is added to their feed to meet the phosphate requirement and to ensure good growth. However, supplemental inorganic phosphate does not diminish the anti-nutritive effect of phytic acid. Therefore, phytase has become an important industrial enzyme and is the object of extensive research. By working efficiently on the substrate in the prevailing conditions, supplemental phytase could diminish the anti-nutritive effects of phytic acid and reduce the cost of diets by removing or reducing the need for supplemental inorganic phosphate.

In addition, phytase would be an environmentally friendly product, reducing the amount of phosphorus entering the environment. Recently, was conducted to determine the effect of superdosing phytase on productive performance and egg quality in laying hens (Jong Hyuk Kim et al., 2017). Hence, Nutritional genomics is expected to improve efficiency of P utilization in livestock (Kebrab et al., 2012). Approaches to problems represented by seed phytic acid include engineering crops to express high levels of phytase enzyme in seeds (Raboy et al., 2000), or breeding crops with reduced levels of seed phytic acid (low-phytate or high-available P (Cichy and Raboy, 2008).

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