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Review Article

Plant phytases: Occurrence, classes, purification strategies and physico-chemical and kinetic properties

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ABSTRACT

Phytases, a subgroup of phosphatases with general preference for phytate, are particularly important in human nutrition for their possible role in the degradation of phytate during both food processing and gastrointestinal transit. High phytate degrading activity is reported in cereals, pseudo-cereals and legumes. Depending on their optimum pH, amino acid residues in the active site and preferential removal of phosphate groups have been classified into different groups. Physico-chemical and kinetic properties of plant phytases vary widely. Occurrence, classes and properties of plant phytases have been presented in this mini review.

Keywords: Phytases, occurrence, classes, kinetic properties

INTRODUCTION

Phosphatases constitute a diverse group of enzymes that catalyze the hydrolysis of phosphomonoester bonds of phosphate esters. Phytases are a subgroup of phosphatases with general preference for phytate, which is hydrolyzed in a stepwise manner generating phosphoric acid and myo-inositol phosphates.

Non-specific acid phosphatases constitute another subgroup of phosphatases, which show high hydrolysis rates with monophosphorylated compounds but lesser activity against phytate.

Phytases are particularly important in human nutrition for their possible role in the degradation of phytate during both food processing and gastrointestinal transit (Sandberg & Andlid, 2002). Studies have demonstrated that the degradation of phytate in the stomach and intestine is mainly due to dietary phytases as well as due to the metabolic activity of the colonic microorganisms (Sandberg & Andlid, 2002).

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OCCURRENCE OF PHYTASES

High phytate degrading activity is reported in cereals (rye, triticale, wheat, barley) and in pseudo-cereals (buckwheat, amaranth). In cereals phytate degrading activity was found to be mainly associated with the aleurone layer (Gabard & Jones, 1986). In legumes phytate degrading activity was reported to be in the cotyledon (Gibson & Ullah, 1988; Hegman & Grabau, 2001).

Viveros et al. (2000) & Steiner (2007) compared phytate-degrading enzyme activities in legume seeds, cereals and cereal by-products. They estimated intermediate phytase activities in cereals which, however, varied over a wide range. Viveros et al. (2000) analyzed many cereals and legumes and found that rye contained the highest activity

of phytase and acid phosphatase (5147 U kg^{-1}; 21955 U g^{-1}, respectively) followed by wheat, rye bran and wheat bran. Legume seeds and oilseeds contained negligible phytase activity and a moderate amount of acid phosphatase activity, except for kidney bean (33433 U g^{-1}) and full-fat linseed meal (13263 U g^{-1}). They further reported that phytase and acid phosphatase were positively correlated with respect to phytate phosphorus in cereals and cereal by products, and negatively correlated in legume seeds and oilseeds. Ratio of acid phosphatase to phytase as low as 3.8 in barley to as high as 633 in beet pulp was found. In millet this ratio was 83.5. Even for one plant species, data for phytase and acid phosphatase activities reported by different authors varied considerably (Viveros et al., 2000; Greiner & Egli, 2003; Steiner et al., 2007; Zarei, 2007). Marounek et al. (2011) compared phytase activity of 26 samples of cereals, legumes and oilseeds and also found that rye had by far the highest phytate dephosphorylation activity followed by wheat. Compared to 2114 units kg^{-1} of activity in wheat, millet sample had only 95 units kg^{-1} of activity.

Azeke et al. (2011) compared rice, maize, millet, sorghum and wheat grown in Nigeria for phytase activity. Phytase activity was high (0.21–0.67 U g^{-1}) in all samples. Phytate content ranged between 5.6 and 6.2 mg g^{-1} while total phosphorus content ranged between 3.3 and 4.3 mg g^{-1}. The variations could be due to differences in the varieties, growth conditions and application of fertilizers (Liu et al., 2006; Steiner et al., 2007; Kaya et al., 2009).

CLASSES OF PHYTASE

Phytate degrading enzyme, phytase have been studied intensively in recent years. Two main types of phytate degrading enzymes have been identified: acid phytate degrading enzymes with pH optimum around pH 5 and alkaline phytate degrading enzymes with pH around pH 8. Based on the amino acid residues in the active site phytase can be histidine acid phosphatase, β-propeller phosphatase, cysteine phosphatase and purple acid phosphates (Mullaney & Ullah, 2007). Two classes of phytate-degrading enzymes are recognized by the International Union of Pure and Applied Chemistry and the International Union of Biochemistry (IUPAC-IUB); 3-Phytase (EC 3.1.3.8) initially removes orthophosphate from the D-3 position of phytate, whereas 6-phytase (EC 3.1.3.26) preferentially initiate the phytate dephosphorylation at the L-6 position of the *myo*-inositol ring. Phytate-degrading enzymes from micro-organisms are considered to be 3-phytases, whereas seeds of higher plants are said to contain 6-phytases. The phytase (*myo*-inositol hexakishosphate 3- and 6-phosphohydrolases; EC 3.1.3.8 and EC 3.1.3.26) are the subfamily of high molecular weight histidine acid phosphatases that can hydrolyze phytic acid to inositol and free orthophosphate (Pointillart, 1984; Wyss et al., 1999). Most of the so far described enzymes belong to the acidic type and the optimal pH ranges from 4.5 to 6.0 (Konietzny & Greiner, 2002).

PURIFICATION OF PHYTASES

Phytase has been extracted either in water or acetate, Tris-HCl or maleate buffer of varying concentration. In most of the studies pH of the extraction media was near 5.0. However, only in few studies buffers of neutral or higher pH have been used. Summary of the extraction buffer/media used by the investigators is given in the Table 1. For extracting the enzyme from wheat flour, navy bean and from pollen additives like $CaCl_2$, β-mercaptoethanol, glutathione and triton has been used. Purification of plant phytases have been carried out using techniques such as ammonium sulphate fraction (Verma et al., 2011; Labourne et al., 1993; Peers, 1953; Gibbins & Norris, 1963; Singh & Sedeh, 1979; Scott & Loewus, 1986; Hûbel & Beck, 1996; Lolas & Markakis, 1997; Greiner et al., 2000; Belho et al., 2016), methanol fractionation (Verma et al., 2011), acetone precipitation (Greiner et al., 2000), gel filtration (Gibbins & Norris, 1963; Hara et al., 1985; Scott & Loewus, 1986; Labourne

Table 1: Summary of extraction media and procedures adopted by investigators for partial purification of phytase by ammonium sulphate fractionation from various sources

Source	Ext buffer/ medium	pH	Amm. sulpahte saturation (%)	Activity in crude extract	Activity in Ammonium. Sulphate fraction	Yield (%)	Reference source
Cereals							
Rye	0.25 M Acetate	5.5	ND	5147 µmol Pi/min	ND	ND	Greiner et al. (1998)
Triticale	Acetate	5.2	95%	1240 µg Pi/hr	2360 µg Pi/hr	ND	Singh & Sedeh (1979)
Wheat (Flour)	Water	ND	ND	ND	274 µg P/hr/ml	ND	Peers (1953)
Wheat (Bran)	Water	ND	50 to 100	100400 µmol Pi/hr	96000 µmol Pi/hr	95	Nagai & Funahashi (1962)
Wheat (Flour*)	0.05 M Acetate [1]	5.3	ND	0.3 to 0.9 µmol Pi/min	ND	ND	Kotber et al. (2003)
Wheat	0.05 M Acetate	5.3	40 to 70	738.9 µmol Pi/min	608.3 µmol Pi/min	82.3	Verma et al. (2011)
Barley	0.02 M Acetate	5	35 to 80	445 µmol Pi/min	468 µmol Pi/min	105	Greiner et al. (2000)
Maize (Germinated)	0.1 M Acetate	4.8	30 to 60	ND	4111 nm Pi/min	100	Labourne et al. (1993)
Maize (Root)	0.05 M Acetate	4.5	0 to 80	758 µmol Pi/min	162 µmol Pi/min	21.3	Hûbel & Beck (1996)
Pulses							
Dwarf bean	10 mM Maleate	6.4	30 to 50	226 µgl Pi/3hr/ml	753 µg Pi/3hr/ml	97	Gibbins & Norris (1963)
Navi beans	2 % $CaCl_2$	5	35 to 80	ND	ND	ND	Lolas & Markakis (1997)
Faba beans *	0.1 M Acetate	5.2	ND	1.63 µM Pi/min/g	ND	ND	Abdel-Gawad et al. (2013)
Pea *	0.1 M Acetate	5.2	ND	1.61 µM Pi/min/g	ND	ND	Abdel-Gawad et al. (2013)
Lentil *	0.1 M Acetate	5.2	ND	1.80 µM Pi/min/g	ND	ND	Abdel-Gawad et al. (2013)
Kidney bean *	0.1 M Acetate	5.2	ND	1.80 µM Pi/min/g	ND	ND	Abdel-Gawad et al. (2013)
Rice bean (germinated)	0.5 M Tris-HCl	7	0 to 50	ND	ND	ND	Belho et al. (2016)
Pollens							
Typha latifolia	10 mM Tris-HCl [2]	7.5	ND	18.26 µmol Pi/min	ND	ND	Hara et al. (1985)
Lilium longiflorum	10 mM Tris-HCl [3]	7.6	25 to 55	2.10 µmol Pi/min	1.82 µmol Pi/min	86.5	Scott and Loewus (1986)

1 β-glucanase and endo-xylanase
2 0.1mM Cacl2, b-marcaptoethanol,0.5M sucrose
3 0.1mM GSH, 0.1mM $CaCl_2$ and 0.1% Triton
** Crude extract*

et al., 1993; Greiner et al., 2000), ion exchange chromatography (Hara et al., 1985; Scott & Loewus, 1986; Labourne et al., 1993; Hûbel & Beck, 1996; Greiner et al., 2000; Verma et al., 2011; Belho et al., 2016). One major problem in the purification of phytase from plant is the separation of phytase from contaminating nonspecific acid phosphatase (Konietzny et al., 1995). The extraction of phytate-degrading activity is strongly enhanced by the presence of triton X 100, suggesting at least an association with membrane structure partly (Scott & Loewus, 1986; Scott 1991; Greiner & Egli, 2001). High purification of phytase from oat (Greiner & Alminger, 1999) and faba beans (Greiner et al., 2001) respectively was achieved using seven-step purification protocol, including ammonium sulphate fractionation, acetone precipitation, ion exchange chromatography and gel filtration.

Table 2: Physicochemical and kinetic properties of phytase purified from various sources

Source	Isoform	Mol. wt. (kDa)	Optimum pH	Optimum Temp.	Thermo-stability	Km value	Reference source
Cereals							
Rye		ND	6.0	45°C	ND	ND	Greiner et al. (1998)
Triticale		ND	5.4	45°C	ND	0.22 x 10^{-3} M	Singh & Sedeh (1979)
Wheat Flour		ND	5.2	55° C	55°C, 10 Min	0.33 x 10^{-3} M	Peers (1953)
Wheat Bran		ND	5.0	35°C	60°C, 2 Min	0.57 x 10^{-3} M	Nagai & Funahashi (1962)
Wheat Flour*		ND	5.3	50° C	60°C, 60 Min	ND	Kotber et al. (2003)
Wheat	PHY 1	64	6.0	55°C	ND	0.123 x 10^{-3} M	Verma et al. (2011)
	PHY 2	62	5.5	55°C	ND	0.123 x 10^{-3} M	
Barley	P1	67	5.0	45°C	60°C, 90 Min	0.072 x 10^{-3} M	Greiner et al. (2000)
	P2	67	6.0	55°C	70°C, 90 Min	0.190 x 10^{-3} M	
Maize (germinated)		76 (38)	4.8	55°C	65°C, 10 Min	0.117 x 10^{-3} M	Labourne et al. (1993)
Maize root	1 (5.0)	76 (38)	5.0	40°C	ND	0.043 x 10^{-3} M	Hûbel & Beck (1996)
	2 (4.9)	76 (38)	5.0	40°C	ND	0.025 x 10^{-3} M	
	3 (4.8)	76 (38)	5.0	40°C	ND	0.024 x 10^{-3} M	
Pulses							
Dwarf bean		ND	5.2	40°C	ND	0.15 x 10^{-3} M	Gibbins & Norris (1963)
Navi beans		ND	5.3	50° C	ND	0.018 x 10^{-3} M	Lolas & Markakis (1997)
Faba beans		ND	5.2	50° C	70°C, 10 Min	0.14 x 10^{-3} M	Abdel-Gawad et al. (2013)
Pea		ND	5.0	45°C	70°C, 10 Min	0.07 x 10^{-3} M	Abdel-Gawad et al. (2013)
Lentil		ND	5.0	50° C	65°C, 10 Min	0.18 x 10^{-3} M	Abdel-Gawad et al. (2013)
Kidney bean		ND	5.2	50° C	70°C, 10 Min	0.07 x 10^{-3} M	Abdel-Gawad et al. (2013)
Rice bean (germinated)		110 (66 & 44)	4.0	40°C	4°C, 35 days	0.197 x 10^{-3} M	Belho et al. (2016)
Pollen							
Typha latifolia		ND	8.0	37°C	ND	0.017 x 10^{-3} M	Hara et al. (1985)
Lilium longiflorum		ND	8.0	55°C	ND	0.07 x 10^{-3} M	Scott & Loewus (1986)

** Crude extract*
ND- Not determined

PHYSICO-CHEMICAL AND KINETIC PROPERTIES OF PLANT PHYTASES

Physico-chemical and kinetic properties of phytase isolated or partially purified from some cereal grains or products, legumes seeds or pollen are summarized in Table 2. Molecular weight of phytase ranges from 76 kDa to 110 kDa. Isoforms have been reported in wheat, maize and barley. The enzyme is a monomer purified from wheat and barley whereas a homodimer from maize and a heterodimer from rice been has been reported. Irrespective of the tissue, optimum pH for most of the plant phytases is in the acidic range 4.0 to 5.5.

The enzyme purified from pollens, however, was optimally active at pH above 7.0. Phytase from pollen of *Lilium longifloram* showed optimum pH of 8.0. Optimum temperature for plant phytases ranges from 35 to 55^{o}C. Plant phytase seem to be thermolabile.

In most of the cases the enzyme started losing its activity at a temperature between 60 and 70^{o}C upon incubation for a short period of 10 min. Km value ranged from 0.017 to 0.57 x 10^{-3} M (Table 2) for the different sources of the enzymes.

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