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Molecular cloning and characterization of a plant α1,3/4-fucosidase based on sequence tags from almond fucosidase I

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Abstract

Our work with almond peptide *N*-glycosidase A made us interested also in the α1,3/4-fucosidase which is used as a specific reagent for glycoconjugate analysis. The enzyme was purified to presumed homogeneity by a series of chromatographic steps including dye affinity and fast-performance anion exchange chromatography. The 63 kDa band was analyzed by tandem mass spectrometry which yielded several partial sequences. A homology search retrieved the hypothetical protein Q8GW72 from *Arabidopsis thaliana*. This protein has recently been described as being specific for α1,2-linkages. However, cDNA cloning and expression in *Pichia pastoris* of the *A. thaliana* fucosidase showed that it hydrolyzed fucose in 3- and 4-linkage to GlcNAc in Lewis determinants whereas neither 2-linked fucose nor fucose in 3-linkage to the innermost GlcNAc residue were attacked. This first cloning of a plant α1,3/4-fucosidase also confirmed the identity of the purified almond enzyme and thus settles the notorious uncertainty about its molecular mass. The α1,3/4-fucosidase from *Arabidopsis* exhibited striking sequence similarity with an enzyme of similar substrate specificity from *Streptomyces* sp. (Q9Z4I9) and with putative proteins from rice.

Keywords: Arabidopsis; Fucosidase; Lewis a; Almond; Plant glycosidase

1. Introduction

In mammalian glycoconjugates, fucose residues are frequently prominent parts of biologically significant determinants (Staudacher et al., 1999). In plant glycoproteins, fucose occurs either in α1,3-linkage to the innermost GlcNAc or in α1,4-linkage to a terminal GlcNAc residue where, together with galactose, it forms the Lewis a determinant (Fitchette-Laine et al., 1997; Melo et al., 1997). Furthermore, plant cell wall glycans contain fucose, e.g., in α1,2-linkage. While the immunogenicity of the core α1,3-fucose is of great interest in allergy and molecular farming (Bencurova et al., 2004; Faye et al., 2005), it appears to have surprisingly little impact on plant physiology as mutant lines without this sugar residue do not exhibit an obvious phenotype (Koprivova et al., 2004; Strasser et al., 2004; von Schaewen et al., 1993). The Lewis a determinants are regularly found on the surfaces of plant cells where they are expected to contribute to tissue cohesion in some way (Fitchette et al., 1999). The fucosyltransferases responsible for the attachment of core α1,3-fucose as well as of Lewis fucose have been studied and the respective genes from several plant species have been cloned and sequenced (Leiter et al., 1999; Leonard et al., 2002; Wilson, 2001; Wilson et al., 2001a). Plant fucosidases have been purified and characterized in the earlier days of glycobiology (Imber et al., 1982; Kobata, 1982; Ogata-Arakawa et al., 1977; Sano et al., 1992; Scudder et al., 1990; Yoshima

Abbreviations: AB, 2-aminobenzamide; AtFuc1, *Arabidopsis thaliana* fucosidase I (α1,3/4 fucosidase); LNFP II, lacto-*N*-fucopentaose II (see Fig. 5); GlcNAc, *N*-acetylglucosamine; MMF^3 etc., *N*-glycan structures (see Fig. 5); PA, pyridylamino-.

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et al., 1979). Almond meal, a rich source of various glycosidases, was found to contain two types of fucosidase; fucosidase I which hydrolyzes α1,3- and α1,4-linkages of fucose to GlcNAc in Lewis-type oligosaccharides or to galactose and fucosidase II which acts upon α1,2-linkages (Kobata, 1982; Ogata-Arakawa et al., 1977). None of these enzymes cleaves *p*-nitrophenyl-α-L-fucopyranoside, which severely complicates the determination of their activities. The molecular nature of the two fucosidases of almonds has not been unravelled so far. On the other hand, an α1,3/4-specific fucosidase from *Streptomyces* sp. has been cloned and sequenced (patent US5637490 by Sano et al., 1997). It was nevertheless unclear, whether the bacterial enzyme has any sequence similarity with plant fucosidases of comparable specificity. A recombinant protein from *Arabidopsis thaliana* (AtFUC1) with similarity to the *Streptomyces* fucosidase was recently reported to exhibit α1,2-fucosidase activity (de La Torre et al., 2002). This fucosidase had no homology to the α1,2-fucosidases from *A. thaliana* (AtFXG1) which acted on fucosylated cell wall xyloglucans (de La Torre et al., 2002) nor to a gene from pea erroneously claimed to encode an α1,2-fucosidase (Augur et al., 1995; Tarrago et al., 2003).

The conflicting results about the protein mass of almond fucosidase and the lack of knowledge about the genetic basis of plant α1,3/4 fucosidases together with our long lasting occupation with glycosidases from almond and other sources (Altmann et al., 1995, 1998; Tretter et al., 1991; Zeleny et al., 1997) prompted us to purify the α1,3/4 specific fucosidase I. from almonds. Peptide sequence tags were generated by tandem mass spectrometry which allowed us to clone an *A. thaliana* homolog, the first plant α1,3/4 fucosidase to be recombinantly expressed and characterized.

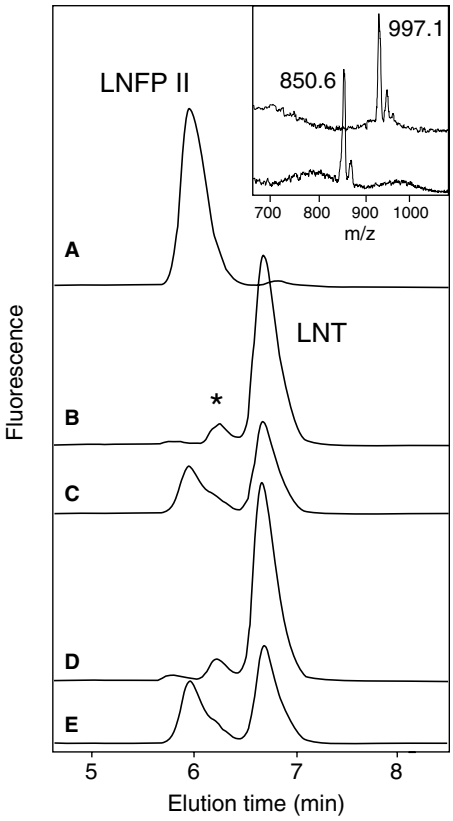

Fig. 1. Determination of fucosidase activity by HPLC. 2-aminobenzamide labelled lacto-*N*-fucopentaose II (LNFP II, 50 pmol, see Fig. 4 for structure) and its degradation product lacto-*N*-tetraose (LNT) were separated by reversed phase HPLC. LNFP was incubated over night with (A): buffer alone; (B and C): with 0.2 and 0.02 μL, respectively, of purified almond fucosidase; (D and E): with 1 and 0.1 μL, respectively, of supernatant of *Pichia* transformed with the *Arabidopsis* fucosidase gene. The asterisk identifies a contaminant. The insert shows MALDI-TOF mass spectra of undigested LNFP II (upper trace) and a sample digested with the *Arabidopsis* fucosidase (as in panel D).

2. Results and discussion

2.1. Purification of almond α1,3/4-fucosidase

The enzymatic activities of fucosidases I and II (nomenclature according to Ogata-Arakawa et al. (1977)) were measured using 2-aminobenzamide labelled oligosaccharides and reversed-phase HPLC for separation of substrate and product (Fig. 1). The α1,3/4-fucosidase I was assayed with lacto-*N*-fucopentaose II and/or 3-fucosyllactose and the α1,2-specific fucosidase II was measured with 2-fucosyllactose. Because confirmation of the success of our purification did not rely on a purification factor, we refrained from acquiring a complete purification protocol with sensible determinations of the enzymatic activities at every step.

Various purification strategies have been applied in previous works on almond fucosidases I and II (Imber et al., 1982; Kobata, 1982; Scudder et al., 1990; Yoshima et al., 1979). We aimed to combine these experiences with the existing protocol for the purification of peptide: *N*-glycosidase (Altmann et al., 1998). In the first chromatographic purification step on DEAE-Sepharose, the fucosidases and most other exoglycosidases were separated from the *N*-glycosidase (Fig. 2). The next step, hydrophobic interaction chromatography, provided separation of the fucosidase from coloured impurities. Gel filtration, usually a less efficient process, resulted in quite a good removal of other proteins and turned out as necessary precaution for the subsequent step. Fucosidase I appeared at about the same elution volume as bovine serum albumin which is in accordance with a report where a mass of 72 kDa was found by gel filtration (Imber et al., 1982). The fucosidase I pool from gel filtration was applied to an Affi-Gel Blue column to which both fucosidases I and II bound, albeit relatively weak. This step separated the two fucosidase activities (Fig. 2). The resulting pool gave a single strong band on SDS–PAGE at 63 kDa (Fig. 3) but it still contained enough β-galactosidase activity to make us doubt that we had already a pure fucosidase I preparation. Therefore we tried additional purification steps such as hydroxylapatite or affinity purification on fucose-agarose to

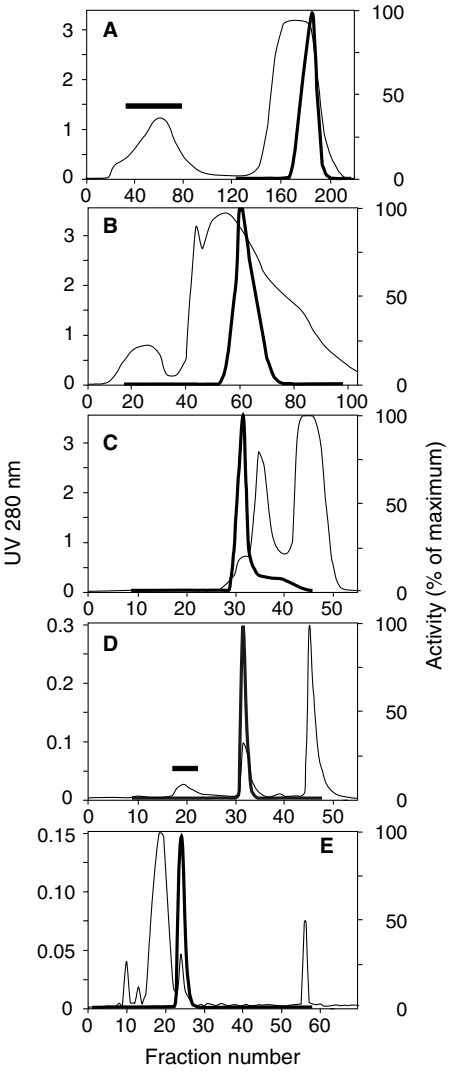

Fig. 2. Purification of almond α1,3/4 fucosidase. Successive purification steps are shown: (A) anion exchange on Sephadex DEAE A-50; (B) hydrophobic interaction chromatography on Phenyl Sepharose; (C) gel filtration on Sepharose S300; (D) dye ligand chromatography on Affigel Blue; (E) anion exchange on Mono Q (for details refer to the Section 3). The finer line depicts the protein concentration of fractions, the bold line that of fucosidase I activity. Enzyme activity in fractions is given in percent of maximum. The bar in panel A shows the elution region of peptide *N*-glycosidase A. In panel D, the bar depicts the elution region of fucosidase II.

which, surprisingly, most of the exoglycosidases but not fucosidase I bound. Anion exchange chromatography on a Mono Q column gave the most convincing result and separated the pool into a large protein peak followed by a small peak with fucosidase I activity (Fig. 2). Like the pool before Mono Q separation, this fraction, which in fact represented fucosidase I as will be shown in the following, gave a band at 63 kDa (Fig. 3) instead of the 54 kDa stated previously (Scudder et al., 1990). Finally, from 750 g of almonds, 20 μg of fucosidase I with a specific activity of 63 mU/mg had been purified. However, this activity was determined with LNFP II at a substrate concentration of 0.1 μM and it would translate to about 30 U/mg when substrate would be supplied at a concentration near the k_M value of 100 μM as estimated previously (Ogata-Arakawa et al., 1977; Scudder et al., 1990). The preparation exhibited no undesirable side-reactions when applied to Lewis a containing plant *N*-glycans (data not shown, but similar to those shown in Fig. 7) and to Lewis x containing human *N*-glycans (Kolarich et al., 2006).

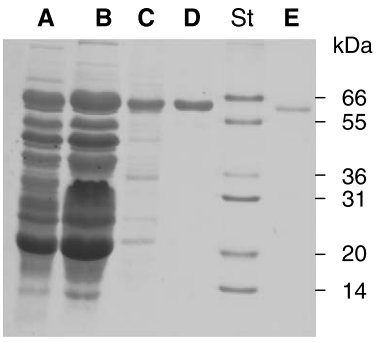

Fig. 3. SDS–PAGE of almond fucosidase I at various stages of purification. Enzyme pools after (A) anion exchange; (B) hydrophobic interaction chromatography; (C) gel filtration; (D) Affigel Blue; and (E) anion exchange on Mono Q. For details see Fig. 1 and text. Lane "St." was the mass standard.

2.2. Sequencing of almond fucosidase I

The presumed fucosidase I band was digested with trypsin and the resulting peptides were subjected to reversed phase chromatography followed by tandem mass spectrometry. Evaluation of the fragment spectra revealed the presence of many glycopeptides which gave no sequence information but, fortunately, also of several peptides which yielded useful sequence tags. The initial idea was to use these partial sequences to clone the respective almond cDNA. The same peptide sequences, however, were also used to screen databanks for homologous entries. Several peptides independently retrieved the hypothetical protein Q8GW72 from *A. thaliana* (Fig. 4). Most peptides could be aligned to this entry despite the considerable phylogenetic distance between almonds and *Arabidopsis* cress and irrespective of the possibility of errors of the de novo sequencing.

2.3. cDNA cloning of A. thaliana fucosidase I and homology searches

Our original idea was to clone the cDNA corresponding to almond fucosidase I. The detection of a close homologue in *Arabidopsis* and the expectable difficulties of cloning the almond cDNA without exactly knowing its nucleotide sequence made us change our mind. The *Arabidopsis* gene could be cloned readily and this endeavour was followed by nucleotide sequencing and expression of the gene product in *Pichia pastoris*.

The predicted protein product of this gene exhibited considerable sequence homologies of 62% and 50% with two hypothetical proteins from rice (TrEMBL entries

```
 1                          ↓                                        60
MNSQITLFFF FFSILSLSQI SNSSSLLKPH PCPILPLPSS QQLQWQLGSM AMFLHFGPNT

 61                                                                 120
FTDSEWGTGK ANPSIFNPTH LNASQWVQIA KDSGFSRVIL TAKHHDGFCL WPSEYTDYSV

121    NGGG DVVEGLAMAA R     GLGLY LS             TLEYNEF YMGQMTELLT R
KSSQWRNGAG DVVAELASAA KEAGIGLGLY LSPWDRHEQC YGKTLEYNEF YLSQMTELLT

161    EVWL DGAK             LGEFS LLHK      V LFSDAGPDTR         240
KYGEIKEVWL DGAKGDGEKD MEYFFDTWFS LIHQLQPKAV IFSDAGPDVR WIGDEAGLAG

241                                                          TLL
STCWSLFNRT NAKIGDTEPS YSQEGDGYGQ DWVPAECDVS IRPGWFWHAS ESPKPAVQLL

DLYYK                              VLKEFSELR                 360
DIYYNSVGRN CLFLLNVPPN SSGLISEQDI KVLEEFSEMK NSIFSNNLAR KAFVNSSSIR

361          FVSEDGLDNV R                                      420
GDQSSQFGPK NVLEEGLDKY WAPEENQNEW VLYLEFKDLV SFNVLEIREP IHMGQRIASF

421                                                            480
HLETRKTGSG EWERVVSGTT VGNKRLLRFL NVVESRSLKL VVDKARTDPL ISYLGLYMDK

481                          506
FSGSSRNTTK ITITRTLKEE QQLHDL
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Fig. 4. Alignment of almond fucosidase sequence tags with *Arabidopsis* protein Q8GW72. The continuous line gives the amino acid sequence of the putative protein Q8GW72. The most probable cleavage site of the signal peptide is indicated by an arrow. The upper line shows the sequences obtained by mass spectrometric sequencing of tryptic peptides from almond fucosidase I with bold letters emphasizing identity. Underlined peptides allowed identification of the gene by similarity search.

Q7XUR3 and Q650T8, respectively) and the protein related to EST DV708359 from *Coffea canephora*, which can be supposed to possess α1,3/4-fucosidase activity. In the BLAST ranking, these entries were followed by 14 hypothetical proteins from diverse bacteria and then by *Streptomyces* sp. α1,3/4-fucosidase (TrEMBL Q9Z4I9) which codes for the enzyme described by Sano et al. (1992). This protein exhibits 40% sequence identity over a stretch of 203 amino acid residues with the *Arabidopsis* enzyme. Such a homology is certainly significant but it appears too low to infer the function of the rice and *Arabidopsis* homologs without experimental evidence. In this context it should be noted that a recombinant protein apparently identical with the *Arabidopsis* α1,3/4-fucosidase described in this paper has recently been reported to be an α1,2-fucosidase (de La Torre et al., 2002). We cannot ultimately resolve this discrepancy but naturally we trust our own experiments performed with HPLC and mass spectrometry (see below) instead of the data obtained with an unusual assay for reducing groups (Lever, 1972).

No sequence similarity of plant α1,3/4-fucosidases with pea α1,2-fucosidase Q41015 could be recognized. The α1,2-fucosidase AtFXG1 acting on fucosylated xyloglucans from *A. thaliana* (translation of NCBI entry AAG28886.1, provided our guesswork about the identity of the described enzyme was correct) had no similarity with either of the two proteins (de La Torre et al., 2002).

2.4. Characterization of the recombinant *A. thaliana* fucosidase I

The cDNA of the presumed *Arabidopsis* fucosidase was cloned and expressed under control of the pGAP promo-

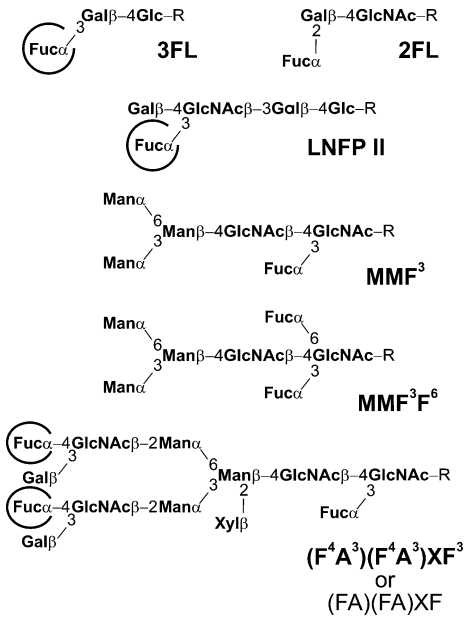

Fig. 5. Structures of oligosaccharides. Fucose residues susceptible to the α1,3/4-fucosidases from almond and *Arabidopsis* are encircled.

tor. The supernatants of transgenic and of wild-type *Pichia* cultures were analyzed for fucosidase activity after five days of induction. LNFP II was readily defucosylated by the transgenic culture as shown by HPLC and MALDI-MS (Fig. 1) but not by wild-type yeast. The transgenic enzyme, called *A. thaliana* fucosidase I (AtFuc1), also hydrolyzed 3-fucosyllactose (Figs. 5 and 6) and plant *N*-glycans containing Lewis a determinants (Figs. 5 and 7). Thus, AtFuc1 acts on both 3- and 4-linked fucose in Lewis x and Lewis a saccharides, respectively. No activity, even with a high enzyme concentration, could be observed with 2-fucosyllactose and *p*-nitrophenol α-fucopyranoside as substrates (Table 1). Likewise, *N*-glycans with a fucose in α1,3-linkage to the reducing-end GlcNAc were not degraded by AtFuc1. The same limitation applied to the almond fucosidase I and it is therefore noteworthy to remember that these plant fucosidases do not cleave all types of fucose linked α1,3 to GlcNAc as insinuated in older publications or product descriptions.

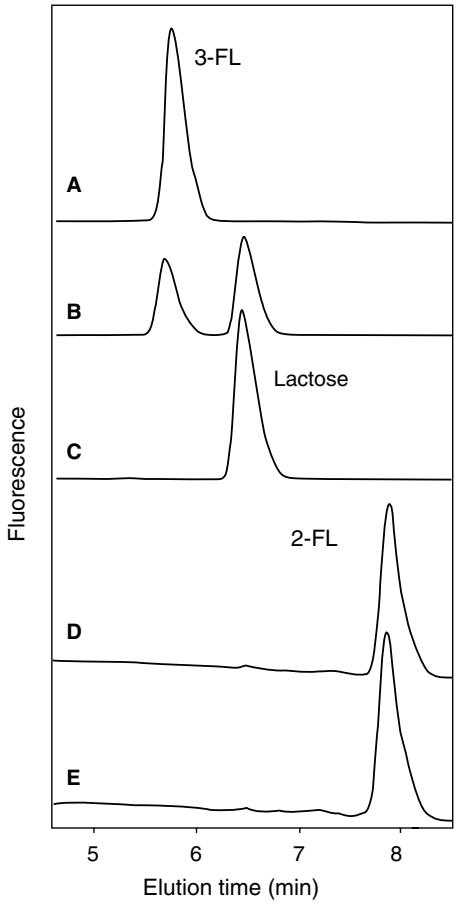

Fig. 6. Fucosidase digestion of 2- and 3-fucosyllactose. 2-Aminobenzamide labelled 3-fucosyllactose (3-FL), 2-fucosyllactose (2-FL, 50 pmol each, see Fig. 4 for structure) and the degradation product lactose were separated by reversed phase HPLC. Panel A: 3-fucosyllactose incubated over night with buffer alone; B and C: with 0.02 and 0.2 μL of *Arabidopsis* fucosidase respectively; D: 2-fucosyllactose with buffer alone and E: with 2 μL of the same enzyme.

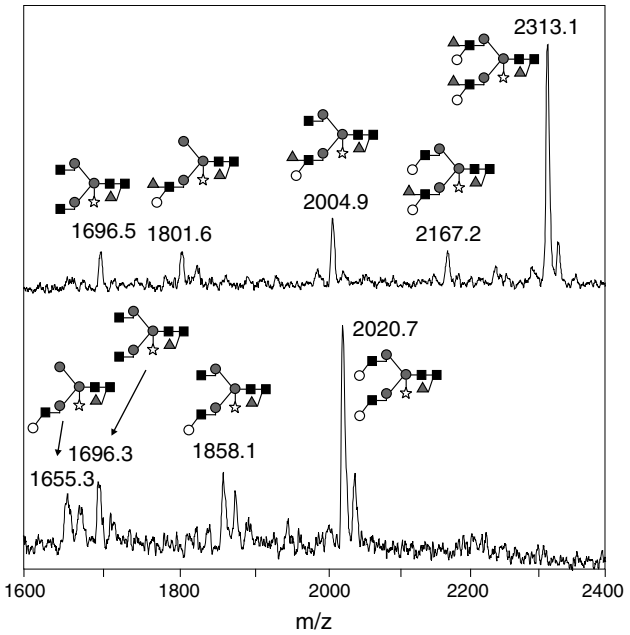

Fig. 7. Fucosidase digestion of Lewis a containing oligosaccharides. A pool of pyridylaminated *N*-glycans from apples which primarily contains the complete complex-type *N*-glycan with two Lewis a determinants (M + Na = 2313.1 Da) was incubated overnight with buffer (upper panel) or with recombinant *Arabidopsis* fucosidase (0.2 μL, lower panel). The Lewis-fucoses have been removed but the core α1,3-fucose resisted the enzyme. The cartoons are drawn in accordance with the "Consortium of Functional Glycomics" (www.functionalglycomics.org). The glycan at mass 2313.1 Da represents the most complex plant *N*-glycan, (FA)(FA)XF, as also shown in Fig. 5.

Table 1
Effect of α1,3/4-fucosidases from almond and mouse ear cress on various substrates

Substrate	Fucosidase I from			
	Almond (purified)		*Arabidopsis* (recombinant)	
	Rate (%)	k_M (μM)	Rate (%)	k_M (μM)
lacto-N-fucopentaose II, AB-	100	95–101[a]	100	28 ± 4.0
3-fucosyllactose, AB-	160		300	6.2 ± 0.4
2-fucosyllactose, AB-	n.d.		n.d.	
Le^a-Glycan, PA-(F^4A^3)(F^4A^3)XF^3, PA-	ca. 100		ca. 100	
MMF^3, PA-	n.d.		n.d.	
MMF^3F^6, PA-	n.d.		n.d.	
p-NP-α-L-fucopyranoside	n.d.		n.d.	

The hydrolysis rates are given in percentage relative to that of LNFP II. n.d. = no degradation could be detected. Substrates were employed at a concentration of 2.5 μM.

[a] As reported previously (Kobata, 1982; Ogata-Arakawa et al., 1977; Scudder et al., 1990).

The k_M value for LNFP II was determined to be 28 μM (Table 1), which is not too different from value of the 100 μM reported by Kobata for the almond enzyme (Kobata, 1982). The k_M for 3-fucosyllactose was found to be about 6 μM, however, maximal velocity was higher by

a factor of ca. 2.5 for the branched substrate LNFP II (data not shown).

The *Arabidopsis* enzyme exhibited a broad pH optimum around pH 5 with a sharp drop towards pH 7. However, no special requirements for high or low ionic strength could be observed (data not shown).

2.5. Physiological role

The ultimate question as to the function of α1,3/4-fucosidases in plants can only be met with the educated guess that exo-glycosidases are mostly found in the vacuolar compartment where they are responsible for the degradation of glycoproteins. The slightly acidic pH-optimum of the almond fucosidases and of AtFuc1 supports this view. As a consequence, plant storage proteins such as phaseolin or phytohemagglutinin carry truncated *N*-glycans (Sturm et al., 1987; Vitale et al., 1984) whereas extracellular and cell-wall associated glycoproteins exhibit "secreted type *N*-glycans" with Lewis a structures (Fitchette et al., 1999; Fitchette-Laine et al., 1997). However, what would be the function of an α1,3/4-fucosidase in *Arabidopsis* which was repeatedly shown to lack complex type antennae with Lewis a determinant (Fitchette et al., 1999; Rayon et al., 1999; Wilson et al., 2001b)? At first, we have to state that these findings may have been misleading as *Arabidopsis* possibly contains Lewis a structures, albeit, only in small amounts and in certain parts of the plant (Strasser, R. and Leonard, R., unpublished data). Furthermore, the fucosidase may have other substrates such as glycolipids or cell wall polysaccharides.

3. Materials and methods

3.1. Determination of fucosidase activity

Lacto-*N*-fucopentaose II (LNFP II) was purchased from Oxford GlycoSystems (UK). 3-fucosyllactose and 2-fucosyllactose were obtained from Sigma–Aldrich. The oligosaccharides were labelled with 2-aminobenzamide (Bigge et al., 1995). The derivatives were purified by HPLC which was also used for the separation of substrate and product. Enzyme was incubated with substrate (usually LNFP II, 30 pmol) in 100 mM sodium citrate/phosphate buffer at pH 5.0 in a total volume of 20 μL at 37 °C for 2 h or longer as required. Enzyme reactions were terminated by dilution and boiling. Half of the sample was subjected to reversed phase HPLC on a 4 × 150 mm column of 5 μm Hypersil ODS using 50 mM ammonium formate buffer of pH 4.4 as the aqueous solvent and a gradient of 2–9% acetonitrile over 9.8 min at a flow rate of 1.4 ml/min.

2-Aminopyridin-labelled *N*-glycans with either core 3-linked or core 6-linked fucose or both (MMF^3, MMF^6, and MMF^3F^6, respectively) and a plant *N*-glycan with two Lewis a antennae, $(F^4A^3)(F^4A^3)XF^3$, were available from previous studies (Kubelka et al., 1993; Wilson et al., 2001b). *p*-Nitrophenyl α-L-fucopyranoside and chromogenic substrates for other exoglycosidases were obtained from Sigma. Enzyme impact on *N*-glycans was monitored by reversed phase HPLC (Kubelka et al., 1993) except in the case of the Lewis a oligosaccharide where MALDI-TOF MS was used (Wilson et al., 2001b).

3.2. Purification of fucosidase I

Extraction of almonds, ammonium sulphate precipitation and fractionation on Sephadex DEAE A-50 were performed as described earlier (Altmann et al., 1998). Phenylmethylsulfonyl fluoride was added at several stages of the purification. The proteins binding to the anion exchange gel were eluted with a salt gradient of 0–1 M NaCl. The effluent was immediately buffered back to pH 5.0. Fractions containing fucosidase I were pooled and mixed with saturated ammonium sulphate to a final concentration of 1.6 M. This solution was applied to a 2.5 × 25 cm Phenyl-Sepharose fast flow 6 low sub (Amersham Biosciences, Uppsala, Sweden), which had been equilibrated with 0.1 M sodium phosphate buffer of pH 6.8 containing 1.6 M ammonium sulphate. Following a quick step down to 0.96 M salt, a 140 min gradient to 0.12 M ammonium sulphate was developed at a flow rate of 5 mL/min. The volume of the pool of fucosidase containing fractions was reduced by ultrafiltration. The concentrate was applied to a 2.5 × 100 cm column filled with Sephacryl S300 (Amersham Biosciences), which was eluted with 50 mM sodium acetate of pH 5.0. Fucosidase containing fractions were concentrated by ultrafiltration and applied to a 1.5 × 20 cm column of AffiGel Blue (Bio-Rad, Richmond, CA) previously equilibrated with 50 mM sodium acetate buffer of pH 5.0. Samples were applied at a flow rate of 0.3 mL/min which was then raised to 0.5 mL/min. Proteins were eluted with a step gradient of 0.05, 0.25 and 1 M NaCl, respectively. Enzyme containing fractions were concentrated and dialyzed against 10 mM TRIS/HCl buffer of pH 7.6. This preparation was applied to a MonoQ HR 5/5 column (Amersham Biosciences) which had been equilibrated in the same buffer. Proteins were eluted during a 120 min gradient from 0 to 0.5 M NaCl at a flow rate of 0.5 mL/min. Fractions of 1.5 mL were assayed for fucosidase activity.

For hydroxylapatite chromatography, an Econo-Pac CHT-II cartridge (Bio-Rad) was used with a sodium acetate buffer gradient. Affinity chromatography on L-fucose agarose (Sigma–Aldrich) was also tried. The latter two methods were not integrated in the finally applied purification scheme.

3.3. Analytical procedures

Protein concentrations were estimated by measuring absorbance at 280 nm (in fractions) or with the micro-BCA test (Pierce, Rockford, IL). Sugar concentrations of

substrate solutions were determined by reversed phase HPLC of UV-absorbing derivatives of monosaccharides released by acid hydrolysis (Fu and O'Neill, 1995). The compositions of the substrates and their derivatives were verified by MALDI-TOF MS (Kolarich and Altmann, 2000).

3.4. Proteomic methods

Proteins were separated on 12.5% polyacrylamide gels, stained, destained, *S*-carbamidomethylated and digested with trypsin as described (Kolarich and Altmann, 2000). Tryptic peptides were analyzed by electrospray tandem mass spectrometry on a Q-TOF Ultima Global (Waters-Micromass, Manchester, UK) coupled to a capillary HPLC as described recently (Kolarich et al., 2005). De novo sequencing of tryptic peptides was done using the peptide sequencing module in MassLynx (Waters-Micromass).

3.5. cDNA cloning and recombinant expression of fucosidase I

mRNA was isolated from leaves of *A. thaliana* (ecotype Landsberg *erecta*) using trizol (Invitrogen, Carlsbad, CA) according to manual instructions. Reverse transcription was performed with the reverse transcriptase III (Promega, Madison, WI). According to the nucleotide sequence of the presumed fucosidase (PubMed NM_128370) the primer pair GCTCTAGAGCTTACAAATCATGTAGTTGCTGCTCTTC (coding *i.a.* for the C-terminus) and GGAATTCCAAATCTCAAATTCATCATCACTACTAAAAC (QISNSSSLLK, corresponding to the N-terminus of the mature protein) containing the underlined restriction sites for Xba I and EcoR I was designed for PCR amplification. The resulting PCR product was, ligated into the plasmid pGAPZαA (Invitrogen) and the resulting construct was used to transform *Pichia pastoris* cells as described recently (Bencurova et al., 2004; Bencurova et al., 2003). Selected clones were grown in YPD medium for five days at 37 °C. Supernatants were diluted five times with citrate buffer pH 5.0 and assayed for enzymatic activity.

Enzyme kinetic data were evaluated by the double-reciprocal plot as well as by the Hanes plot and the direct linear plot method (Cornish-Bowden and Eisenthal, 1974).

Similarity searches were performed with the BLAST software provided by the Expasy proteomics server.

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