

# Molecular cloning and functional expression of $\beta$ 1,2-xylosyltransferase cDNA from *Arabidopsis thaliana*<sup>1</sup>

R. Strasser<sup>a</sup>, J. Mucha<sup>a</sup>, L. Mach<sup>a</sup>, F. Altmann<sup>b</sup>, I.B.H. Wilson<sup>b</sup>, J. Glössl<sup>a,\*</sup>, H. Steinkellner<sup>a</sup>

<sup>a</sup>Zentrum für Angewandte Genetik, Universität für Bodenkultur Wien, Muthgasse 18, A-1190 Vienna, Austria

<sup>b</sup>Institut für Chemie, Universität für Bodenkultur Wien, Muthgasse 18, A-1190 Vienna, Austria

Received 7 March 2000

Edited by Marc Van Montagu

**Abstract** The transfer of xylose from UDP-xylose to the core  $\beta$ -linked mannose of *N*-linked oligosaccharides by  $\beta$ 1,2-xylosyltransferase (XylT) is a widespread feature of plant glycoproteins which renders them immunogenic and allergenic in man. Here, we report the isolation of the *Arabidopsis thaliana* XylT gene, which contains two introns and encodes a 60.2 kDa protein with a predicted type II transmembrane protein topology typical for Golgi glycosyltransferases. Upon expression of *A. thaliana* XylT cDNA in the baculovirus/insect cell system, a recombinant protein was produced that exhibited XylT activity in vitro. Furthermore, the recombinant enzyme displayed XylT activity in vivo in the insect cells, as judged by the acquired cross-reaction of cellular glycoproteins with antibodies against the  $\beta$ 1,2-xylose epitope. The cloned XylT cDNA as well as the recombinant enzyme are essential tools to study the role of  $\beta$ 1,2-xylose in the immunogenicity and allergenicity of plant glycoproteins at the molecular level.

© 2000 Federation of European Biochemical Societies.

**Key words:**  $\beta$ 1,2-Xylosyltransferase; Glycosyltransferase; *N*-Glycan; *Arabidopsis thaliana*

## 1. Introduction

*N*-Glycosylation is one of the major posttranslational modifications of proteins in eukaryotes. In both plant and animal cells, a highly conserved multistep biosynthetic pathway covalently links carbohydrate side-chains to asparagine residues of newly synthesised proteins. Animal and plant glycoproteins contain both oligomannosidic and complex-type *N*-linked glycans. However, complex plant *N*-glycans are generally smaller than their animal counterparts, lack sialic acid but contain instead  $\beta$ 1,2-xylose and/or core  $\alpha$ 1,3-fucose residues. The antigenicity of complex plant *N*-glycans is well documented [1–5]. Since both  $\beta$ 1,2-xylose and/or core  $\alpha$ 1,3-fucose are not found on mammalian glycoproteins, they constitute epitopes for carbohydrate-reactive antibodies. Due to the ubiquitous occurrence of complex plant *N*-glycans throughout the plant kingdom, these antibodies are probably responsible for the frequently observed cross-reactivity of otherwise unrelated plant glycoproteins [2,6]. It is also likely that complex plant *N*-glycans constitute a major class of the so-called ‘carbohydrate cross-reactive determinants’ reactive with IgE antibodies in the sera of many allergic patients [5,7–12]. While the clin-

ical role of carbohydrate cross-reactive determinants remains controversial, they frequently obscure (at least in vitro) the identification of the cause of allergic symptoms. The specific importance of  $\beta$ 1,2-xylose to the allergenicity of plant glycoproteins has been reported [4,13].

The enzyme that catalyses the transfer of xylose to the *N*-linked oligosaccharides of glycoproteins is  $\beta$ 1,2-xylosyltransferase (XylT). Although the characterisation of this enzyme from *Acer pseudoplatanus* [13] and its purification from soybeans [14] have been reported, the isolation of XylT cDNAs has so far remained elusive. In the present paper however, we report the cloning of the cDNA containing the entire coding sequence of *Arabidopsis thaliana* XylT, its predicted amino acid sequence and demonstrate its authentic enzymatic activity in vitro and in vivo.

## 2. Materials and methods

### 2.1. Plant material, reverse transcription PCR and sequencing

*A. thaliana* (var. Columbia) plants were cultivated in a controlled growth chamber at 22°C (day and night), a 16 h photoperiod and 50% humidity.

Total RNA was isolated from *A. thaliana* leaves using the TRIzol reagent (Life Technologies). The RNA was treated with DNase (Promega, RQ1 RNase-free DNase) to remove traces of genomic DNA. First-strand cDNA was synthesised from 2  $\mu$ g of total RNA using AMV reverse transcriptase (Promega) and oligo(dT) primers (Sigma). PCR amplification was carried out using *Pfu* polymerase (Promega), primer XT1, 5'-ATGAGTAAACGGAATCCGAAG-3', and primer XT2, 5'-TTAGCAGCCAAGGCTCTTCAT-3'. After a first denaturation step at 94°C for 2 min, 30 cycles of 1 min at 92°C, 40 s at 53°C and 3.5 min at 72°C were performed. The final extension step was carried out at 72°C for 8 min. PCR products were subcloned into pUC19. Plasmid DNA was purified with the aid of the Qiagen plasmid purification kit and subjected to cycle sequencing using the Big Dye Terminator Sequencing Ready Mix and an ABI PRISM 310 Genetic analyser (Applied Biosystems, Perkin Elmer). DNA and protein sequence analysis was performed using the DNASTAR Lasergene-software package. Rapid amplification of cDNA ends (RACE) was carried out with the 5'-RACE system (Life Technologies) according to the manufacturer's instructions and Strasser et al. [15].

### 2.2. Expression of XylT in insect cells

The *A. thaliana* XylT cDNA was subcloned into the baculovirus transfer vector pVL1393 (PharMingen). *Spodoptera frugiperda* Sf-9 cells were grown in IPL-41 medium (Sigma) supplemented with 5% heat-inactivated foetal calf serum at 27°C. 1  $\mu$ g of recombinant baculovirus transfer vector was cotransfected with 200 ng linear Baculo-Gold DNA (PharMingen) into  $1 \times 10^6$  Sf-9 cells using lipofectin (Life Technologies) according to the manufacturer's protocol. Cells were incubated for 6 days at 27°C. The supernatant was used to infect  $2 \times 10^6$  Sf-21 cells cultured as above. Cells were incubated for 4 days at 27°C, harvested and washed twice with phosphate-buffered saline solution. The cells were resuspended and lysed in the following buffer (1 ml per  $10^7$  cells): 100 mM MES buffer, pH 7.0, 1% Triton X-100,

\*Corresponding author. Fax: (43)-1-36006 6392.

E-mail: gloessl@edv2.boku.ac.at

<sup>1</sup> EMBL database accession numbers: AJ272121, ATH272121



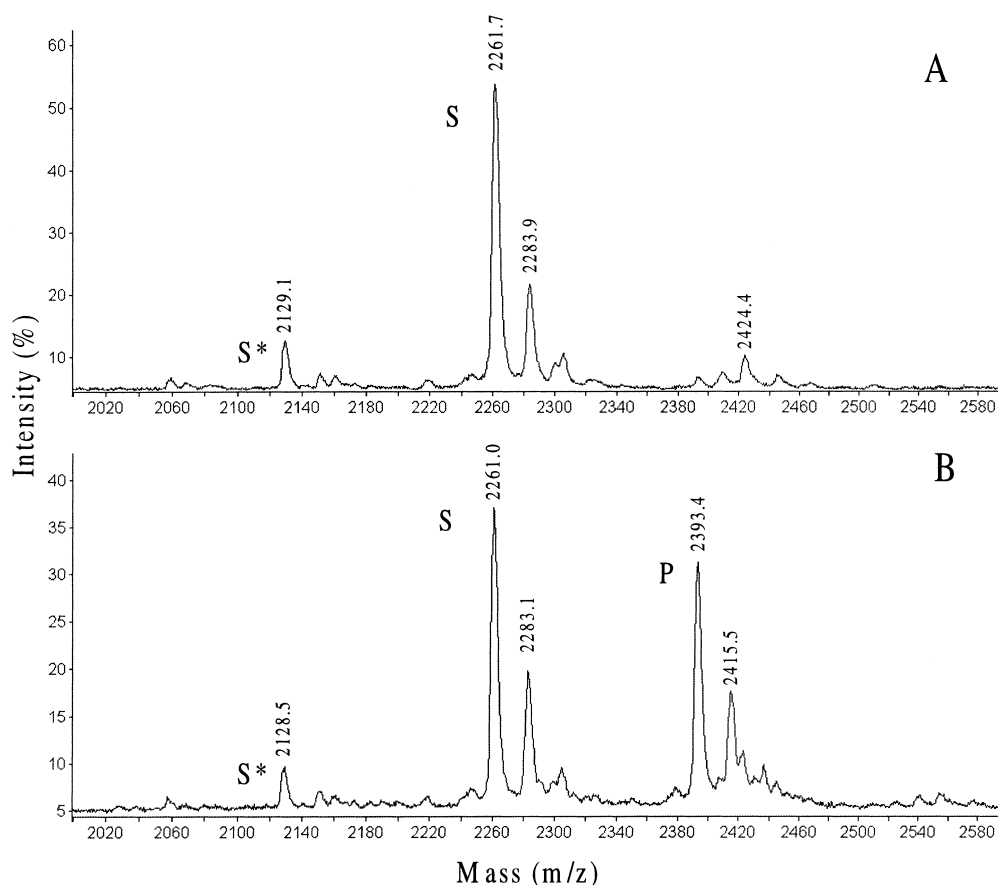


Fig. 2. Demonstration of XylT activity of the recombinant enzyme. Cell extracts of *A. thaliana* XylT expressing insect cells were incubated with dabsylated GnGn-hexapeptide as described in Section 2. The mixtures were analysed by MALDI-TOF mass spectrometry. A: Extract of  $1.3 \times 10^5$  uninfected *Sf*-21 cells. The peaks at 2261.7 and 2283.9 Da represent the  $[M+H]^+$  and the  $[M+Na]^+$  ions, respectively, of the acceptor substrate (S) which is accompanied by an in-source fragmentation product ( $S^*$ ). B: Extract of approximately  $1.3 \times 10^5$  *Sf*-21 cells expressing *A. thaliana* XylT. The xylosylated product (P) is detected by means of peaks at 2393.4 and 2415.5 Da corresponding to the  $[M+H]^+$  and the  $[M+Na]^+$  ions, respectively.

number: AB015479) revealed homology to two putative internal peptides (GLETHAIN and SQVQAIHDASVIIGAH-GAGLTHIVSAL) derived from soybean XylT [14,18]. Comparative analysis of these sequences using the Genefinder program (Phil Green, University of Washington, <http://dot.imgen.bcm.tmc.edu>) revealed an open reading frame of 1602 nucleotides (two putative introns excluded). To isolate the cDNA clone corresponding to this genomic sequence, the oligonucleotide primers XT1 and XT2 were designed which include the putative start and stop codons of the gene, respectively. Using these primers, we obtained a PCR fragment of 1605 bp from *A. thaliana* leaf cDNA. This PCR fragment revealed upon sequence analysis 100% identity to the genomic sequence. The cDNA sequence also confirmed the presence of two introns in the genomic clone. The open reading frame encodes a 534 amino acid protein with a calculated molecular mass of 60.2 kDa (Fig. 1). RACE analysis at the 5'-end of the cDNA confirmed the predicted start codon of the gene.

Hydrophobicity analysis of the deduced amino acid sequence of XylT, using SOSUI software package (<http://azusa.proteome.bio.tuat.ac.jp/>) with manual refinements, revealed a type II transmembrane protein topology typical of all previously cloned Golgi-located glycosyltransferases, exhibiting a putative cytoplasmic domain of 10 residues at the

N-terminus followed by a transmembrane region and a C-terminal putative catalytic domain. GenBank and SwissProt database searches did not reveal significant sequence similarities to known DNA or protein sequences except to a hybrid aspen EST clone (accession number A162640).

### 3.2. Detection of *in vitro* activity of XylT

Recombinant baculoviruses were constructed to express the entire coding sequence of *A. thaliana* XylT cDNA in *S. frugiperda* *Sf*-21 cells. When assayed for XylT activity with specific substrates, the lysates of cells infected with the recombinant viruses were found to display significant enzymatic activity, whereas no activity was detectable in uninfected cells (Fig. 2).

The authenticity of the reaction product obtained with GnGn-PA as acceptor substrate was verified by reversed-phase HPLC and exoglycosidase digestion. After sequential removal of terminal GlcNAc- and 1,3-linked  $\alpha$ -mannosyl residues, the product of XylT action co-eluted as anticipated with M0X-PA and was sensitive to treatment with  $\beta$ 1,2-xylosidase (data not shown). Taken together, these data provide good evidence that the recombinant *A. thaliana* enzyme had transferred xylose in  $\beta$ 1,2-linkage to the  $\beta$ -mannosyl residue of the core pentasaccharide moiety of *N*-linked oligosaccharides.

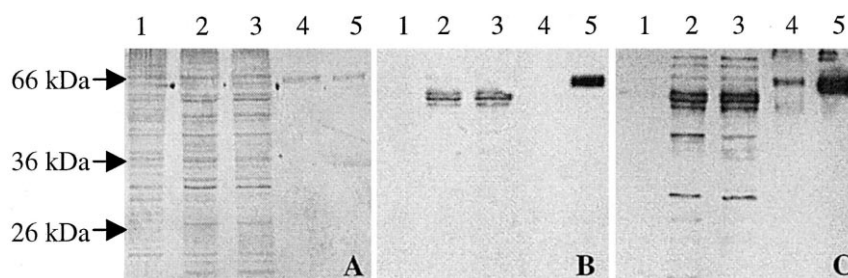


Fig. 3. Detection of XylT in vivo activity in insect cells. A: silver-stained gel; B and C: corresponding Western blot analyses. B: Anti-HRP pre-incubated with BSA-M0X, C: anti-HRP pre-incubated with BSA-MM. Lane 1: uninfected *Sf*-21 cells ( $2 \times 10^5$ ), lanes 2 and 3: *Sf*-21 cells ( $2 \times 10^5$ ) infected with recombinant baculoviruses containing XylT cDNA, lane 4: BSA-M0X (200 ng), lane 5: BSA-M0XF<sup>3</sup> (200 ng).

### 3.3. Determination of in vivo activity of XylT

To date, no endogenous  $\beta$ 1,2-XylT activity has been reported in insect cells. Consequently, cellular glycoproteins are devoid of xylosylated *N*-glycans (see [19] for review). Indeed, protein extracts from uninfected *Sf*-21 cells did not cross-react with a polyclonal anti-HRP antiserum which contains antibodies to *N*-glycans containing  $\beta$ 1,2-xylose and core  $\alpha$ 1,3-fucose [2]. However, cross-reaction was observed upon baculoviral expression of XylT cDNAs in these cells. The specificity of the anti-glycan antibodies was assessed by Western blotting of the neoglycoconjugates BSA-M0X and BSA-M0XF<sup>3</sup>. When the antiserum was preadsorbed with the control neoglycoprotein BSA-MM, both BSA-M0X (containing  $\beta$ 1,2-xylose) and BSA-M0XF<sup>3</sup> (containing both  $\beta$ 1,2-xylose and core  $\alpha$ 1,3-fucose) exhibited reactivity with the anti-glycan antibodies. Preadsorption with BSA-M0X completely abolished binding to this neoglycoprotein. However, BSA-M0XF<sup>3</sup> was detected to a significant extent even after the latter pretreatment. In contrast, the acquired cross-reactivity of XylT expressing *Sf*-21 cells with the anti-HRP antibodies was strongly reduced after preadsorption with BSA-M0X (compare B to C in Fig. 3). Taken together, these experiments indicate that expression of *A. thaliana* XylT cDNA in insect cells results in the  $\beta$ 1,2-xylosylation of endogenous glycoproteins, suggesting proper targeting of the recombinant enzyme to the insect Golgi apparatus and the utilisation of intracellular pools of UDP-xylose.

In conclusion, the present data indicate that we have identified the gene that encodes XylT of *A. thaliana*. We did not detect significant homologies to any other nucleotide or protein sequence. Since the core  $\alpha$ 1,3-fucosyltransferase from mung beans has recently also been cloned and functionally expressed in insect cells [16], we now have recombinant forms of two important plant glycosyltransferases whose products are involved in the allergenicity and immunogenicity in humans. Large quantities of purified enzyme can now be produced thus allowing the in vitro synthesis of homogeneous *N*-glycans containing  $\beta$ 1,2-xylose and/or core  $\alpha$ 1,3-fucose. Such 'vegetabilised' structures will aid the further elucidation of the role of these compounds in the immunogenicity and allergenicity of plant glycoproteins. Together with the recently identified genes of tobacco *N*-acetylglucosaminyl-transferase I (GnT I) [15] and *A. thaliana* *N*-acetylglucosaminyltransferase II (Strasser, R., Steinkellner, H., Borén, M., Altmann, F., Mach, L., Glössl, J. and Mucha, J., submitted for publication), there are now four glycosyltransferases from the plant *N*-glycosylation pathway characterised at a molecular level.

Thus, we now have the genetic tools to specifically modify the *N*-glycosylation pathway with the aim to produce more 'mammalianised' glycoproteins in plants. Furthermore, we can now start to study the subcellular localisation of these glycosyltransferases as it has already been reported for GnT I [20], in an effort to characterise the architecture of the plant Golgi apparatus.

**Acknowledgements:** We would like to thank Barbara Svoboda, Daniel Kolarich and Thomas Dalik for excellent technical support. This work was supported in part by the EC-Project No. BIO4CT-98-0255.

### References

- [1] McManus, M.T., McKeating, J., Secher, D.S., Osborne, D.J., Ashford, D., Dwek, R.A. and Rademacher, T.W. (1988) *Planta* 175, 506–512.
- [2] Wilson, I.B.H., Harthill, J.E., Mullin, N., Ashford, D. and Altmann, F. (1998) *Glycobiology* 8, 651–661.
- [3] Faye, L., Gomord, V., Fitchette-Lainé, A.C. and Chrispeels, M.J. (1993) *Anal. Biochem.* 209, 104–108.
- [4] Garcia-Casado, G., Sanchez-Monge, R., Chrispeels, M.J., Armentia, A., Salcedo, G. and Gomez, L. (1996) *Glycobiology* 6, 471–477.
- [5] Petersen, A., Vieths, S., Aulepp, H., Schlaak, M. and Becker, W.M. (1996) *J. Allergy Clin. Immunol.* 98, 805–815.
- [6] Faye, L. and Chrispeels, M.J. (1988) *Glycoconjug. J.* 5, 245–256.
- [7] Tretter, V., Altmann, F., Kubelka, V., März, L. and Becker, W.M. (1993) *Int. Arch. Allergy Immunol.* 102, 259–266.
- [8] Aalberse, R.C. and van Ree, R. (1997) *Clin. Rev. Allergy Immunol.* 15, 375–387.
- [9] Aalberse, R.C. (1998) *Allergy* 53, 54–57.
- [10] Jankiewicz, A., Aulepp, H., Altmann, F., Fötisch, K. and Vieths, S. (1998) *Allergo J.* 7, 87–95.
- [11] Hansen, M.Y., Wold, J.K., Smestad Paulsen, B., Cohen, E.H. and Karlsson-Borga, A. (1994) *Allergy* 49, 235–241.
- [12] Vieths, S., Mayer, M. and Baumgart, M. (1994) *Food Agric. Immunol.* 6, 453–563.
- [13] Tezuka, K., Hayashi, M., Ishihara, H., Akazawa, T. and Takahashi, N. (1992) *Eur. J. Biochem.* 203, 401–413.
- [14] Zeng, Y., Bannon, G., Thomas, V.H., Rice, K., Drake, R. and Elbein, A. (1997) *J. Biol. Chem.* 272, 31340–31347.
- [15] Strasser, R., Mucha, J., Schwihla, H., Altmann, F., Glössl, J. and Steinkellner, H. (1999) *Glycobiology* 9, 779–785.
- [16] Leiter, H., Mucha, J., Staudacher, E., Grimm, R., Glössl, J. and Altmann, F. (1999) *J. Biol. Chem.* 274, 21830–21839.
- [17] Wilson, I.B.H. and Altmann, F. (1998) *Glycoconjug. J.* 15, 1055–1070.
- [18] Elbein, A.D. and Bannon, G.A., Patent Number: WO9929835A1.
- [19] Altmann, F., Staudacher, E., Wilson, I.B. and März, L. (1999) *Glycoconjug. J.* 16, 109–123.
- [20] Essl, D., Dirnberger, D., Gomord, V., Strasser, R., Faye, L., Glössl, J. and Steinkellner, H. (1999) *FEBS Lett.* 453, 169–173.