Phytases: attributes, catalytic mechanisms, and applications

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Phytic acid, which was discovered in 1903, has been found to be a nearly ubiquitous component in cereals and grains (Posternak, 1903). The widespread occurrence of phytate in the plant kingdom is accompanied by the need for plants, microorganisms and some animals to hydrolyze the compound. A number of phytate-degrading enzymes have now been reported and studied. The detailed characterization of some of these enzymes has revealed that nature did not develop a single catalytic mechanism to cleave phosphate groups from phytate in all these diverse organisms. The recognition that not all phytases are structurally similar or share a common active site has become important for anyone who wishes to fully comprehend research in this field. Today, four distinctly different classes of phytases are recognized: histidine acid phosphatase, β-propeller phytase, cysteine phosphatase, and purple acid phosphatase.

Histidine acid phosphatase (HAP). Phytases belonging to this class are the most widely studied and utilized today. Representatives of this large class of enzymes are known to occur in animals, plants and microorganisms (Wodzinski and Ullah, 1996). A common active site motif, RHGXRXP, is shared by all HAPs (Ullah *et al*., 1991). The catalytic histidine in this sequence initiates a two-step reaction that results in the hydrolysis of phosphate monoesters. The term Histidine Acid Phytase (HAPhy) has been advanced to designate the HAPs that can accommodate phytate as a substrate (Oh *et al*., 2004). Both prokaryotic and eukaryotic HAPhys are known and they share little sequence homology other than the conserved active site motif. Among prokaryotic phytases, the one produced by *Escherichia coli* is the best-characterized HAPhy (Greiner *et al*., 1993). A 3-D molecular model of its structure is available, and a eukaryotic version of the enzyme from *Aspergillus niger* has been advanced for use as an animal feed additive. In eukaryotes, HAPhys have been cloned in maize and in a number of fungal isolates. The most widely studied fungal phytases are from *A. niger* and *A. fumigatus*. Theses studies revealed that there are two classes of HAPhys. The first class has broad substrate specificity but a lower specific activity for phytic acid; the second class has narrow substrate specificity but a high specific activity for phytase (Wyss *et al*., 1999). Evidence from site-directed mutagenesis studies established the importance of certain amino acid residues that make up the substrate specificity site in fungal HAPhys. Mutating these key amino acids leads to changes in substrate affinity and the pH profile (Mullaney *et al*., 2002). While not directly involved in the catalytic mechanism of HAPhys, the conservation of an eight-cysteine motif appears to be essential to maintain the proper molecular structure necessary for the enzyme activity in fungal phytases (Mullaney and Ullah, 2005). Today, the major application for HAPhys is in the hydrolysis of phytate in cereal and grains in animal feed. Future applications extend from the development of plant cultivars that require less P fertilizer to modification for use as a peroxidase.

β–Propeller Phytase (BPP). A wide range of catalytic functions has been ascribed to proteins possessing the β-propeller molecular architecture (Pons *et al*., 2003). A 3-D drawing of BPP molecule shows a shape that resembles a propeller with six blades (Ha *et al*., 2000). A

novel calcium dependent *Bacillus* phytase that has this configuration has been cloned and characterized. It lacks the RHGXRXP sequence motif and therefore, it is not a member of HAP. It requires Ca^{2+} for both activity and thermostability (Kim *et al*., 1998). This phytase employs two phosphate-binding sites, a cleavage site for substrate hydrolysis and an affinity site to bind the substrate (Shin *et al*., 2001). β–propeller phytases share an optimum pH range with some alkaline plant phytases. The molecular structure of these plant phytases has yet to be determined, but they display some common traits with β-propeller phytases. They both have a narrow substrate range while requiring calcium for activity and only remove three phosphates from phytic acid to yield inositol trisphospahte as a final product. No commercial applications are available thus far for BPP, but it has been advanced as an animal feed additive and as a means to promote plant growth under phosphate limiting conditions.

Cysteine Phosphatase (CP). Recently, another class of phytase has been reported from an anaerobic ruminal bacterium, *Selenomonas ruminantium* (Chu *et al*., 2004). Its optimum temperature ranged between 50–55^{o}C with optimal activity in the pH range of 4.0–5.0 depending on the buffer used. Lead cations enhance activity, while Fe^{2+}, Fe^{3+}, Hg^{2+}, and Zn^{2+} ions strongly inhibited the enzyme. Sequence homology studies support similarities between this phytase and the catalytic domain found in the cysteine phosphatase superfamily. Its 3-D structure's accession number is 1U24. The structure of this phytase consists of one large and one small domain. Towards the C-terminal, near the edge of the large domain is a shallow pocket containing a two loop structure similar to the active site found in protein tyrosine phosphatase with the catalytically important HCXXGXXR(T/S). This enzyme catalyzes dephosphorylation of phytic acid to *myo*-inositol monophosphate.

Purple acid phosphatase (PAP). Characterization of a soybean (*Glycine max* L. Merr.) phytase has revealed the purple acid phosphatases sequence motif, DXG..GDXXY. .GNH(E,D)..VXXH..GHXH. The *GmPhy* phytase, found in germinating soybean seedlings, apparently contains the catalytic mechanism associated with this large class of metalloenzymes (Hegeman and Grabau, 2001). This and a putative rice (*Oryza sativa*) phytase are the only PAP phytases currently deposited in GenBank. As compared to fungal phytase, this soybean seed phytase has a relatively low specific activity for phytic acid. It has been proposed that the low catalytic activity of *GmPhy* may be advantageous in plant seed where a slow and balanced breakdown of phytate during germination could be efficacious. No 3-D model of soybean phytase is available, and no commercial applicant is envisioned.

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