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Purification and characterization of a β-xylosidase from potatoes (*Solanum tuberosum*)

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Abstract

Potatoes are a cheap and easily available source for the preparation of β1,2-xylosidase. The soluble enzyme was purified from potato tubers by ammonium sulfate precipitation, hydrophobic interaction chromatography, affinity gel blue chromatography, ion exchange and size exclusion chromatography yielding a glycoprotein with a molecular weight of 39–40 kDa, an isoelectric point of 5.1 and a typical plant N-glycosylation pattern. The enzyme releases xylose residues β1,2-linked to the β-mannose of an N-glycan core, if the 3-position of this mannose is not occupied. It showed an optimal enzymatic activity at pH 4.0–4.5 and at a temperature of 50 °C. The activity was reduced in the presence of Ni^{2+} and Cu^{2+} and slightly increased by the addition of Mn^{2+} or Ca^{2+}. At 37 °C the cleavage of xylose from *p*-nitrophenyl-β-xylopyranoside or appropriate pyridylaminated N-glycans was proportional to the time of incubation over a period of 8 h and increased with time for at least 24 h. *N*-Methoxycarbonylpentyl-1,5-dideoxy-1,5-iminoxylitol inhibits the enzyme effectively. Sequencing of the N-terminus showed a high homology to a number of isoforms of patatin, the main protein of potato tubers. This enzyme will be an important tool for the analysis of N-glycans and in the modification of N-glycans for immunological studies.

Keywords: β-Xylosidase; Exoglycosidase; Potato enzyme

1. Introduction

Carbohydrates of defined structures are a prerequisite for the investigation of glycoprotein glycan biosynthesis, for determining the fine binding specificity of novel carbohydrate-binding proteins of interest in immunology (i.e. galectins or selectins) or for the development of new analytical and preparative tools such as affinity matrices. Whereas organic synthesis of complex N-glycans requires a considerable number of consecutive usually low-yield steps, the purification of glycans from natural sources and their modification, by glycosidases and glycosyltransferases with known specificity, has been developed during the last years as an interesting alternative in order to obtain homogenous oligosaccharides in yields sufficient for further studies.

Xylose is a common monosaccharide of plant derived N-glycans [1]. Furthermore, it has been found in structures of gastropod and schistosome origin [2–4]. As far as it is known today, when a component of N-glycans, it is linked exclusively by a β1,2-linkage to the β-mannose of the inner core. In a number of studies dealing with plant allergy, the xylose residue has been found to take part in immunologically relevant phenomena. Together with the α1,3-linked core-fucose it forms a carbohydrate based epitope [1,5,6]. The modification of the potential allergens by specific enzymes is extremely helpful in the investigation of minimum structural requirements of a glycan to serve as an allergen or immune modulator.

A number of xylose-releasing exo- and endo-enzymes have been described so far. Most of them have been found in microorganisms and fungi [7–13]. In vivo they seem to

Abbreviations: MALDI-TOF MS, matrix assisted laser desorption ionisation-time of flight mass spectrometry; MUXF, Manα1,6(Xylβ1,2)Manβ1,4GlcNAcβ1,4(Fucα1,3)GlcNAc; MMXF, Manα1,6(Manα1,3)[Xylβ1,2]Manβ1,4GlcNAcβ1,4(Fucα1,3)GlcNAc and GnGnXF,GlcNAcβ1,2Manα1,6(GlcNAcβ1,2Manα1,3)[Xylβ1,2]Manβ1,4GlcNAcβ1,4(Fucα1,3)-GlcNAc; PA, pyridylaminated; PLA_2, phospholipase A_2

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be involved in the degradation of hemicelluloses and are part of the biological recycling of plant material. Some of them are in commercial use in pulp and paper industry or for softening processes in food industry [14].

However, those described thus far are not suitable for the modification of N-glycans as they have a high preference for the cleavage of β1,4-linkages and a number of them are bifunctional for the release of xylose and glucose or arabinose [15–18]. Hydrolysis of β1,2-linkages is a minor, if at all present, side effect activity. The only commercially available β1,2-xylosidase is from *Xanthomonas* sp. and displays a mixture of several β-xylosidase activities. Enzyme preparations from sycamore cells (*Acer pseudoplatanus* L.) [19] or apple snails (*Pomacea canaliculata*) [20] are not easy available and the use of the 0.003% xylosidase impurity of the commercial available jack been α-mannosidase [21] is also far from being optimal. In order to contribute to solving this far from optimal situation, we screened several plant tissues for their β-xylosidase activities to find a cheap and easily available source for the preparation of a β1,2-specific xylosidase.

Here we present the purification of a β1,2-xylosidase isolated from potatoes which is specific for xylose and does not act on other sugars commonly found in N-glycans. We have also characterized several properties of this enzyme. The enzyme can be used in N-glycan structure analysis as well as for the modification of glycans in binding specificity and immunological studies.

2. Materials and methods

2.1. Materials

Octyl-Sepharose CL4B and Sephacryl S-200 superfine were from Amersham Pharmacia, Affi-Gel Blue Gel 100–200 mesh was from Bio-Rad Laboratories and DE 52 (Diethylaminoethyl cellulose) was from Whatman. Oligosaccharides and pyridylaminated reference oligosaccharides, Manα1,6(Xylβ1,2)Manβ1,4GlcNAcβ1,4(Fucα1,3GlcNAc(MUXF), Manα1,6(Manα1,3)[Xylβ1,2] Manβ1,4GlcNAcβ1,4(Fucα1,3)GlcNAc (MMXF) and GlcNAcβ1,2Manα1,6(GlcNAcβ1,2Manα1,3)[Xylβ1,2]Manβ1,4GlcNAcβ1,4(Fucα1,3)-GlcNAc (GnGnXF) were available from previous studies [1]. Xylobiose from Sigma was pyridylaminated according to Hase et al. [22]. *N*-Methoxycarbonylpentyl-1,5-dideoxy-1,5-iminoxylitol was kindly provided by Dr. Arnold Stütz (Institute of Organic Chemistry, Technical University Graz, Austria). N-Glycosidase A was from Roche. All other materials purchased were of the highest quality available from Merck or Sigma.

2.2. Purification of the enzyme

Unless otherwise indicated, all operations were performed at room temperature. An Amicon stirred cell with a YM10 membrane was used for concentration and buffer exchange. Each step of the preparation and purification was monitored by assessing protein concentration and exoglycosidase activities.

2.2.1. Homogenization and protein precepitation

Fresh potatoes (320 g) (Ditta I, Tulln, Austria) were cut into small pieces and homogenized with 300 ml of 50 mM Tris/HCl, pH 7.5, containing 3 mM dithiothreitol and 0,02% of sodium azide with a Blender and centrifuged at 3000×*g* for 10 min using a Sorvall GSA rotor. The pellet was resuspended in 300 ml of the same buffer, homogenized with an IKA Ultra Turrax T25 at 15 000 rpm for 2×20 s and centrifuged at 10 000×*g* for 10 min. The combined supernatants of both centrifugations were adjusted to achieve a 40% saturation in terms of ammonium sulfate and centrifuged at 27 500×*g* for 25 min. The supernatant was further adjusted to be 80% of ammonium sulfate and centrifuged at 27 500×*g* for 40 min.

2.2.2. Octyl-sepharose CL4B chromatography

The precipitate of the 80%-step was dissolved in 40 ml of 10 mM sodium phosphate, pH 7.0, containing 1.2 M ammonium sulfate and applied onto an Octyl-Sepharose column containing 45-ml gel, equilibrated in the same buffer. Elution was carried out first by 50 ml of starting buffer and then by 400 ml of a linear gradient from 1.2–0 M ammonium sulfate. Enzyme containing fractions were pooled, concentrated, adjusted to 1.2 M ammonium sulfate and rechromatographied on the same column under the same conditions.

2.2.3. Affi-gel blue gel chromatography

Enzyme containing fractions of the second Octyl-Sepharose step were concentrated, adjusted to 50 mM Tris/HCl buffer, pH 7.5 and applied to a column of 5 ml of Affi-Gel Blue. The column was eluted with 20 ml of the same buffer, followed by 40 ml of a linear gradient from 0–1 M sodium chloride in the same buffer.

2.2.4. Ion exchange on DE52

Enzyme-containing fractions of the Affi-Gel Blue chromatography were concentrated, adjusted to Tris/HCl, pH 8.3 and applied to a column of 5 ml of DE 52. The column was eluted with 30 ml of the same buffer, followed by 40 ml of a linear gradient from 0–1 M sodium chloride in the same buffer.

2.2.5. Sephacryl S-200 gel filtration

The enzyme preparation after ion exchange chromatography was concentrated and applied onto a Sephacryl S-200 column (1.5×120 cm) at 4 °C equilibrated with 50 mM Tris/HCl, pH 7.5, containing 0.02% NaN_3. Fractions of 3.5 ml were collected and analysed for enzyme activities. This column was also used for the determination of molecular weight of the native β-xylosidase.

The purified protein was concentrated and stored in 50 mM Tris/HCl, pH 7.5 at 4 °C.

2.3. Assay procedures

For the determination of exoglycosidases, the standard incubation mixture contained in a total volume of 50 μl, 5 mM *p*-nitrophenyl-sugar (β-D-xylopyranoside, α-D-mannopyranoside, β-D-glucopyranoside, α-D-galactopyranoside, β-D-galactopyranoside, α-L-fucopyranoside, 2-acetamido-2-deoxy-β-D-galactopyranoside, 2-acetamido-2-deoxy-β-D-glucopyranoside), 50 mM Citrate/NaOH-buffer, pH 4.0 and 5–25 μl of the appropriate fraction. Incubations were carried out for 2 h at 37 °C and terminated by the addition of 250-μl 0.4 M glycine/NaOH, pH 10.0 and the absorbance was measured at 405 nm. One unit of activity is defined as the amount of enzyme releasing 1.0 μmol of sugar per minute.

For substrate specificity studies, 5 nmol of substrate (MUXF, MMXF, GnGnXF-oligosaccharides for MALDI-TOF and MUXF-PA, MMXF-PA, GnGnXF-PA and pyridylaminated xylobiose for HPLC) was incubated in a total volume of 10 μl with 5-μl pure enzyme in the presence of 50 mM citrate/NaOH, pH 4.0, for 24 h at 37 °C. Product analysis was carried out by MALDI-TOF MS (1 μl of sample) and two-dimensional HPLC (ODS, Palpak N, 3 μl of each sample).

For the determination of the pH-optimum, the activity was analysed using the standard assay conditions varying the pH from 3.5 to 9.0 in 0.5-pH-unit steps using the following buffer systems: from pH 3.5–5.5 acetate/NaOH; from 3.5–6.5 citrate/NaOH; from 5.5–7.0 MES (2-(*N*-morpholino)ethanesulfonic acid); from 6.0–7.5 phosphate; and from 7.0–9.0 Tris/HCl buffer. The overlapping points show the influence of the different buffer salts at a certain pH.

Cation requirement was analysed by the standard incubation assay without addition of any divalent cation or in the presence of 10 mM EDTA, Mn^{2+}, Mg^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, or Ba^{2+}. A time course was established by varying the incubation time of the standard assay from 10 min to 24 h. For the determination of the stability the enzyme was stored at temperatures from −20 to +80 °C for 48 h before performing the standard assay. Furthermore the enzyme was stored for 48 h at 37 °C in the presence of 10% (v/v) of methanol, acetonitrile or glycerol.

Inhibition experiments were performed by mixing 10 μl of *N*-methoxycarbonylpentyl-1,5-dideoxy-1,5-iminoxylitol in a concentration varying from 1 μM to 100 mM in 50 mM citrate/NaOH pH 4.0 with the enzyme solution. After an incubation of 30 min at room temperature, *p*-nitrophenyl-β-D-xylopyranoside was added to a final concentration of 5 mM. Further incubation and termination were carried out according to the standard assay.

All these assays were carried out in duplicate with appropriate controls.

2.4. Analysis of the purified protein

2.4.1. Determination of protein content

Protein concentrations were determined by the Micro-BCA protein assay (Pierce) with bovine serum albumin as the standard.

2.4.2. SDS-PAGE

SDS-polyacrylamide gel electrophoresis was carried out using a Bio-Rad Mini-Protean II Cell with gels containing 12.5% (w/v) acrylamide and 1% (w/v) bisacrylamide [23] and determination of the isoelectric point was done according to Ref. [24].

2.4.3. Amino acid composition

Amino acid analysis was carried out with *o*-phthalaldehyde as previously described [25].

2.4.4. N-glycan analysis

N-glycan preparation by peptic digest of the protein, N-glycosidase A, release of the glycan and fluorescence labelling of the glycan with 2-aminopyridine were done as previously described [26]. The analysis of the fluorescently labelled glycan was carried out on HPLC (data not shown) and MALDI-TOF mass spectrometry.

Normal phase HPLC was performed using a modification of the procedure of Khoo et al. [4] using a Palpak type N column (4.6×250 mm, TaKaRa, Japan) at a flow rate of 1 ml/min. Solvent A was 75:25 (v/v) acetonitrile/stock solution (3% w/v acetic acid–triethylamine buffer at pH 7.3 with 10% (v/v) acetonitrile). Solvent B was 50:50 (v/v) acetonitrile/stock solution. The run was started with 5 min at 10% solvent B in solvent A followed by a linear gradient of 2.8% per minute to 80% solvent B in solvent A, and terminated by 8 min at 80% solvent B. Reverse-phase chromatography was carried out according to Ref. [26]. Fluorometric detection was carried out at Ex/Em 320/400 nm.

MALDI-TOF mass spectrometry was done according to Ref. [27]. One microliter of sample (0.2–0.8 pmol) was spotted onto the target and dried, followed by the addition of 0.8 μl of matrix (2% 2,5-dihydroxybenzoic acid in water containing 30% acetonitrile). The platen was immediately transferred to a desiccator and vacuum was applied until all solvent had evaporated. All spectra were recorded on a DYNAMO linear MALDI-TOF mass spectrometer (Thermo BioAnalysis, Hemel Hempstead, UK) operated with a dynamic extraction setting of 0.1. External mass calibration was performed with pyridylaminated N-glycans derived from bovine fibrin. About 20 individual laser shots were summed.

2.4.5. Sequencing of the N-terminus

The N-terminal sequencing was carried out as described previously [28]. A sample of purified enzyme was separated on a SDS-PAGE using Tris–Tricine

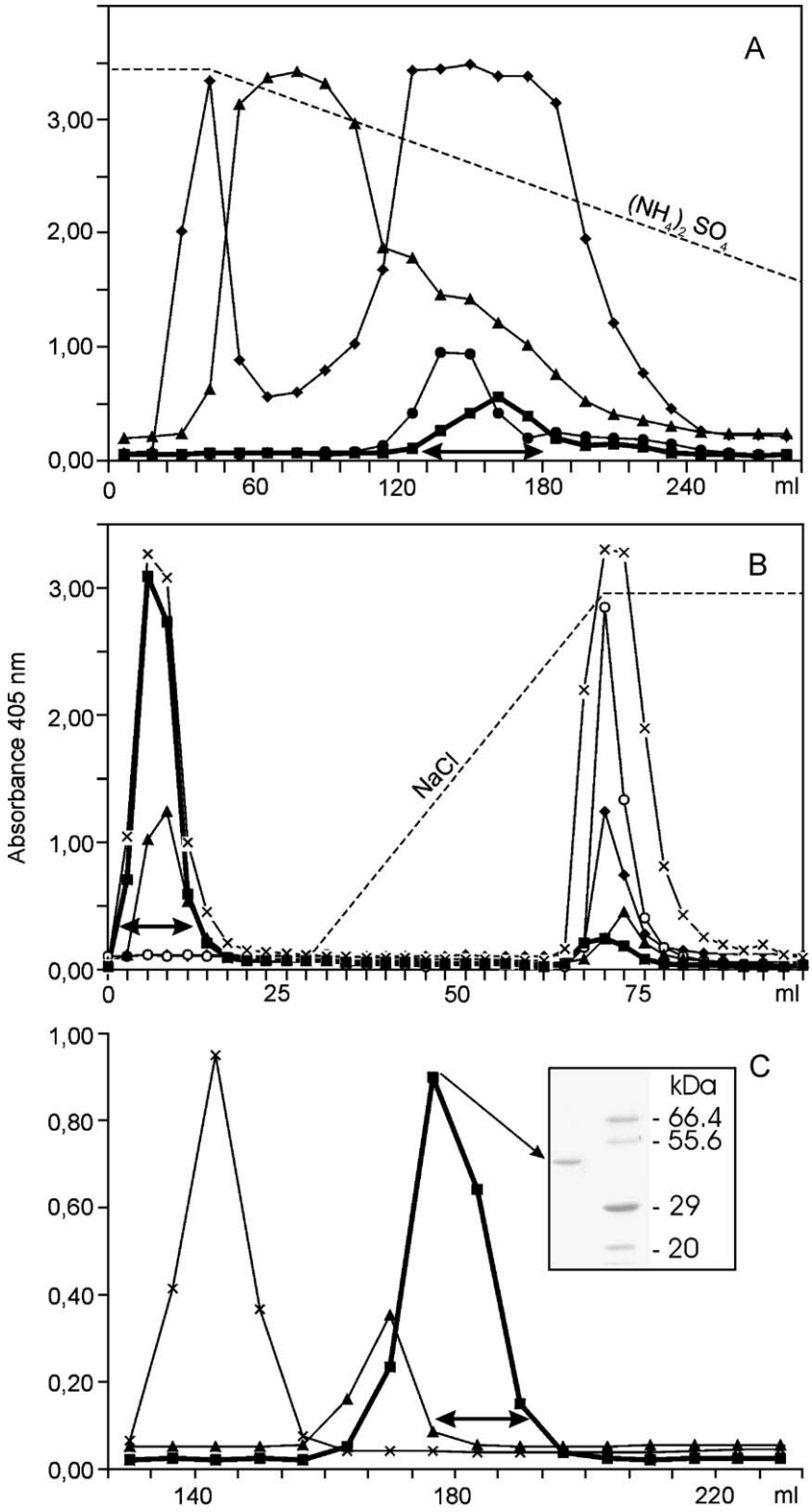

Fig. 1. Purification of β-xylosidase from potato by (A) octylsepharose CL4B (second step), (B) anion exchange chromatography on DE52, (C) gel filtration on Sephacryl S 200. Fractions were pooled as indicated. (■—■) β-xylosidase (bold line), (♦—♦) β-*N*-acetylglucosaminidase, (▲—▲) β-galactosidase, (●—●) β-glucosidase, (○—○) β-*N*-acetylgalactosaminidase, (×—×) α-mannosidase. The dotted lines in (A) and (B) indicate the ammonium sulfate and the sodium chloride gradients, respectively. In (C) the Coomassie-stained SDS-PAGE of the purified enzyme in comparison with a molecular weight ladder is shown.

Table 1
Purification protocol of β-xylosidase from potato

Steps	Total protein (mg)	Total activity (mU)	Specific activity (mU/mg)	Recovery (%)	Purification-fold
(1) Homogenate	7622	1904	0.250	100	1
(2) 40% supernatant	2635	1800	0.683	94.5	2.7
(3) 80% precipitation	123.6	1429	11.56	75	46.24
(4) Octyl-Sepharose Cl 4B	11.52	256	22.22	13.44	88.88
(5) Affi-Gel Blue Gel	3.6	120	33.33	6.3	133.3
(6) DE52	1.2	86.1	71.75	4.5	287
(7) Sephacryl S-200	0.3	25.6	85.33	1.34	341

buffer [29], electroblotted onto Immobilon P, detected by fluorescamine staining, excised and placed into the sequencer cartridge. Automated sequencing was done on a model 473A sequencer (Applied Biosystems, Foster City, CA).

3. Results

3.1. Purification of the enzyme

Preliminary work on several plant tissues revealed that potatoes are a good and easily available starting material for the purification of a β-xylosidase. Following homogenisation and the separation of insoluble material by centrifugation, a two-step salt-precipitation of the proteins was carried out by means of which most of the starch could be removed. The final precipitate was further purified by hydrophobic interaction chromatography removing a large amount of protein and coloured compounds. Fractions were rechromatographed to obtain better resolution under the same conditions. This rechromatographic step is extremely important in order to reduce the amount of other closely related exoglycosidases (Fig. 1A). The desalted sample was then applied to Affi-Gel Blue. The exoglycosidases do not bind to the column but a number of other proteins are removed by this step. The anion-exchanger is used mainly in order to reduce the amount of remaining contaminant glycosidases. *N*-Acetylglucosaminidase and *N*-acetylgalactosaminidase are removed here completely (Fig. 1B). Finally, the xylosidase was separated from α-mannosidase and β-galactosidase by Sephacryl S200 gel filtration, which provided an enzyme preparation suitable for preparative applications (Fig. 1C, Table 1). Under the conditions used, the final product did not contain any other exoglycosidase activities detectable with *p*-nitrophenyl-substrates and appeared as a single band on SDS-PAGE.

Table 2
Sequence of the putative N-terminus of potato β-xylosidase

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Reference
Xylosidase potato*	Q/E	L	G/E	D/E	M	V	T	V	L	S	I	D	G	G	G	I/G	K	G	I/L	I	P/Q	A/P	T/I	I	L/G	
	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	
Q41487 patatin	S	L	E	E	M	V	T	V	L	S	I	D	G	G	G	I	K	G	I	I	P	G	T	I	L	TrEMBL
Q41478 patatin	T	L	G	E	M	V	T	V	L	S	I	D	G	G	G	I	K	G	I	I	P	A	T	T	L	TrEMBL
Q41443 patatin	K	L	E	E	M	V	T	V	L	S	I	D	G	G	G	I	K	G	I	I	P	A	I	I	L	TrEMBL
Q41467 patatin	K	L	E	E	M	V	T	V	L	S	I	D	G	G	G	I	K	G	I	I	P	A	I	I	L	TrEMBL
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22				
PLA_2 "Irish cobbler"	K	L	G	E	M	V	T	V	L	S	I	D/G	G	G	G	I/G	K	G	I	I	P	A				[30]
PLA_2 "Superior"	T	L	G	E	M	V	T	V	L	S	I	D	G	G	G	I	K	G	I	I	P	A				[31]
PLA_2 "La Chipper"	T	L	G	E	M	V	T	V	L	S	I	D	G	G	G	I	K	G	I	I	P	A				[31]
	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	
Hev b 7	T	Q	G	K	K	I	T	V	L	S	I	D	G	G	G	I	R	G	I	I	P	G	I	I	L	[32]
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	
PLA_2 tobacco leave	T	K	G	K	I	V	T	V	L	S	I	D	G	G	G	I	R	G	I	I	P	G	T	L	L	[33]

Characteristic amino acids are highlighted in black (strictly conserved) or in grey.
(*) In positions where two amino acids are shown, the first one represents more than 95% of the total area obtained in the cycle.

3.2. Characterization of the enzyme

The molecular weight was estimated from SDS-PAGE as well as by the elution position upon gel filtration as being 39–40 kDa; therefore, the enzyme is a monomer. The isoelectric point was found to be 5.1. Glycosylation analysis revealed a typical plant glycan pattern with MMXF as the main N-linked oligosaccharide (>85%) and some minor compounds of oligomannosidic (with four to six mannoses) and paucimannosidic (e.g. MUXF, MMX) structures. The amino terminal was determined to be

Gln/Glu – Leu – Gly/Glu – Asp/Glu – Met – Val – Thr
–Val – Leu – Ser – Ile – Asp – Gly – Gly – Gly
–Ile/Gly – Lys – Gly – Ile/Leu – Ile – Pro/Gln
–Ala/Pro – Thr/Ile – Ile – Leu/Gly–

Comparing this sequence with already published sequences from databanks, a high homology to a number of isoforms of patatin, the main protein of potato tubers was found (Table 2 [30–33]). To confirm these data, patatin (molecular weight about 41 kDa, isoelectric point 4.4–4.7) was prepared exactly according to Pots et al. [34] from the same potato source. No xylosidase activity could be determined in this preparation. The molar amino acid compositions of the xylosidase and the patatin preparations were very similar (Table 3) even though their molecular weights and their isoelectric points were not the same.

3.3. Specificity of the enzyme

The intermediate and final products of 24-h incubation assays in the presence of MUXF, MMXF, GnGnXF and xylobiose were analysed by two-dimensional HPLC (data not shown) and MALDI-TOF mass spectrometry (Fig. 2).

Table 3
Amino acid composition of β-xylosidase and patatin of potato

Amino acids	Amino acid composition (molar %)	
	β-Xylosidase	Patatin
Asp	10.66	8.55
Glu	8.60	7.87
Ser	7.71	6.15
His	1.55	1.35
Gly	7.38	7.72
Thr	8.09	7.77
Arg	2.44	2.78
Ala	9.36	9.89
Tyr	2.08	1.16
Val	3.78	5.57
Ile	2.56	4.13
Phe	4.20	4.12
Leu	9.94	9.16
Lys	21.63	23.78

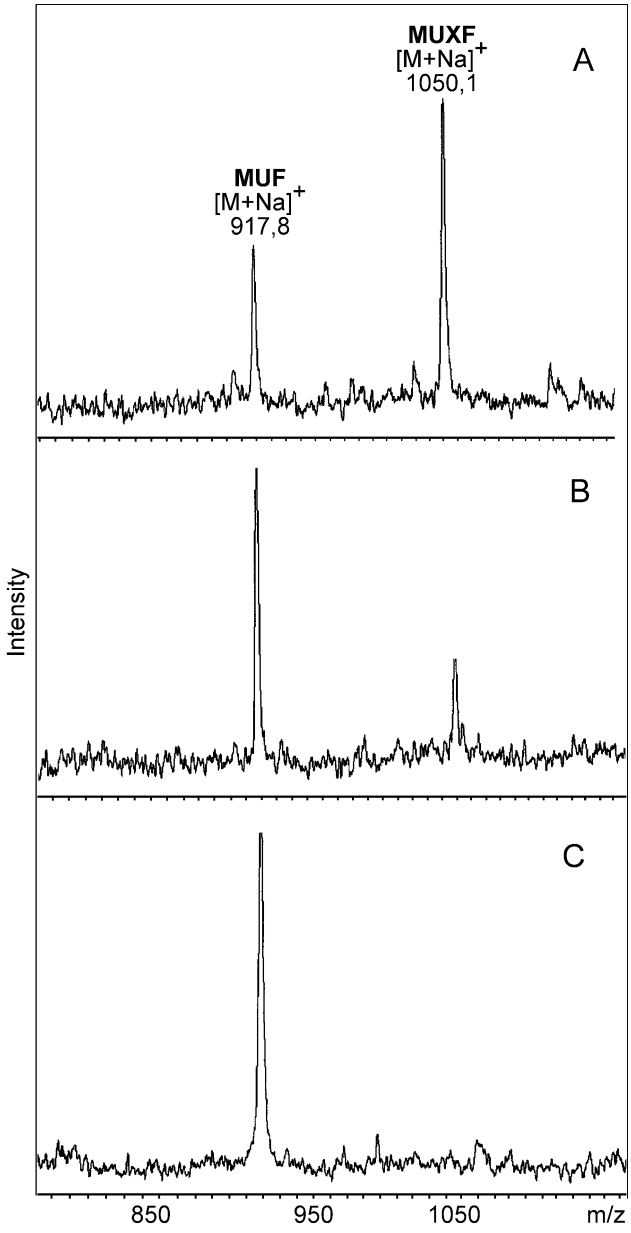

Fig. 2. MALDI-TOF analysis of MUXF-oligosaccharide after incubation with β-xylosidase for (A) 2 h, (B) 6 h and (C) 24 h.

The enzyme cleaved the β1,2-linked xylose from MUXF but could not act on substrates with intact α1,3-antenna (MMXF, GnGnXF). A minor activity against xylobiose could be detected. Cleavage of xylose from MUXF and *p*-nitrophenyl-β-xylopyranoside was proportional to the time of incubation over a period of 8 h and increased with time for at least 24 h (Fig. 3A).

3.4. Properties of the enzyme

β-Xylosidase activity was not affected by a storage for 48 h in a range from −20 to 37 °C, or by the addition of 10% of methanol or glycerol. The activity was slightly reduced to about 80% by the addition of acetonitrile and

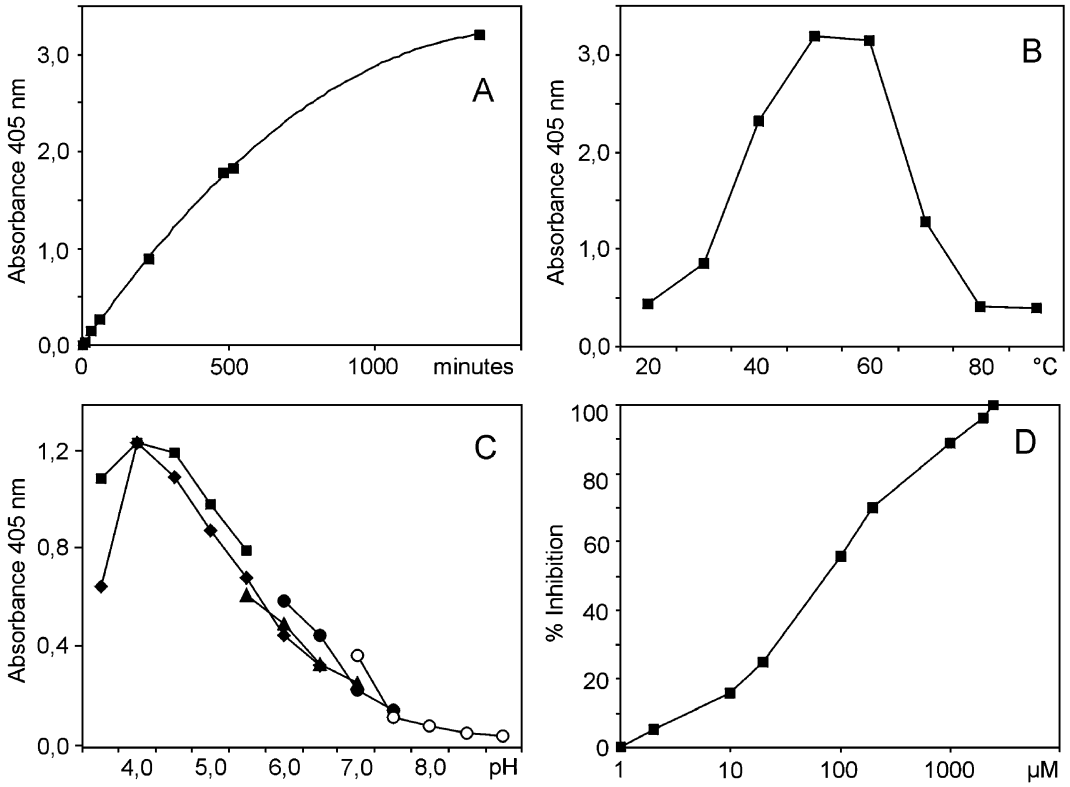

Fig. 3. Properties of β-xylosidase from potato. (A) Time course for 24 h. (B) Influence of incubation temperature. (C) pH Pattern of β-xylosidase using the following buffer systems: (■—■) acetate/NaOH, (◆—◆) citrate/NaOH, (▲—▲) MES, (●—●) phosphate, (○—○) Tris/HCl. (D) Inhibition by *N*-Methoxycarbonylpentyl-1,5-dideoxy-1,5-iminoxylitol.

more drastically by temperatures above 40 °C. After 48 h at 50 °C, only 25% of the previous activity was detected. However, 50 °C was the optimal incubation temperature for short assays up to 2 h (Fig. 3B).

The β-xylosidase showed a pH-optimum at 4.0–4.5 (Fig. 3C). Enzyme activity was increased with Mn^{2+} or Ca^{2+} at a concentration of 10 mM and reduced in the presence of Ni^{2+} and Cu^{2+}.

N-Methoxycarbonylpentyl-1,5-dideoxy-1,5-iminoxylitol, which has been described in a previous study to be an inhibitor for β-xylosidases [35], effectively inhibited the potato xylosidase at a concentration of 2 mM (Fig. 3D).

4. Discussion

Xylose, a common component of many plant and some trematode and gastropod N-glycans, has been shown to play an important role in forming allergenic epitopes. A specific xylosidase for the removal of xylose is a necessary requisite for the analysis of these structures and for the modification of potential allergens.

In this study we report the functional purification of a soluble β1,2-xylosidase from potato tubers by various precipitation and chromatography steps. Similarly to many exoglycosidases, the enzyme shows an acidic pH optimum, suggesting a vacuolar localization and a role in breakdown of N-glycans in the plant cell. The xylosidase requires no divalent cations, even though the activity can be slightly increased by the addition of Mn^{2+} or Ca^{2+}.

The purified enzyme is specific for xylose residues β1,2-linked to the β-mannose of the inner core of N-glycans. Core fucosylation did not prevent the activity of the enzyme; however, substitution of the 3-hydroxyl of the β-linked mannose prevented the release of xylose, therefore a potential pathway of degradation can be deduced in which removal of xylose from N-glycan depends on the prior action of at least α-mannosidase. The β1,2-xylosidase from potatoes shares this specificity with the corresponding enzymes purified from sycamore cells [19] and apple snails [20]. The α-1,3-linked mannose seems to be an important sterical hindrance for the action of β1,2-xylosidase. However, it has to be kept in mind that any enzyme activity against artificial substrates may not accurately reflect its action in vivo against natural substrates.

The N-terminus of the potato β1,2-xylosidase is highly homologous to internal sequences of a number of isoforms of patatin and its molar amino acid composition is similar to a patatin preparation from the same potato source. Patatin has been shown to be a family of glycoproteins that contributes about 40% of the total soluble protein in potato tubers. The protein is encoded by a multigene family of 50–70 genes [36–38]. Several enzyme activities, such as lipid acid hydrolase, acyltransferase, wax synthase or phospholi-

pase have been identified to correspond with the patatin isoforms [30,39,40]. The finding of a hydrolytic activity against a part of an oligosaccharides is a not very surprising extension to this list. All these enzyme activities may have an important function in sprouting or pathogen defence of the tuber [40,41].

The β1,2-xylosidase from potato tubers can be easily prepared and is convenient to handle due to its stability against temperature and chemicals. It can be used for analysing N-glycans or for the modification of substrates for immunological studies.

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