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Trans-α-xylosidase, a widespread enzyme activity in plants, introduces (1→4)-α-D-xylobiose side-chains into xyloglucan structures

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ABSTRACT

Angiosperms possess a retaining trans-α-xylosidase activity that catalyses the inter-molecular transfer of xylose residues between xyloglucan structures. To identify the linkage of the newly transferred α-xylose residue, we used [*Xyl*-^{3}H]XXXG (xyloglucan heptasaccharide) as donor substrate and reductively-aminated xyloglucan oligosaccharides (XGO–NH_2) as acceptor. *Asparagus officinalis* enzyme extracts generated cationic radioactive products ([^{3}H]Xyl·XGO–NH_2) that were Driselase-digestible to a neutral trisaccharide containing an α-[^{3}H]xylose residue. After borohydride reduction, the trimer exhibited high molybdate-affinity, indicating xylobiosyl-(1→6)-glucitol rather than a di-xylosylated glucitol. Thus the trans-α-xylosidase had grafted an additional α-[^{3}H]xylose residue onto the xylose of an isoprimeverose unit. The trisaccharide was rapidly acetolysed to an α-[^{3}H]xylobiose, confirming the presence of an acetolysis-labile (1→6)-bond. The α-[^{3}H]xylobiitol formed by reduction of this α-[^{3}H]xylobiose had low molybdate-affinity, indicating a (1→2) or (1→4) linkage. In NaOH, the α-[^{3}H]xylobiose underwent alkaline peeling at the moderate rate characteristic of a (1→4)-disaccharide. Finally, we synthesised eight non-radioactive xylobioses [α and β; (1↔1), (1→2), (1→3) and (1→4)] and found that the [^{3}H]xylobiose co-chromatographed only with (1→4)-α-xylobiose. We conclude that *Asparagus* trans-α-xylosidase activity generates a novel xyloglucan building block, α-D-Xyl*p*-(1→4)-α-D-Xyl*p*-(1→6)-D-Glc (abbreviation: 'V'). Modifying xyloglucan structures in this way may alter oligosaccharin activities, or change their suitability as acceptor substrates for xyloglucan endotransglucosylase (XET) activity.

1. Introduction

Xyloglucan is the major hemicellulose in the primary cell walls of most land plants. It binds to and probably tethers microfibrils, consequently restraining cell expansion [Hayashi, 1989; Fry, 2011a]. Xyloglucan also acts as a source of oligosaccharins (biologically active oligosaccharides) [York et al., 1984; McDougall and Fry, 1989; Darvill et al., 1992; Aldington and Fry, 1993; Takeda et al., 2002]. It thus serves both structural and signalling roles, and enzymes that modify xyloglucan and/or its oligosaccharides are highly significant in plant biology.

The backbone of xyloglucan is a cellulose-like chain of (1→4)-linked β-D-glucose (Glc) residues. Those Glc residues that are not further substituted are abbreviated by the one-letter code 'G', but most carry an α-D-xylose (Xyl) residue on position 6, forming an isoprimeverose unit (X) (Fry et al., 1993). In other building-blocks of xyloglucan, the Xyl and/or Glc residue of an isoprimeverose unit is further substituted: currently there are fifteen known xyloglucan repeat-units besides G and X, given the one-letter codes listed in Table 1. Major examples in most vascular plant species include F and L, and common sequences of these units in primary cell-wall xyloglucan include XXXG, XXFG and XLFG (the repeat-units being listed from non-reducing to reducing end). Rarer and more taxonomically restricted units include three (B, C and U) that contain a β-D-Xyl residue in addition to the α-D-Xyl residue of isoprimeverose (Hisamatsu et al., 1992; Ray et al., 2004; Hilz et al., 2007), and recently discovered units that contain a β-D-galacturonic acid (P and Q) or an α-L-arabinopyranose (D, E, M and N) residue (Peña et al., 2008).

Plant enzyme activities that can modify xyloglucan in the cell wall include a glycanase [XEH, catalysing endo-cleavage of xyloglucan via a retaining hydrolytic mechanism (Rose et al., 2002;

Abbreviations: BAW, butan-1-ol/acetic acid/water; G, glucose residue in xyloglucan; HVPE, high-voltage paper electrophoresis; MXE, mixed-linkage glucan : xyloglucan endotransglucosylase; PC, paper chromatography; $R_{Orange\ G}$, chromatographic mobility relative to that of Orange G; TLC, thin-layer chromatography; V, α-D-Xyl*p*-(1→4)-α-D-Xyl*p*-(1→6)-D-Glc unit in xyloglucan; X, isoprimeverose residue in xyloglucan; XET, xyloglucan endotransglucosylase; XGO, xyloglucan oligosaccharide; XGO–NH_2, reductively aminated xyloglucan oligosaccharide; XXFG, major nonasaccharide of xyloglucan; XXXG, major heptasaccharide of xyloglucan.

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Table 1
Nomenclature of xyloglucan oligosaccharides. Updated from Fry et al. (1993) and Peña et al. (2008). The following code letters are used for describing concisely the sequence of side-chains and unbranched glucose residues along the (1→4)-β-D-glucan backbone of xyloglucan. Oligosaccharides are named by listing these building blocks in sequence from non-reducing to reducing end. All residues are pyranose except arabinose which may be pyranose (Ara*p*) or furanose (Ara*f*). Square brackets indicate branching. For example, in 'C' an α-Xyl residue is linked to the 6-position of the Glc* and an α-Ara-(1→3)-β-Xyl group is linked to the 2-position of the same Glc*; thus 'C' could be represented as α-Xyl→β-Glc*←β-Xyl←α-Ara. Likewise, in 'N' an Ara*p* residue is linked to the 2-position of the Xyl and a β-Gal-(1→6)-β-Gal group is linked to the 4-position of the same Xyl.

Code	Structure
A	α-D-Xyl-(1→6)-[α-L-Ara*f*-(1→2)]-β-D-Glc*
B	α-D-Xyl-(1→6)-[β-D-Xyl-(1→2)]-β-D-Glc*
C	α-D-Xyl-(1→6)-[α-L-Ara*f*-(1→3)-β-D-Xyl-(1→2)]-β-D-Glc*
D	α-L-Ara*p*-(1→2)-α-D-Xyl-(1→6)-β-D-Glc*
E	α-L-Fuc-(1→2)-α-L-Ara*p*-(1→2)-α-D-Xyl-(1→6)-β-D-Glc*
F	α-L-Fuc-(1→2)-β-D-Gal-(1→2)-α-D-Xyl-(1→6)-β-D-Glc*
G	β-D-Glc* with no side-chain attached
Gol	Glucitol (the former reducing terminus after reduction with $NaBH_4$)
J	α-L-Gal-(1→2)-β-D-Gal-(1→2)-α-D-Xyl-(1→6)-β-D-Glc*
L	β-D-Gal-(1→2)-α-D-Xyl-(1→6)-β-D-Glc*
M	α-L-Ara*p*-(1→2)-[β-D-Gal-(1→4)]-α-D-Xyl-(1→6)-β-D-Glc*
N	α-L-Ara*p*-(1→2)-[β-D-Gal-(1→6)-β-D-Gal-(1→4)]-α-D-Xyl-(1→6)-β-D-Glc*
P	β-D-GalA-(1→2)-[β-D-Gal-(1→4)]-α-D-Xyl-(1→6)-β-D-Glc*
Q	β-D-Gal-(1→4)-β-D-GalA-(1→2)-[β-D-Gal-(1→4)]-α-D-Xyl-(1→6)-β-D-Glc*
S	α-L-Ara*f*-(1→2)-α-D-Xyl-(1→6)-β-D-Glc*
T	β-L-Ara*f*-(1→3)-α-L-Ara*f*-(1→2)-α-D-Xyl-(1→6)-β-D-Glc*
U	β-D-Xyl-(1→2)-α-D-Xyl-(1→6)-β-D-Glc*
V	α-D-Xyl-(1→4)-α-D-Xyl-(1→6)-β-D-Glc* (present work)
X	α-D-Xyl-(1→6)-β-D-Glc* (=isoprimeverose)

* The β-D-Glc in each structure is part of the (1→4)-β-glucan backbone of the xyloglucan.

Eklöf and Brumer, 2010)], several glycosidases [especially α-fucosidase, α-xylosidase, β-glucosidase and β-galactosidase, catalysing exo-hydrolysis (Fanutti et al., 1991; Edwards et al., 1985; Koyama et al., 1983; de Alcântara et al., 1999)], and xyloglucan endotransglucosylase (XET) [Fry et al., 1992; Nishitani and Tominaga, 1992; Fanutti et al., 1993; Steele and Fry, 2000; Thompson and Fry, 2001; Rose et al., 2002] — all of which have been found in most land-plant species studied although with considerable phylogenetic variation in their quantitative activities (Franková and Fry, 2011). In addition to these broadly distributed enzymes, *Equisetum* almost uniquely possesses a relatively specific hetero-endotransglucosylase, called mixed-linkage glucan : xyloglucan endotransglucosylase (MXE) (Fry et al., 2008), and there is evidence for certain additional hetero-endotransglycosylase activities acting with xyloglucan as either the donor or the acceptor substrate (Hrmová et al., 2007; Kosík et al., 2010; Popper and Fry, 2008). Endotransglycosylase activities acting on pectic polysaccharides have been postulated but shown to be absent in plants (García-Romera and Fry, 1994; Popper and Fry, 2008). Trans-β-mannanase (Schröder et al., 2006,2009) and trans-β-xylanase (Franková and Fry, 2011) activities have also been detected in plants — enzymes that catalyse the endo-transglycosylation of (gluco)mannans and xylans respectively.

Some of the glycosidases that catalyse exo-hydrolysis of xyloglucan can also act as transglycosidases (Crombie et al., 1998; Franková and Fry, 2011, 2012; Opassiri et al., 2003; Sampedro et al., 2010). At high substrate concentrations, this ability is a general characteristic of all 'retaining' glycosidases [i.e., those which transiently form a glycosyl–enzyme ester bond and, after reaction with H_2O, release the sugar in a form retaining the same anomeric configuration (α or β) as it possessed in the original substrate]. Retaining glycosidases that potentially attack xyloglucan in plant cell walls include β-glucosidase (Crombie et al., 1998), α-xylosidase (Sampedro et al., 2001, 2010; Günl and Pauly, 2011) and β-galactosidase (Edwards et al., 1988; de Alcântara et al., 2006). In contrast, α-fucosidase is an inverting enzyme (CAZy; Cantarel et al., 2009): it is assumed not to form a transient fucosyl–enzyme ester bond, it releases β-L-fucose rather than α-L-fucose (although these anomers will quickly interconvert once released), and it lacks the ability to catalyse transglycosylation (Franková and Fry, 2012).

Although all retaining glycosidases can in principle catalyse both transglycosylation and hydrolysis, the ratio between these two competing reactions usually favours hydrolysis unless unphysiologically high acceptor substrate concentrations are used, e.g. 0.1–2.0 M (Dey, 1979; Hrmová et al., 1998; Nari et al., 1983). In the cases of certain enzymes from some plant species, however, there is a pronounced ability to catalyse transglycosylation even at relatively low (~1 mM) acceptor substrate concentrations, for example in the cases of trans-α-xylosidase, trans-β-xylosidase and trans-α-L-arabinosidase (Franková and Fry, 2011, 2012; Sampedro et al., 2010) — three activities that act on xyloglucan, (1→4)-β-xylan and (1→5)-α-L-arabinan model-substrates respectively.

We recently devised a simple and highly sensitive 'dual labelling' assay for xyloglucan-oligosaccharide-acting trans-α-xylosidase and trans-β-galactosidase activities (Franková and Fry, 2012). In the former case, [*Xyl*-^{3}H]XXXG (i.e., a xyloglucan heptasaccharide, $Xyl_3{\cdot}Glc_4$, radiolabelled specifically in its α-D-Xyl residues) was used as donor substrate, and a mixture of reductively aminated oligosaccharides of xyloglucan (XGO–NH_2; such as XXLG–NH_2, where G–NH_2 is 1-amino-1-deoxyglucitol) as acceptor substrate. Thus, the donor substrate is neutral and radioactively labelled while the acceptor substrate is cationic (amino-labelled) and non-radioactive. After the transxylosylation reaction,

$$\mathbf{Xyl}_3 \bullet Glc_4 + XGO{-}NH_3^+ \rightarrow \mathbf{Xyl}_2 \bullet Glc_4 + \mathbf{Xyl} \bullet XGO{-}NH_3^+$$

(where **Xyl** is a radiolabelled xylose residue), a product is formed that is both cationic and radioactive and can therefore be assayed by scintillation-counting of material that migrates towards the cathode during high-voltage paper electrophoresis (HVPE) (Franková and Fry, 2012).

By analysing the products formed during this reaction, we showed that the transferred [^{3}H]Xyl residue remained as the α-anomer, indicating that the plant transxylosidase was a retaining enzyme activity (Franková and Fry, 2012), as expected of a GH31-family enzyme (http://www.cazy.org/GH31.html). We also showed that the trans-α-xylosidase is capable of using not only oligosaccharides but also high-M_r xyloglucan as the acceptor substrate (Franková and Fry, 2012). However, we did not determine whether the [^{3}H]xylose residue was transferred onto a Gal, Xyl or Glc residue of the acceptor substrate (e.g. XXLG–NH_2). To answer this question, we have now used a range of highly sensitive radiochemical assays to elucidate the structure of the radioactive transxylosylation product. We report that the plant trans-α-xylosidase generates a novel xyloglucan unit. A second aim of the present work was to develop a battery of highly sensitive methods by which sub-picomole quantities of radiolabelled xyloglucan structures, generated *in vivo* or *in vitro* by novel enzyme activities, can be characterised. An overview of the main approaches used (Fig. 1) will assist with navigating the manuscript.

2. Results

2.1. Characterisation of radiolabelled substrates

Portions of [*Xyl*-^{3}H]XXXG and [*Xyl*-^{3}H]XXFG (prepared after *in-vivo* [^{3}H]arabinose feeding) were run by thin-layer chromatography (TLC) in butan-1-ol/acetic acid/water (BAW) (2:1:1); some lanes were fluorographed (Supplementary data Fig. S1a) and others