Purification and Catalytic Properties of a Phytase from Scallion (*Allium fistulosum* L.) Leaves

Brian Q. Phillippy†

U.S. Department of Agriculture, Agricultural Research Service, Southern Regional Research Center, 1100 Robert E. Lee Boulevard, New Orleans, Louisiana 70124

Levels of phytase comparable to that in wheat germ were detected in a variety of fresh vegetables. Scallion leaf phytase was purified 31 000-fold to an activity of 500 μmol min^{-1} mg^{-1} at 37 °C and had a K_m of 200 μM for inositol hexakisphosphate and maximum activity at pH 5.5. The enzyme had a temperature optimum of 51 °C and was inactivated by preincubation for 10 min at 65 °C. The product specificity appeared similar to that of wheat phytase and was not altered by a 10-fold excess of magnesium. Vegetable phytases may be an alternative to seed and microbial phytases for use in the processing of food and feed products.

Keywords: *Phytase; phosphatase; phytate; inositol phosphates; Allium*

INTRODUCTION

Yeast, cereal, and fungal phytases are utilized to enhance the absorption of minerals such as iron, calcium, and zinc from food (Turk et al., 1996; Sandberg et al., 1996). Phytases are also employed commercially as feed additives to increase the bioavailability of phosphorus from *myo*-inositol hexakisphosphate ($InsP_6$, phytate) and thereby decrease the potential for phosphate pollution of surface water. *Aspergillus niger phy*A phytase is added to feeds most frequently, but cereal phytases may be almost as effective (Han et al., 1997). Phytases cloned from thermophilic fungi are now being evaluated for their efficiency at high temperatures and their ability to withstand the heat generated during industrial processing (Mitchell et al., 1997; Pasamontes et al., 1997).

Although phytases are currently used with the goal of liberating inorganic phosphate from the inositol moiety of phytate, it is becoming evident that the inositol phosphate intermediates have beneficial biological activities. Phytate is an antioxidant that prevents iron-catalyzed hydroxyl radical formation and may be useful to protect against the deterioration of foods (Graf et al., 1984; Lee and Hendricks, 1997). Recently it was shown that the breakdown products of phytate containing the 1,2,3-trisphosphate configuration also have antioxidant functions while forming mineral complexes that are more soluble than those containing phytate (Spiers et al., 1995; Phillippy and Graf, 1997). Inositol 1,2,3,6-tetrakisphosphate also has second messenger activity in mobilizing calcium (DeLisle et al., 1994; Burford et al., 1997) and increases the absorption of calcium from the diet in rats (Shen et al., 1998).

Phytases are classified according to their specificity of removal of the first phosphate group to form inositol pentakisphosphate. *A. niger* phytase first removes the D-3 phosphate (Irving and Cosgrove, 1972), whereas wheat bran phytase hydrolyzes at the D-4 position (Johnson and Tate, 1969), paramecium phytase at D-6 (Van der Kaay and Van Haastert, 1995) and alkaline phytase from lily pollen at position 5 (Barrientos et al., 1994). Seeds have more phytate than vegetative plant tissue and have traditionally been used, along with yeast, as a source of phytase in foods. The objective of the present work was to determine the concentrations and catalytic properties of phytase in unprocessed vegetables.

EXPERIMENTAL PROCEDURES

Materials. Scallions (green onions) and other vegetables were purchased at local supermarkets. Untreated wheat germ, heparin type II−S agarose, and reactive red 120 type 3000-CL agarose were from Sigma Chemical Company (St. Louis, MO). Toyopearl SP-650M and Toyopearl DEAE−650M ion exchange packings were purchased from Supelco, Inc. (Bellefonte, PA). AG 1-X8, 200−400 mesh, chloride form anion-exchange resin was from Bio-Rad Laboratories (Hercules, CA), and Microcon 10 microconcentrators were obtained from Amicon, Inc. (Beverly, MA). Quantigold reagent and protein standards for electrophoresis were obtained from Diversified Biotech (Boston, MA). Inositol hexakisphosphate, *myo*[inositol-2-^{3}H(N)]- (21 Ci/mmol) was purchased from NEN Life Science Products (Boston, MA). ScintiSafe Plus 50% liquid scintillation cocktail was from Fisher Scientific (Pittsburgh, PA).

Tissue Extractions. Vegetables were homogenized with five parts of 20 mM HEPES pH 7.8 for 60 s in a Waring blender. One milliliter aliquots of the homogenates were centrifuged 5 min at 10000*g* and assayed for phytase activity as described below.

Purification of Scallion Phytase. Fifty grams of scallion leaves was torn into approximately one inch long pieces and homogenized with 250 mL of 20 mM sodium acetate pH 5.0 containing 0.1 mM PMSF at 4 °C for 60 s in a Waring blender. Following centrifugation 30 min at 20000*g* and 4 °C, the supernatant was pumped through a 2.5 × 5.0 cm Toyopearl SP-650M column at 1.5 mL/min. The unbound protein was loaded on a 1.5 × 3.0 cm Toyopearl DEAE−650M column using a peristaltic pump at 1 mL/min and eluted with ten 10 mL fractions of 0−0.3 M KCl in 20 mM sodium acetate pH 5.0 (buffer A). The fractions with the highest activity were combined and dialyzed overnight at 4 °C against 1 L of buffer A. The retentate was loaded on a 1.5 × 3.0 cm heparin agarose column using a Pasteur pipet and eluted with ten 5 mL

† Phone (504) 286-4385; fax (504) 286-4419; e-mail bqphil@nola.srrc.usda.gov.

S0021-8561(98)00317-3 This article not subject to U.S. Copyright. Published 1998 by the American Chemical Society
Published on Web 08/11/1998

Table 1. Phytase Content of Crude Vegetable Extracts[a]

vegetable	phytase activity, nmol P_i min^{-1} g^{-1} (n)	vegetable	phytase activity, nmol P_i min^{-1} g^{-1} (n)
spinach leaves	126 ± 5 (4)	tomato fruits	6 ± 1 (4)
endive leaves	59 ± 19 (4)	Romaine lettuce leaves	51 ± 5 (4)
whole green onions	153 ± 20 (3)	button mushrooms	78 ± 3 (4)
yellow onion bulbs	47 ± 20 (3)	radish roots	52 ± 3 (4)
cabbage leaves	62 ± 18 (4)	bell pepper fruits	51 ± 4 (4)

[a] Data represent the means ± SD of phytase activity per gram of wet tissue.

fractions of 0−0.5 M KCl in buffer A at 1.0 mL/min. The fractions with the highest activity were diluted with four volumes of buffer A and loaded on a 1.5 × 3.0 cm reactive red 120 agarose column using a Pasteur pipet. Purified phytase was eluted with ten 5 mL fractions of 0−0.5 M KCl in buffer A at 1 mL/min.

Determination of Phytase Activity and Protein. Phytase activity was assayed in 200 μL of 100 mM sodium acetate, 0.001% Triton X-100, and 1 mM sodium phytate pH 5.5 incubated for 30 min at 37 °C. The reaction was terminated by the addition of 800 μL of freshly prepared acetone/5 N H_2SO_4/10 mM ammonium molybdate (2:1:1), and inorganic phosphate was estimated from the absorption at 355 nm (Heinonen and Lahti, 1981). Protein was routinely determined according to Bradford (1976) using ovalbumin as the standard. Protein in the reactive red 120 agarose fractions was determined with Quantigold following concentration in Micron 10 microconcentrators in the presence of 0.01% Triton X-100. The recovery of phytase activity from the microconcentrators averaged 98%. Ten microliters of each concentrated fraction was analyzed by sodium dodecyl sulfate−polyacrylamide electrophoresis according to Laemmli (1970).

Product Specificity of Scallion Phytase. To remove the ammonium phosphate, which inhibited the phytase reaction, 50 μL of [^{3}H]InsP_6 (0.5 μCi) in 50 mM ammonium phosphate was diluted with 200 μL of H_2O and loaded on a column constructed with 50 mg of AG 1X8 200−400 mesh resin in the chloride form. Inorganic phosphate was eluted with five 200 μL of aliquots of 0.1 M KCl. [^{3}H]InsP_6 was eluted with ten 200 μL fractions of 1 M KCl, and the fractions with the most cpm were used to determine phytase specificity. Thirty-five nanograms of purified phytase was incubated in 130 μL of 15 mM sodium acetate, 77 μM sodium phytate, approximately 3000 cpm [^{3}H]InsP_6, 0.001% Triton X-100, pH 5.5 for 30 min at 37 °C. Following the addition of 80 μg of InsP_6 hydrolysate a 50 μL aliquot was separated by gradient ion chromatography on a Dionex AS3 plus AG3 analytical plus guard column combination with a 25 mL gradient of 0−0.155 N HNO_3 followed by 7 mL of 0.155 N HNO_3 at 1 mL/min (Phillippy and Bland, 1988). Fractions were collected at 12 s intervals and counted with 5 mL of ScintiSafe Plus 50% liquid scintillation cocktail. Retention times were compared with those of standards prepared by nonenzymatic hydrolysis of InsP_6 as described previously (Phillippy and Bland, 1988).

RESULTS

The recently reported data on inositol phosphates in fresh vegetables (Harland and Morris, 1995) led to an investigation of the phytases in these tissues. A brief survey showed that some vegetables purchased at a local supermarket contained surprisingly high amounts of phytase activity at pH 5.5 (Table 1). In similar experiments no alkaline phytase activity was detected at pH 7.5 in the crude extracts from whole scallions, yellow onion bulbs, spinach leaves, or endive leaves. To further investigate the distribution of phytase within the whole green onions (scallions), the tissue was divided into three sections. As shown in Table 2, the level of phytase activity in the leaves was approximately double that in the stalk and bulb, although all three tissues had a specific activity of about 7 nmol min^{-1} per mg protein. In comparison, untreated wheat germ analyzed in a similar experiment was found to have a phytase activity at pH 5.0 of 10 nmol min^{-1} per mg protein.

Table 2. Distribution of Phytase Activity at pH 5.5 in Whole Green Onion (Scallion) Tissues[a]

tissue	activity, nmol P_i min^{-1} g^{-1}	protein, mg/g	specific activity, nmol P_i min^{-1} mg^{-1}
leaf	209 ± 30	29 ± 6	7.2
stalk	98 ± 32	15 ± 3	6.5
bulb	132 ± 26	17 ± 2	7.8

[a] Data represent the means ± SD (n = 3).

Purification of the scallion leaf phytase was performed using a procedure similar to that used by Nogimori et al. (1991) to purify from rat liver a phytase that has been named multiple inositol polyphosphate phosphatase or MIPP (Craxton et al., 1995). The amounts of phytase activity extracted with 20 mM HEPES pH 7.8 or 20 mM sodium acetate pH 5.0 were similar, but 8-fold less total protein was extracted with the latter, resulting in a higher initial specific activity. The results of a typical purification are shown in Table 3 and Figure 1. The SP cation exchange step achieved little increase in purity but was critical for the successful enrichment of activity in the following DEAE anion exchange step. Since the small scale of the process resulted in a purified product too dilute for electrophoresis, the protein was concentrated 20-fold in a Micron 10 microconcentrator in the presence of 0.01% Triton X-100, which was needed to achieve a good recovery. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis revealed multiple bands, but the phytase activity appeared to correspond to a band with a molecular mass of 72 000 (Figure 2). The specific activity of the final preparation was 500 μmol min^{-1} mg^{-1} compared to 262 μmol min^{-1} mg^{-1} for spelt phytase (Konietzny et al., 1995) and less than 10 μmol min^{-1} mg^{-1} for maize phytase (Labore et al., 1993; Hübel and Beck, 1996).

Kinetic experiments were performed to determine the optimum composition of the assay medium. Following the observation that the addition of 0.01% Triton X-100 greatly increased the recovery of the purified enzyme during concentration as described above, 0.001% Triton X-100 was included in the assay mix. Phytase activity determined with InsP_6 levels from 10 μM to 1 mM at pH 5.5 and 37 °C followed typical Michaelis−Menton kinetics, and the resulting Lineweaver−Burk plot gave K_m and V_{max} values of 200 μM and 44 μmol min^{-1} mg^{-1}, respectively. When the assay was conducted over the range of pH 3.0 to pH 7.0, maximum activity was observed at pH 5.5 (Figure 3).

The effect of temperature was examined on the reaction rate and on the stability of the scallion phytase. In the presence of Triton X-100, optimal activity was obtained at 51 °C (Figure 4A). The 30 min length of incubation was chosen to correspond to the time used in all other experiments, with the resulting data not

Table 3. Purification of Scallion Phytase

step	total activity, μmol min^{-1}	protein, mg	specific activity, μmol min^{-1}mg^{-1}	purification, -fold	yield, %
crude	4.97	311	0.016	1.0	100
SP-650M	3.37	135	0.025	1.6	68
DEAE−650M	2.97	12.3	0.241	15	60
heparin	1.94	1.33	1.46	91	39
reactive red 120	2.00	0.004	500	31 000	40

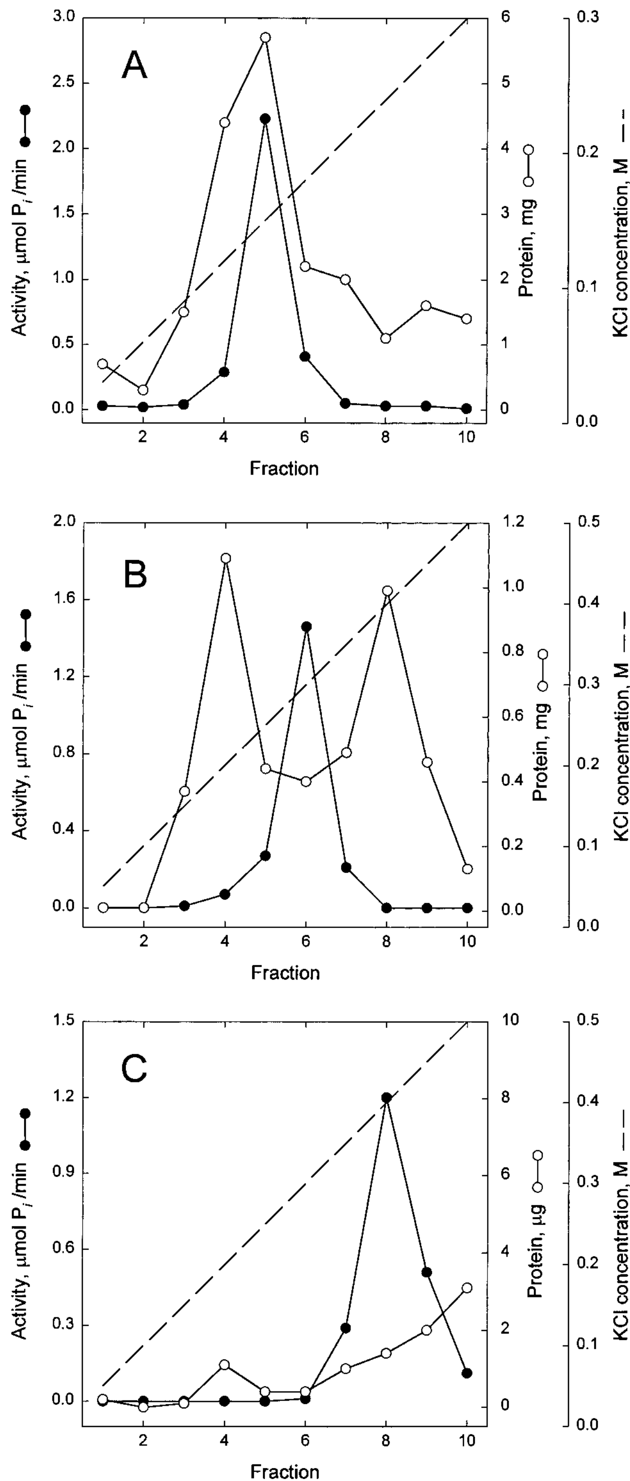

Figure 1. Chromatography of scallion phytase. Unbound activity from the SP-650M column was purified by sequential separations on DEAE−650M (A), heparin agarose (B), and reactive red 120 agarose (C) columns.

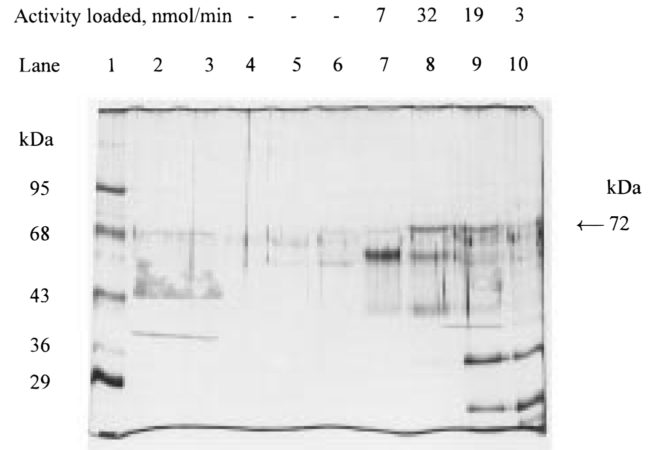

Figure 2. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis of fractions from the reactive red 120 agarose column. Lane 1: standards (phosphorylase B, 95 000; bovine serum albumin, 68 000; ovalbumin, 43 000; lactate dehydrogenase, 36 000; carbonic anhydrase, 29 000). Lanes 2−10: reactive red 120 agarose column fractions 2−10.

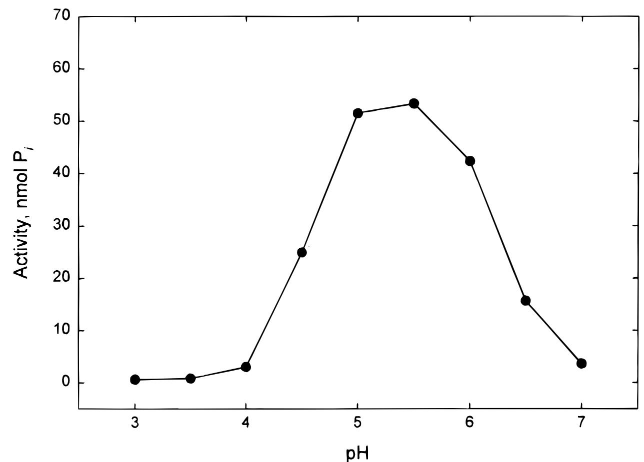

Figure 3. Effect of pH on scallion phytase activity. Purified phytase was assayed 30 min at 37 °C in buffers containing 19 mM acetic acid, 19 mM formic acid, 19 mM 2-[*N*-morpholino]-ethanesulfonic acid (MES), 0.001% Triton X-100, and 0.9 mM sodium phytate at the indicated pH. Data represent the means of two determinations.

completely independent of the effects of thermostability. When the enzyme was preincubated 10 min at various temperatures prior to the assay, little activity was detected above 58 °C (Figure 4B). Phytase activity was completely stable during storage at 0 °C for two months at a protein concentration of 5 μg/mL.

When scallion phytase was incubated with 77 μM $InsP_6$ spiked with a trace of $[^3H]InsP_6$, the breakdown pattern, shown in Figure 5, appeared similar to that of the phytase from wheat kernels (Phillippy et al., 1994). The major pentakisphosphate product of scallion phytase eluted with the retention time of the enantiomeric pair $Ins(1,2,3,4,5)P_5$/$Ins(1,2,3,5,6)P_5$. Wheat phytase is known to form the latter isomer (Johnson and Tate, 1969), and it is likely that scallion phytase does as well. The retention times of the secondary products corresponded

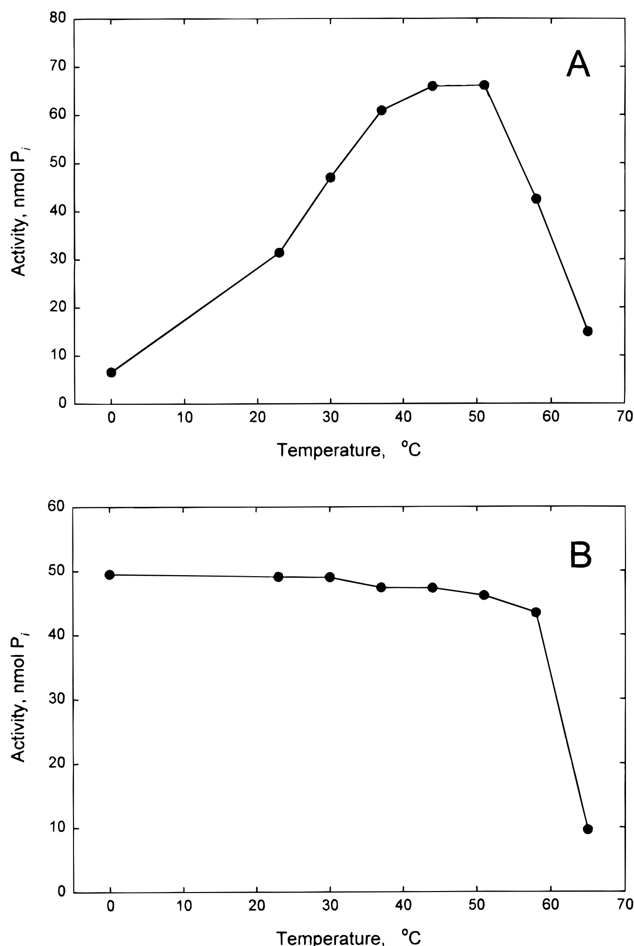

Figure 4. Effect of temperature on scallion phytase activity. Purified phytase was assayed 30 min at the indicated temperatures (A) or 30 min at 37 °C following 10 min preincubation at the indicated temperatures (B). Data represent the means of two determinations.

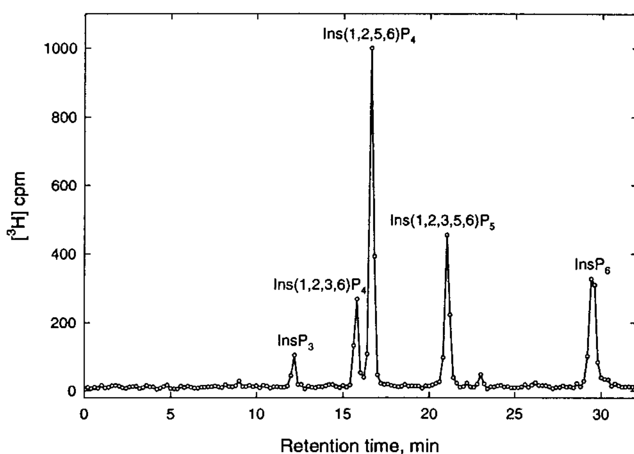

Figure 5. Specificity of scallion phytase. Products of $InsP_6$ hydrolysis by scallion phytase were identified by comparison of retention times in gradient ion chromatography with those of standards prepared from a nonenzymatic hydrolysate of $InsP_6$.

to those of authentic Ins(1,2,3,6)P_4 and Ins(1,2,5,6)P_4, which are the isomers formed by the wheat enzyme (Phillippy, 1989). Since phytate in seeds is present as a potassium and magnesium salt, the effect of the divalent cation magnesium on the product specificity was examined. However, when 820 μM $MgCl_2$ was included in the assay with 82 μM $InsP_6$, no change in specificity was observed. Iron can bind tightly to the 1,2,3-trisphosphate array of $InsP_6$, but when 40 nM $InsP_6$ was hydrolyzed by scallion phytase, 400 nM $FeCl_3$ did not appear to significantly change the product specificity, whereas 820 nM $FeCl_3$ almost completely inhibited the reaction (results not shown). It is noteworthy that due to the high K_m of scallion phytase, the use of [^{3}H]$InsP_6$ in the preceding experiments did not increase the sensitivity of the method but was used primarily to avoid the precipitation known to occur at higher concentrations of phytate−mineral complexes.

DISCUSSION

The presence of appreciable amounts of phytase in fresh vegetables is a discovery with numerous implications. Since excessive amounts of undegraded $InsP_6$ in the diet can lead to mineral deficiencies, $InsP_6$ was branded an antinutrient that should be avoided and eliminated by extraneous processing efforts. The dearth of fresh vegetables in some diets may have been partly responsible for this development. It is known that seed and microbial phytases can degrade phytate in the human gut (Sandberg et al., 1996; Sandberg and Andersson, 1988). Test diets fed to animals to investigate the effects of $InsP_6$ ordinarily do not include unprocessed vegetables and therefore do not reflect the type of balanced diet that is recommended for humans. Nothing is currently known about the effectiveness of vegetable phytases when consumed with $InsP_6$ derived from seeds, and this will be an important topic for future research. A related area of concern will be the postharvest changes in the levels of phytase and inositol phosphates in vegetables. The storage history of the vegetables analyzed in the present study was unknown, and the phytase levels may have been different at earlier times postharvest. The high amounts of phytase observed in the present study also provide the potential for significant inositol polyphosphate degradation in the vegetables.

Phytase was purified from scallion leaves because they contained higher levels than the other vegetables that were analyzed. The activity in scallion leaves was comparable to that in wheat germ, which means that vegetable phytases may be an alternative to seed and microbial phytases for use in food and feed processing. The thermostability and response to pH of scallion leaf phytase was similar to that of seed phytases from spelt (Konietzny et al., 1995), maize (Labore et al., 1993), and soybean (Gibson and Ullah, 1988). Although microbial phytases are generally more thermostable than plant phytases and are more active in low acid environments between pH 2 and pH 4, wheat bran phytase performed adequately to increase phosphorus utilization in pigs, possibly as a result of a superior resistance to intestinal proteases (Han et al., 1997). In human diets wheat phytase was less effective than *A. niger* phytase in increasing iron bioavailability, but it was not clear whether the total phytase activities of the test meals were similar (Sandberg et al., 1996).

Although phytases with different specificities in product formation from $InsP_6$ have been identified in various organisms, the significance of these differences is not yet known. What is becoming clear is that some of the breakdown products probably have useful chemical and biological activities. Inositol phosphates containing the 1,2,3-trisphosphate cluster are iron-binding antioxidants (Phillippy and Graf, 1997; Spiers et al., 1995), and

$Ins(1,2,3,6)P_4$ has a structure that is moderately effective in opening calcium channels (Burford et al., 1997; DeLisle et al., 1994). In addition inositol 1,2,6-trisphosphate, which is also a product of wheat phytase, has antiinflammatory and antisecretory properties (Hansen et al., 1996). Whether these naturally occurring isomers have nutritional significance in foods is for the most part speculative. Nevertheless, the absorption of calcium was significantly increased in rats fed diets supplemented with 16.8 mg of $Ins(1,2,3,6)P_4$ in a test meal (Shen et al., 1998), and diets supplemented with 0.1% $Ins(1,2,3,6)P_4$ and fed to rats counteracted the effects of oxidative stress nearly as well as diets with 0.1% $InsP_6$ (J. R. Burgess and B. Q. Phillippy, unpublished results). Similar in vivo antioxidant effects of dietary $InsP_6$ were reported recently in mice (Singh et al., 1997). The predominant initial product derived from $InsP_6$ by fungal phytase is $Ins(1,2,4,5,6)P_5$, whereas that from plant phytases is $Ins(1,2,3,5,6)P_5$ (Irving and Cosgrove, 1972; Johnson and Tate, 1969). Thus plant phytases give a higher proportion of breakdown products containing a phosphate at the 3-position with the bioactive attributes described above. Although fungal phytase satisfactorily improves the growth and development of young chickens and pigs, the bioactive inositol phosphate isomers obtained with plant phytases may provide a nutritional advantage in human foods when consumed throughout adulthood.

Just as $InsP_6$ seems to occur in living cells of all types, phytases appear to be widely distributed in plants, animals, and microorganisms. In microbes phytase functions to hydrolyze phytate as a source of nutrients. In liver it may play a role in protein metabolism or the detoxification of harmful substances (Craxton et al., 1995). Intestinal mucosal cells contain phytase to assist the digestive process and facilitate nutrient absorption. Seed phytases liberate phosphorus during germination for growth of the juvenile plant. The substantial levels of phytase that have now been measured in the leaves of nearly full-grown plants may suggest a rather widespread role of $InsP_6$ and phytase in tissue growth and development in plants and perhaps animals as well.

Since $InsP_6$ has positive as well as negative properties relating to foods and nutrition, a pertinent issue may be how much enzymatic hydrolysis is desirable. $InsP_6$ is an important component within animal cells as evidenced by an increasing number of reports (Efanov et al., 1997; Huang et al., 1997; Larsson et al., 1997). However, the main functions of dietary phytate may be as an antioxidant (Graf and Eaton, 1990) and in the prevention of cancer (Shamsuddin, 1995). Partial rather than complete $InsP_6$ degradation may be preferred to avail the consumer of the variety of bioactive and antioxidant inositol phosphates isomers found in diets containing fermented foods such as whole wheat bread and those that combine phytate from grains and legumes with phytase from fresh vegetables.

ABBREVIATIONS USED

myo-Inositol phosphates are depicted as $Ins(X)P_Y$, where X represents the positions of the phosphates according to the D configuration and Y is the number of phosphates.

LITERATURE CITED

Barrientos, L.; Scott, J. J.; Murthy, P. P. N. Specificity of hydrolysis of phytic acid by alkaline phytase from lily pollen. *Plant Physiol.* **1994**, *106*, 1489−1495.

Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248- 254.

Burford, N. T.; Nahorski, S. R.; Chung, S.-K.; Chang, Y. T.; Wilcox, R. A. Binding and activity of the nine possible regioisomers of *myo*-inositol tetrakisphosphate at the inositol 1,4,5-trisphosphate receptor. *Cell Calcium* **1997**, *21*, 301−310.

Craxton, A.; Ali, N.; Shears, S. B. Comparison of the activities of a multiple inositol polyphosphate phosphatase obtained from several sources: a search for heterogeneity in this enzyme. *Biochem. J.* **1995**, *305*, 491−498.

DeLisle, S.; Radenberg, T.; Wintermantel, M. R.; Tietz, C.; Parys, J. B.; Pittet, D.; Welsh, M. J.; Mayr, G. W. Second messenger specificity of the inositol trisphosphate receptor: reappraisal based on novel inositol phosphates. *Am. J. Physiol.* **1994**, *266* (Cell Physiol. 35), C429−436.

Efanov, A. M.; Zaitsev, S. V.; Berggren, P.-O. Inositol hexakisphosphate stimulates non-Ca^{2+}-mediated and primes Ca^{2+}-mediated exocytosis of insulin by activation of protein kinase C. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 4435−4439.

Gibson, D. M.; Ullah, A. H. J. Purification and characterization of phytase from cotyledons of germinating soybean seeds. *Arch. Biochem. Biophys.* **1988**, *260*, 503−513.

Graf, E.; Eaton, J. W. Antioxidant functions of phytic acid. *Free Radical Biol. Med.* **1990**, *8*, 61−69.

Graf, E.; Mahoney, J. R.; Bryant, R. G.; Eaton, J. W. Iron-catalyzed hydroxyl radical formation. Stringent requirement for free iron coordination site. *J. Biol. Chem.* **1984**, *259*, 3620−3624.

Han, Y. M.; Yang, F.; Zhou, A. G.; Miller, E. R.; Ku, P. K.; Hogberg, M. G.; Lei, X. G. Supplemental phytases of microbial and cereal sources improve dietary phytate phosphorus utilization by pigs from weaning through finishing. *J. Anim. Sci.* **1997**, *75*, 1017−1025.

Hansen, M. B.; Tindholdt, T. T.; Elbrond, V. S.; Makinde, M.; Cassuto, J.; Beubler, E.; Westerberg, E. J.; Skadhauge, E. The effect of α-trinositol on cholera toxin-induced hypersecretion and morphological changes in pig jejunum. *Pharmacol. Toxicol.* **1996**, *78*, 104−110.

Harland, B. F.; Morris, E. R. Phytate: a good or a bad food component? *Nutr. Res.* **1995**, *15*, 733−754.

Heinonen, J. K.; Lahti, R. J. A new and convenient colorimetric determination of inorganic orthophosphate and its application to the assay of inorganic pyrophosphatase. *Anal. Biochem.* **1981**, *113*, 313−317.

Huang, C.; Ma, W.-Y.; Hecht, S. S.; Dong, Z. Inositol hexaphosphate inhibits cell transformation and activator protein 1 activation by targeting phosphatidylinositol-3′ kinase. *Cancer Res.* **1997**, *57*, 2873−2878.

Hübel, F.; Beck, E. Maize root phytase. Purification, characterization, and localization of enzyme activity and its putative substrate. *Plant Physiol.* **1996**, *112*, 1429−1436.

Irving, G. C. J.; Cosgrove, D. J. Inositol phosphate phosphatases of microbial origin: the inositol pentaphosphate products of *Aspergillus ficuum* phytases. *J. Bacteriol.* **1972**, *112*, 434−438.

Johnson, L. F.; Tate, M. E. The structure of *myo*-Inositol pentaphosphates. *Ann. N. Y. Acad. Sci.* **1969**, *165*, 526−532.

Konietzny, U.; Greiner, R.; Jany, K.-D. Purification and characterization of a phytase from spelt. *J. Food Biochem.* **1995**, *18*, 165−183.

Laboure, A.-M.; Gagnon, J.; Lescure, A.-M. Purification and characterization of a phytase (*myo*-inositol-hexakisphosphate phosphohydrolase) accumulated in maize (*Zea mays*) seedlings during germination. *Biochem. J.* **1993**, *295*, 413−419.

Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680−685.

Larsson, O.; Barker, C. J.; Sjoholm, A.; Carlqvist, H.; Michell, R. H.; Bertorello, A.; Nilsson, T.; Honkanen, R. E.; Mayr, G. W.; Zwiller, J.; Berggren, P.-O. Inhibition of phosphatases

and increased Ca^{2+} channel activity by inositol hexakisphosphate. *Science* **1997**, *278*, 471−474.

Lee, B. J.; Hendricks, D. G. Metal-catalyzed oxidation of ascorbate, deoxyribose and linoleic acids as affected by phytic acid in a model system. *J. Food Sci.* **1997**, *62*, 935−938, 984.

Mitchell, D. B.; Vogel, K.; Weimann, B. J.; Pasamontes, L.; van Loon, A. P. G. M. The phytase subfamily of histidine acid phosphatases: isolation of genes for two novel phytases from the fungi *Aspergillus terreus* and *Myceliophthora thermophila*. *Microbiology* **1997**, *143*, 245−252.

Nogimori, K.; Hughes, P. J.; Glennon, M. C.; Hodgson, M. E.; Putney, J. W., Jr.; Shears, S. B. Purification of an inositol (1,3,4,5)-tetrakisphosphate 3-phosphatase activity from rat liver and the evaluation of its substrate specificity. *J. Biol. Chem.* **1991**, *266*, 16499−16506.

Pasamontes, L.; Haiker, M.; Wyss, M.; Tessier, M.; van Loon, A. P. G. M. Gene cloning, purification, and characterization of a heat-stable phytase from the fungus *Aspergillus fumigatus*. *Appl. Environ. Microbiol.* **1997**, *63*, 1696−1700.

Phillippy, B. Q.; Bland, J. M. Gradient ion chromatography of inositol phosphates. *Anal. Biochem.* **1988**, *175*, 162−166.

Phillippy, B. Q. Identification by two-dimensional NMR of inositol tris- and tetrakis(phosphates) formed from phytic acid by wheat phytase. *J. Agric. Food Chem.* **1989**, *37*, 1261−1265.

Phillippy, B. Q.; Graf, E. Antioxidant functions of inositol 1,2,3-trisphosphate and inositol 1,2,3,6-tetrakisphosphate. *Free Radical Biol. Med.* **1997**, *22*, 939−946.

Phillippy, B. Q.; Ullah, A. H. J.; Ehrlich, K. E. Purification and some properties of inositol 1,3,4,5,6-pentakisphosphate 2-kinase from immature soybean seeds. *J. Biol. Chem.* **1994**, *269*, 28393−28399.

Sandberg, A.-S.; Andersson, H. Effect of dietary phytase on the digestion of phytate in the stomach and small intestine of humans. *J. Nutr.* **1988**, *118*, 469−473.

Sandberg, A.-S.; Hulthen, L. R.; Turk, M. Dietary *Aspergillus niger* phytase increases iron absorption in humans. *J. Nutr.* **1996**, *126*, 476−480.

Shamsuddin, A. M. Inositol phosphates have novel anticancer function. *J. Nutr.* **1995**, *125*, 725S-732S.

Shen, X.; Weaver, C. M.; Kempa-Steczko, A.; Martin, B. R.; Phillippy, B. Q.; Heaney, R. P. An inositol phosphate aa a calcium absorption enhancer in rats. *J. Nutr. Biochem.* **1998**, *9*, 298−301.

Singh, A.; Singh, S. P.; Bamezai, R. Modulatory influence of arecoline on the phytic acid-altered hepatic biotransformation system enzymes, sulfhydryl content and lipid peroxidation in a murine system. *Cancer Lett.* **1997**, *117*, 1−6.

Spiers, I. D.; Freeman, S.; Poyner, D. R.; Schwalbe, C. H. The first synthesis and iron binding studies of the natural product, *myo*-inositol 1,2,3-trisphosphate. *Tetrahedron Lett.* **1995**, *36*, 2125−2128.

Turk, M.; Carlsson, N.-G.; Sandberg, A.-S. Reduction in the levels of phytate during wholemeal bread making; effect of yeast and wheat phytases. *J. Cereal Sci.* **1996**, *23*, 257- 264.

Van der Kaay, J.; Van Haastert, P. J. M. Stereospecificity of inositol hexakisphosphate dephosphorylation by *Paramecium* phytase. *Biochem. J.* **1995**, *312*, 907−910.

Received for review March 27, 1998. Revised manuscript received June 25, 1998. Accepted July 1, 1998.

JF9803177