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Degradation of *myo*-inositol Hexakisphosphate by a Phytate-degrading Enzyme from *Pantoea agglomerans*

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High-pressure liquid chromatography (HPLC) analysis established *myo*-inositol pentakisphosphate as the final product of phytate dephosphorylation by the phytate-degrading enzyme from *Pantoea agglomerans*. Neither product inhibition by phosphate nor inactivation of the *Pantoea* enzyme during the incubation period were responsible for the limited phytate hydrolysis as shown by addition of phytate-degrading enzyme and phytate, respectively, after the observed stop of enzymatic phytate degradation. In additon, the *Pantoea* enzyme did not possess activity toward the purified *myo*-inositol pentakisphosphate. Using a combination of High-Performance Ion Chromatography (HPIC) analysis and kinetic studies, the nature of the generated *myo*-inositol pentakisphosphate was established. The data demonstrate that the phytate-degrading enzyme from *Pantoea agglomerans* dephosphorylates *myo*-inositol hexakisphosphate in a stereospecific way to finally D-*myo*-inositol(1,2,4,5,6)pentakisphosphate.

KEY WORDS: Glucose-1-phosphatase; *myo*-inositol phosphate isomers; *Pantoea agglomerans*; phytase; phytate; phytate-degrading enzyme.

1. INTRODUCTION

All microbial acid phosphatases acting upon phytate [*myo*-inositol (1,2,3,4,5,6) hexakisphosphate] belong to the subfamily of histidine acid phosphatase (Berka *et al*., 1998; Kostrewa *et al*., 1997, 1999; Lee *et al*., 2003; Lim *et al*., 2000; Mitchell *et al*., 1997; Pasamontes *et al*., 1997a, b; Piddington *et al*., 1993; Rodriguez *et al*., 1999; Sajidan *et al*., 2004). A common feature of all these enzymes is the highly conserved N-terminal sequence motif RH(G/N)*XRXP* considered to be the phosphate acceptor site as well as an R and a HD-motif located at almost identical positions in the active site. With the exception of the *Escherichia coli* glucose-1-phosphatase, all microbial acid phytate-degrading enzymes studied so far in respect to their phytate dephosphorylation pathway release five of the six phosphate residues of phytate generating *myo*-inositol(2)monophosphate as the final product (Cosgrove, 1970; Greiner *et al*., 2001; Sajidan *et al*., 2004; Wyss *et al*., 1999). The glucose-1-phosphatase from *Escherichia coli* was reported to degrade phytate to a *myo*-inositolpentakisphosphate identified as D/L-*myo*-inositol (1,2,4,5,6) pentakisphosphate by High-pressure liquid chromatography (HPLC)-analysis (Cortrill *et al*., 2002). From a docking model it was concluded that the phytate molecule could only fit into the substrate binding pocket in such a way, that the phosphate at position D-3 in the *myo*-inositol ring is cleaved (Lee *et al*., 2003). Therefore, D-*myo*-inositol (1,2,4,5,6) pentakisphosphate was expected to be the end product of phytate dephosphorylation by the glucose-1-phosphatase from *Escherichia coli*, which has not been

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[2] Abbreviations: HPIC: high performance ion chromotography: HPLC: high pressure liquid chromotography; TBAH; tetrabutyl ammonium hydroxide.

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confirmed experimentally so far. However, the validity of the glucose-1-phosphatase-phytate binding model was confirmed by an excellent agreement between the experimentally obtained and the expected hydrolysis products for various other *myo*-inositol phosphates (Lee *et al*., 2003).

The recently purified phytate-degrading enzyme from *Pantoea agglomerans* (Greiner, 2004) shares many properties with the glucose-1-phosphatase from *Escherichia coli* (Cottrill *et al*., 2002). Optimal conditions for phytate dephosphorylation were determined to be pH 4.5 and 60°C and both enzymes were virtually inactive below pH 3.0 or above pH 7.0 while acting upon phytate. Furthermore, both enzymes showed are very similar pH-stability. High activity toward glucose-1-phosphate and phytate as well as the release of in average only one phosphate residue per phytate molecule are further properties both enzymes have in common. In addition, both proteins are produced as periplasmatic enzymes from gram-negative Enterobacteriaceae family members and no regulation of enzyme activity by inorganic phosphate was observed (Greiner, 2004; Pradel and Boquet, 1991). Therefore the so called phytate-degrading enzyme could be assumed to be the pendant of the *Escherichia coli* glucose-1-phosphatase in *Pantoea agglomerans*. To further confirm this assumption, the questions on the catalytic mechanism and the final product(s) of phytate dephosphorylation by the phytate-degrading enzyme from *Pantoea agglomerans* have to be answered and the amino acid sequence as well as the three dimensional structure of the enzyme have to be elucidated.

The aim of this study was to identify the end product(s) of phytate dephosphorylation by the phytate-degrading enzyme from *Pantoea agglomerans* and to investigate, if the docking model established for the *Escherichia coli* glucose-1-phosphatase (Lee *et al*., 2003) is also applicable for the *Pantoea* enzyme.

2. MATERIALS AND METHODS

2.1. Chemicals

Aspergillus niger phytase was obtained from Novo Nordisk (Copenhagen, Denmark). Phytic acid, as a dodecasodium salt, was purchased from Aldrich (Steinheim, Germany), Ultrasep ES 100 RP18 from Bischoff (Leonberg, Germany) and HPIC Carbo-Pac PA-100 column from Dionex (Sunnyvale, CA, USA).

2.2. Purification of the Phytate-degrading Enzymes

Purification of the phytases of *Aspergillus niger* (Greiner *et al*., 2001) and *Pantoea agglomerans* (Greiner, 2004) were performed as described previously. Both phytate-degrading enzymes were purified to apparent homogeneity according to denaturing and non-denaturing polyacrylamide gel electrophoresis.

2.3. Assay of Phytate-degrading Activity

Phytase activity measurements were carried out at 35°C. The enzymatic reactions were started by the addition of 10 µl enzyme to the assay mixtures. The incubation mixture for phytate-degrading activity determination consisted of 350 µl 0.1 *M* sodium acetate, pH 4.5 containing 875 nmol sodium phytate.

After incubating for 30 min at 35°C, the liberated phosphate was measured according to the ammonium molybdate method (Heinonen and Lahti, 1981) with some modifications.

To determine the substrate selectivity of the phytate-degrading enzyme from *Pantoea agglomerans*, several phosphorylated compounds in addition to phytate were used for K_M and v_{max} estimation. The incubation mixture consisted of 350 µl 0.1 *M* sodium acetate buffer, pH 4.5, containing the phosphorylated compound in a serial dilution of a concentrated stock solution (10 m*M*). The enzymatic reactions were started by adding 10 µl of enzyme to the assay mixtures. The kinetic constants (K_M, v_{max}) were calculated from Lineweaver-Burk plots of the data. For calculation of k_{cat} the following molecular masses were used: 85 kDa for the *Aspergillus niger* phytase (Ullah and Gibson, 1987) and 42 kDa for the *Pantoea* enzyme (Greiner, 2004).

2.4. Quantification of the Liberated Phosphate

Added to the assay mixture were 1.5 ml of a freshly prepared solution of acetone/5 N H_2SO_4/10 m*M* ammonium molybdate (2:1:1 v/v) and 100 µl 1.0 *M* citric acid. Any cloudiness was

removed by centrifugation prior to the measurement of absorbance at 355 nm. To calculate the enzyme activity, a calibration curve was produced over the range of 5–600 nmol phosphate (= 8.7 cm^2/nmol). Activity (units) was expressed as 1 μmol phosphate liberated per min. Blanks were run by addition the ammonium molybdate solution prior to adding the enzyme to the assay mixture.

2.5. Production of Enzymatically Formed Hydrolysis Products

The enzymatic reaction was started at 35°C by addition of 10 μl of the suitable diluted enzyme solution to the incubation mixtures (2 U/ml). The incubation mixture consisted of 1250 μl 0.1 *M* sodium acetate buffer, pH 4.5 containing 3.125 μmol sodium phytate or one of the purified individual lower *myo*-inositol phosphates. From the incubation mixture, 100 μl samples were removed periodically and the reaction was stopped by heat treatment (95°C, 10 min). The liberated phosphate was measured according to the ammonium molybdate method (Heinonen and Lahti, 1981) with some modifications. *Myo*-inositol hexakis- and pentakisphosphate concentrations were determined by HPLC ion-pair chromatography (Sandberg and Ahderinne, 1986). For the identification of *myo*-inositolphosphate isomers, High-Performance Ion Chromatography (HPIC) was used (Skoglund *et al.*, 1998).

2.6. Determination of *myo*-inositol Hexakis- and Pentakisphosphate Concentrations

Heat treated samples of 20 μl were chromatographed on Ultrasep ES 100 RP18 (2 × 250 mm). The column was run at 45°C and 0.2 ml/min with an eluant consisting of formic acid:methanol:water:TBAH (tetrabutylammonium hydroxide) (44:56:5:1.5 v/v), pH 4.25 (Sandberg and Ahderinne, 1986). A mixture of the individual *myo*-inositol phosphate esters ($InsP_3$–$InsP_6$) was used as a standard.

2.7. Identification of Enzymatically Formed Hydrolysis Products

Heat-treated samples of 50 μl were chromatographed on a HPIC system using a Carbo Pac PA-100 (4 × 250 mm) analytical column and a gradient of 5–98% HCl (0.5 *M*, 0.8 ml/min) (Skoglund *et al.*, 1998). The eluants were mixed in a post-column reactor with 0.1% $Fe(NO_3)_3 \times 9H_2O$ in a 2% $HClO_4$ solution (0.4 ml/min) (Phillippy and Bland, 1988). The combined flow rate was 1.2 ml/min.

2.8. Preparation of Individual *myo*-inositolphosphate Isomers

D-Ins(1,2,4,5,6)P_5, D-Ins(1,2,3,4,5)P_5, D-Ins(1,2,3,5,6)P_5, and D-Ins(1,2,5,6)P_4 were obtained as described previously (Greiner *et al.*, 2001). For the production of the *myo*-inositol pentakisphosphate isomer generated by the phytate-degrading enzyme from *Pantoea agglomerans*, *myo*-inositol hexakisphosphate (1000 μmol) was incubated at 35°C in a mixture containing 50 m*M* NH_4-acetate, pH 4.5 and 10 U of the purified enzyme in a final volume of 200 ml. After an incubation period of 30 min, the reaction was stopped by heat treatment (95°C, 10 min). The incubation mixture was lyophilised and the dry residues were dissolved in 10 ml 1.0 *M* NH_4-formate, pH 2.5. The solution was loaded onto a Q-Sepharose column (2.6 × 90 cm) equilibrated with 1.0 *M* NH_4-formate, pH 2.5 at a flow rate of 2.5 ml/min. The column was washed with 500 ml of 1.0 *M* NH_4-formate, pH 2.5; the bound *myo*-inositol pentakisphosphates were eluted with a linear gradient from 1.0 to 1.4 *M* NH_4-formate, pH 2.5 (1000 ml) at 2.5 ml/min. Fractions of 10-ml were collected. From even-numbered tubes, 100 μl aliquots were lyophilised. The residues were dissolved in 3 N sulfuric acid and incubated for 90 min at 165°C to hydrolyse the eluted *myo*-inositol phosphates completely. The liberated phosphate was measured according to the ammonium molybdate method (Heinonen and Lahti, 1981) with some modifications. The content of the fraction tubes corresponding to the *myo*-inositol pentakisphosphate were pooled and lyophilised until only a dry residue remained. Ten milliliters of water were used to redissolve the residues. Lyophilisation and redissolving were repeated twice. *Myo*-inositol pentakisphosphate concentration was determined by HPLC ion-pair chromatography (Sandberg and Ahderinne, 1986). The purity of the *myo*-inositol pentakisphosphate prep-

Table 1. Time Course of Phosphate Release from Phytate by a Phytate-degrading Enzyme from *Pantoea agglomerans*

Time [min]	Phosphate [nmol]
0	0
10	211 ± 12
20	405 ± 24
30	599 ± 21
40	786 ± 37
50	861 ± 41
60	878 ± 31
70	875 ± 29
80	881 ± 34
90	879 ± 42
120	884 ± 35

Note: Temperature: 35°C; buffer: 100 m*M* sodium acetate, pH 4.5; phytate: 875 nmol; enzyme activity: 20 mU; after 90 min another 20 mU were added. The data are mean values of four independent experiments.

aration was determined on a HPIC system (Skoglund *et al.*, 1998).

3. RESULTS

3.1. Phosphate Release from Phytate

In order to study phytate degradation by the phytate-degrading enzyme from *Pantoea agglomerans*, the time dependence of phosphate release was quantitatively determined using formation of a soluble phospho-molybdate complex in an acidic water–acetone mixture. The results are summarised in Table 1. Under assay conditions (35°C, pH 4.5), phosphate concentration increased linearly with time in the first 40 min of incubation. A linear release of phosphate within a time period of 30 min was expected, since this fact was the requirement for the development of an enzyme assay for the phytate-degrading enzyme from *Pantoea agglomerans* (Greiner, 2004). Above 40 min of incubation, phosphate release decreased steadily with time and after about 60 min the maximum amount of phosphate (about 880 nmol) was detected in the incubation mixture. Even addition of another 20 mU of enzyme activity to the incubation mixture did not result in a further release of phosphate from phytate. Therefore, in average a maximum of only one phosphate residue per phytate molecule could be released by the action of the phytate-degrading enzyme from *Pantoea agglomerans*. The data suggest *myo*-inositol pentakisphosphate as the final degradation product of phytate.

3.2. Time Course of Phytate Degradation

In order to examine the action of the phytate-degrading enzyme on phytate, the hydrolysis products were separated and quantified by HPLC

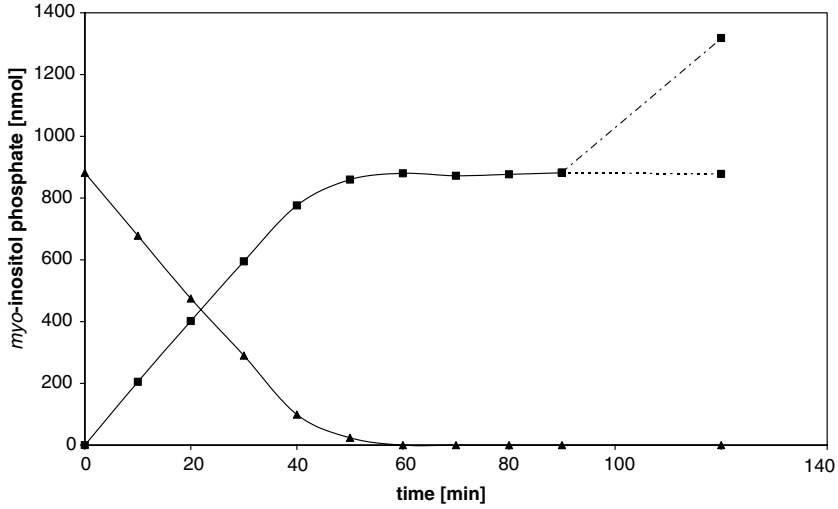


Fig. 1. Time course of the action of the phytate-degrading enzyme from *Pantoea agglomerans* on phytate Temperature: 35°C; buffer: 100 m*M* sodium acetate, pH 4.5; phytate: 875 nmol; enzyme activity: 20 mU; after 90 min either 20 mU enzyme activity (.....) or 437.5 nmol phytate (-·-·-·-) were added. Enzymatic reaction products were separated by ion-pair chromatography. phytate (▲), *myo*-inositol pentakisphosphate (■).

ion-pair chromatography (Figure 1). A marked decrease in *myo*-inositol hexakisphosphate (phytate) content of the reaction mixture with a concomitant increase in *myo*-inositol pentakisphophate content has been observed during incubation. No *myo*-inositol phosphates with less than five phosphate residues have been detected throughout the incubation period of 90 min. The data demonstrate that the phytate-degrading enzyme from *Pantoea agglomerans* dephosphorylates phytate quantitatively to *myo*-inositol pentakisphosphate. Neither product inhibition by phosphate nor inactivation of the *Pantoea* enzyme during the incubation period were responsible for the limited phytate hydrolysis as shown by addition of phytate-degrading enzyme (20 mU) and phytate (437.5 nmol), respectively, after 90 min. Addition of enzyme did not result in any detectable effect within 30 min, whereas the added phytate was completely degraded to *myo*-inositol pentakisphosphate (Figure 1).

3.3. Identification of the Generated *myo*-inositol Pentakisphosphate

3.3.1. HPIC Analysis of Enzymatic myo-inositol Hexakisphoshate Dephosphorylation

The identification of the hydrolysis products of *myo*-inositol hexakisphosphate generated by the phytate-degrading enzymes from *Pantoea agglomerans* purified to apparent homogeneity was performed by isomer-specific HPIC analysis. As a degradation product, only one single *myo*-inositol pentakisphosphate isomer could be detected (Figure 2). Since all theoretically existing *myo*-inositol pentakisphosphate isomers are well resolved on the HPIC system used (Figure 2a), the identity of

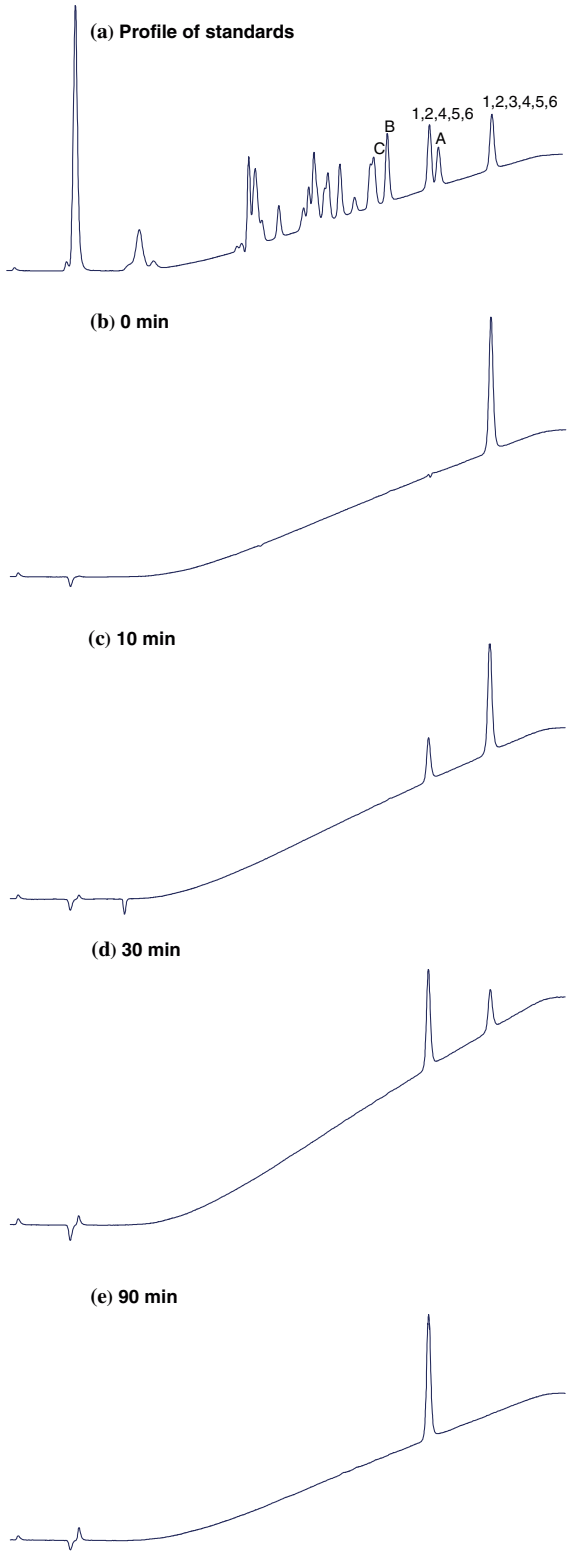


Fig. 2. HPIC analysis of hydrolysis products of *myo*-inositol hexakisphosphate by a purified phytate-degrading enzyme from *Pantoea agglomerans* Purified phytate-degrading enzyme from *Pantoea agglomerans* and Na-phytate were incubated at 35°C and pH 4.5. From the incubation mixture samples were removed periodically and the reaction was stopped by heat treatment. 50 μl of the heat treated samples were chromatographed on a HPIC Carbo Pac PA-100 (4 × 250 mm) analytical column using a gradient of 5–98% HCl (0.5 *M*) (0.8 ml/min).The eluants were mixed in a post-column reactor with 0.1% $Fe(NO_3)_3 \times 9H_2O$ in a 2% $HClO_4$ solution (0.4 ml/min). The combined flow rate was 1.2 ml/min. Reference sample (a) The source of the reference *myo*-inositol phosphates is as indicated in Skoglund *et al*., (1998); phytate-degrading enzyme from *Pantoea agglomerans*, incubation time: (b) 0 min; (c) 10 min; (d) 30 min; (e) 90 min; Peaks:(A) Ins(1,3,4,5,6)P_5; (B) D/L-Ins(1,2,3,4,5)P_5; (C) Ins(1,2,3,4,6)P_5.

the *myo*-inositol pentakisphosphate isomer produced by the *Pantoea* enzyme as D/L-Ins(1,2,4,5,6)P_5 is well established.

3.3.2. Kinetic Studies

To determine the absolute configuration of the *myo*-inositol pentakisphosphate isomer generated by the phytate-degrading enzyme from *Pantoea agglomerans*, kinetic studies with the purified *myo*-inositol pentakisphosphate isomers generated either by the *Aspergillus niger* or the *Pantoea* enzyme were performed. The enzymes were added to sequentially diluted solutions of the purified *myo*-inositol pentakisphosphate isomers and the kinetic parameters (K_M, v_{max}) were calculated from Lineweaver-Burk plots of the data (Table 2). K_M, v_{max} and k_{cat} for the enzymatic hydrolysis of the *myo*-inositol pentakisphosphate isomer generated by the phytate-degrading enzyme from *Pantoea agglomerans* were almost identical with the kinetic constants for the dephosphorylation of the *myo*-inositol pentakisphosphate intermediate generated by the *Aspergillus niger* phytase. Thus, both *myo*-inositol pentakisphosphates are identical. In agreement with data, the *Pantoea* enzyme did not posses activity toward both *myo*-inositol pentakisphosphate isomers. Since it is known that the *Aspergillus niger* phytase predominantly generates the D-Ins(1,2,4,5,6)P_5 isomer (Ullah and Phillippy, 1988), D-Ins(1,2,4,5,6)P_5 is the first and final degradation product of *myo*-inositol hexakisphosphate dephosphorylation by the phytate-degrading enzyme from *Pantoea agglomerans*.

Table 2. Kinetic Constants for Enzymatic *myo*-inositol Pentakisphosphate Dephosphorylation

enzyme origin	kinetic constants	*myo*-inositol pentakisphosphate generated by the	
		Pantoea enzyme	*Aspergillus* enzyme
Pantoea agglomerans	K_M [μmol/l]	not determined	
	v_{max}[U/mg]	both *myo*-inositolpentakisphosphates	
	k_{cat}[s^{-1}]	were not hydrolysed	
Aspergillus niger	K_M[μmol/l]	149 ± 11	155 ± 10
	v_{max}[U/mg]	94 ± 5	98 ± 6
	k_{cat}[s^{-1}]	132 ± 5	139 ± 9

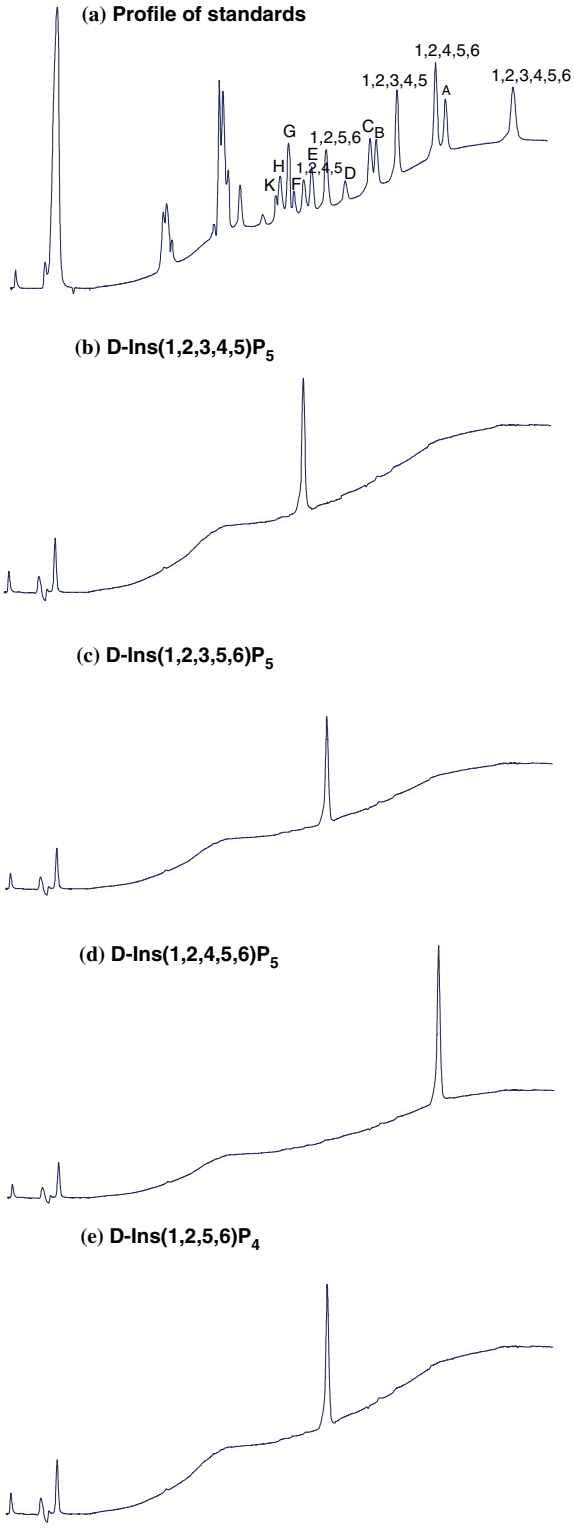
10 mU of the enzymes were added to sequentially diluted solutions of the purified *myo*-inositol pentakisphosphate isomers either generated by the phytate-degrading enzyme from *Pantoea agglomerans* or the *Aspergillus niger* phytase in 400 μl 0.1 *M* sodium acetate buffer, pH 4.5 at 35°C. After an incubation period of 30 min, the liberated phosphate was quantified by a modification of the ammonium molybdate method (Heinonen and Lahti, 1981) with some modification. Activity (U) was expressed as 1 μmol phosphate liberated per min. The kinetic constants (K_M, v_{max}) were calculated from Lineweaver-Burk plots of the data. For calculation of k_{cat} the following molecular masses were used: 85 kDa for the *Aspergillus niger* phytase (Ullah and Gibson, 1987) and 42 kDa for the *Pantoea* enzyme (Greiner, submitted). The data are mean values of three independent experiments.

3.4. Investigation of the Applicability of the Docking Model

To investigate if the docking model established for the *Escherichia coli* glucose-1-phosphatase (Lee *et al*., 2003) is also applicable for the *Pantoea* enzyme, dephosphorylation of the four *myo*-inositolphosphate isomers D-Ins(1,2,4,5,6)P_5, D-Ins(1,2,3,4,5)P_5, D-Ins(1,2,3,5,6)P_5, and D-Ins(1,2,5,6)P_4 by the phytate-degrading enzyme from *Pantoea agglomerans* was studied (Figure 3). As predicted by the model, D-Ins(1,2,4,5,6)P_5 and D-Ins(1,2,5,6)P_4 were not substrates of the *Pantoea* enzyme and D-Ins(1,2,3,4,5)P_5 and D-Ins(1,2,3,5,6)P_5 were specifically hydrolysed to D-Ins(1,2,4,5)P_4 and D-Ins(1,2,5,6)P_4, respectively. In comparison to *myo*-inositol hexakisphosphate ($K_M = 0.34$ mM, $k_{cat} = 21$ s^{-1}), the affinity of D-Ins(1,2,3,4,5)P_5 and D-Ins(1,2,3,5,6)P_5 for the phytate-degrading enzyme from *Pantoea agglomerans* and their maximal rates of hydrolysis were lower (Table 3). K_M and k_{cat} for both *myo*-inositolphosphates were found to be about 0.5 m*M* and 16 s^{-1}.

4. DISCUSSION

This is the second report of an enzyme generating *myo*-inositol pentakisphosphate as the final product of enzymatic phytate degradation. HPLC analysis of enzymatic phytate hydrolysis demonstrated that glucose-1-phosphatase from *Escherichia coli* (Cottrill *et al*., 2002) as well as the *Pantoea* enzyme hydrolysed phytate to D/L-Ins(1,2,4,5,6)P_5. Since HPLC analysis does not allow differentiation of cleavage of phosphate residues from D-1 and D-3 position of the *myo*-inositol ring, Cottrill *et al*., (2002) used two observations to support their conclusion of D-Ins(1,2,4,5,6)P_5 as the final product of phytate dephosphorylation by the *Escherichia coli*



glucose-1-phosphatase. First, when incubated with D-Ins(1,2,3,4,5)P_5, the enzyme specifically removed only the phosphate in the D-3 position and secondly, D-Ins(1,2,5,6)P_4, which does not have a phosphate group in the D-3 position, was not hydrolysed by the glucose-1-phosphatase. In addition, these experimentally obtained hydrolysis products were in excellent agreement with those expected from a glucose-1-phosphatase-phytate binding model (Lee *et al.*, 2003). Since the suitability of a combination of HPIC and kinetic studies to reveal the stereospecificity of enzymatic phytate degradation has already been demonstrated (Greiner *et al.*, 2000, 2001, 2002a,b; Greiner and Larsson Alminger, 2001), this methodology was applied to identify the absolute configuration of the *myo*-inositol pentakisphosphate generated by the phytate-degrading enzyme from

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Fig. 3. HPIC analysis of hydrolysis products of individual *myo*-inositol phosphate isomers by a purified phytate-degrading enzyme from *Pantoea agglomerans* Purified phytate-degrading enzyme from *Pantoea agglomerans* and the individual *myo*-inositol phosphate isomer were incubated at 35°C and pH 4.5 for 90 min. The reactions were stopped by heat treatment. 50 μl of the heat treated samples were chromatographed on a HPIC Carbo Pac PA-100 (4 × 250 mm) analytical column using a gradient of 5–98% HCl (0.5 *M*) (0.8 ml/min).The eluants were mixed in a post-column reactor with 0.1% $Fe(NO_3)_3 \times 9H_2O$ in a 2% $HClO_4$ solution (0.4 ml/min). The combined flow rate was 1.2 ml/min. Reference sample (a). The source of the reference *myo*-inositol phosphates is as indicated in Skoglund *et al.*, (1998); phytate-degrading enzyme from *Pantoea agglomerans*, substrate:(b) D-Ins(1,2,3,4,5)P_5; (c) D-Ins(1,2,3,5,6)P_5; (d) D-Ins(1,2,4,5,6)P_5; (e) D-Ins(1,2,5,6)P_4; Peaks:(A) Ins(1,3,4,5,6)P_5; (B) Ins(1,2,3,4,6)P_5; (C) D/L-Ins(1,4,5,6)P_4; (D) Ins(2,4,5,6)P_4; (E) D/L-Ins(1,3,4,5)P_4; (F) Ins(1,3,4,6)P_4; (G) D/L-Ins(1,2,3,4)P_4; (H) D/L-Ins(1,2,4,6)P_4; (K) Ins(1,2,3,5)P_4.

Table 3. Kinetic Constants for the Hydrolysis of Phosphorylated Compounds by the Phytate-degrading Enzyme from *Pantoea agglomerans* at pH 4.5

Substrate	K_M (m*M*)	k_{cat} (s^{-1})	k_{cat}/K_M (s^{-1} M^{-1})
Phytate	0.34 ± 0.014	21 ± 0.7	61765
D-Ins(1,2,3,4,5)P_5	0.49 ± 0.02	16 ± 0.5	32742
D-Ins(1,2,3,5,6)P_5	0.51 ± 0.025	16 ± 0.7	31515
D-Ins(1,2,4,5,6)P_5	no degradation		
D-Ins(1,2,5,6)P_4	no degradation		
glucose-1-phosphate	0.26 ± 0.01	101 ± 2.3	384615
glucose-6-phosphate	1.3 ± 0.1	2.9 ± 0.15	282
fructose-1-phosphate	1.9 ± 0.12	1.6 ± 0.12	152
fructose-1,6-diphosphate	2.2 ± 0.15	1.1 ± 0.12	103
ribose-5-phosphate	9.8 ± 0.4	1.1 ± 0.1	96

Note: Temperature: 37°C; buffer: 100 m*M* sodium acetate, pH 4.5; enzyme concentration: 25 mU/ml.

Pantoea agglomerans. The data indeed indicate that the *Pantoea* enzyme dephosphorylates phytate in a stereospecific way to D-*myo*-inositol(1,2,4,5,6)pentakisphosphate as the sole hydrolysis product. As the *Escherichia coli* glucose-1-phosphatase (Cottrill *et al*., 2002), the phytate-degrading enzyme from *Pantoea agglomerans* dephosphorylated D-Ins(1,2,3,4,5)P_5 specifically to D-Ins(1,2,4,5)P_4 and both enzymes did not act upon D-Ins(1,2,4,5,6)P_5 and D-Ins(1,2,5,6)P_4. Also in excellent agreement with the docking model developed for the glucose-1-phosphatase from *Escherichia coli* (Lee *et al*., 2003), the *Pantoea* enzyme removes only the phosphate residue in D-3 position of D-Ins(1,2,3,5,6)P_5 to yield D-Ins(1,2,5,6)P_4 as the sole degradation product. The validity of the docking model to describe dephosphorylation of different *myo*-inositol phosphates by the phytate-degrading enzyme from *Pantoea agglomerans* implies a very similar folding architecture of the *Pantoea* enzyme and the glucose-1-phosphatase from *Escherichia coli*.

As outlined above, a series of physico-chemical and enzymatic parameters, such as molecular mass, periplasmatic localisation, pH profile as well as temperature optimum for phytate degradation, and high activity towards glucose-1-phosphate already pointed to a close similarity between the phytate-degrading enzyme from *Pantoea agglomerans* and the glucose-1-phosphatase from *Escherichia coli*. The data given in this paper even prolong the list of similarities. Both enzymes exhibit an identical hydrolysis profile for different *myo*-inositol phosphates, which is in contrast to all other acid phytate-degrading enzymes studies so far with respect to their phytate degradation pathway. All these enzymes are capable of sequentially dephosphorylate five from six phosphate residues from phytate, leaving myo-inositol(2)monophosphate as the final product (Cosgrove, 1970; Greiner *et al*., 2000, 2001; Greiner *et al*., 2002a,b; Greiner and Larsson Alminger, 2001; Hayakawa *et al*., 1990; Nakano *et al*., 2000; Sajidan *et al*., 2004; Wyss *et al*., 1999). In addition, both enzymes have strong preference for glucose-1-phosphate over other small monosaccharide phosphates (Table 3) and hydrolyse glucose-1-phosphate at an unusually high pH (*Escherichia coli*: pH 6.5 (Cottrill *et al*., 2002), *Pantoea agglomerans:* pH 6.25 (data not shown)) compared with other histidine acid phosphatases. Therefore both enzymes are expected to have identical physiological functions. The reported primary function of the glucose-1-phosphatase from *Escherichia coli* is to scavenge glucose from glucose-1-phosphate (Pradel and Boquet, 1991), but a role in inositol phosphate metabolism or perhaps microbial pathogenesis was also discussed (Cottrill *et al*., 2002).

In contrast to *Escherichia coli*, where two enzymes with phytate-degrading activity were identified: the *appA*-encoded phytase (Greiner *et al*., 1993) and the *agp*-encoded glucose-1-phosphatase (Cottrill *et al*., 2002), only the pendant to the glucose-1-phosphatase, but no phytase-like enzyme could be found in *Pantoea agglomerans* (Greiner, 2004). Therefore, a role of the *Pantoea* enzyme in phytate breakdown could not fully be ruled out, even if glucose-1-phosphate seems to be its most likely substrate. Phytate degradation *in vivo* is not necessarily due to the action of a single enzyme, but could be realised by the concerted action of several phosphatases. Thus, the phytate-degrading enzyme from *Pantoea agglomerans* could conceivably be active in phytate breakdown in a co-operative role initiating the stepwise release of phosphate from phytate, whereas other phosphatases present in *Pantoea agglomerans* proceed with the hydrolysis of the generated *myo*-inositol pentakisphosphate.

Independent of its *in vivo* function and due to its special hydrolysis profile for different *myo*-inositol phosphates, the phytate-degrading enzyme from *Pantoea agglomerans* is a good candidate for the production of individual *myo*-inositol phosphate isomers. The major interest in individual breakdown products of phytate results from physiological effects which have been attributed to their action (Ohkawa *et al*., 1984; Potter, 1995; Vucenik and Shamsuddin, 2003). Up to now, the diversity and practical unavailability of most of the individual phytate breakdown products preclude their being studied. Attempts to produce individual *myo*-inositol phosphate isomers non-enzymatically have resulted in mixtures of *myo*-inositol pentakis-, tetrakis-, tris-, and bisphosphate isomers. Purification of these isomers from the mixtures is arduous and uneconomical. The use of phytate-degrading enzymes has been seen as a way to get easily access to the huge variety of individual *myo*-inositol phosphate isomers, since these enzymes generate predominantly only one or at maximum two major isomers of the different lower *myo*-inositol phosphate groups (*myo*-inositol pentakis to monophosphates) (Greiner and Konietzny, 1996).

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