Widespread Occurrence of a Covalent Linkage Between Xyloglucan and Acidic Polysaccharides in Suspension-cultured Angiosperm Cells

ZOE A. POPPER and STEPHEN C. FRY*

The Edinburgh Cell Wall Group, Institute of Molecular Plant Sciences, School of Biological Sciences, The University of Edinburgh, Daniel Rutherford Building, The King’s Buildings, Mayfield Road, Edinburgh EH9 3JH, UK

Received: 19 January 2005 Returned for revision: 17 February 2005 Accepted: 3 March 2005 Published electronically: 18 April 2005

INTRODUCTION

Xyloglucan and pectins are quantitatively and dynamically important primary cell wall (PCW) polymers. Xyloglucan, a neutral hemicellulose, has a widespread occurrence in land plant PCWs (Popper and Fry, 2003), typically contributing 20–25 % of the dry weight of the dicot PCW (Keegstra et al., 1973) and 2–5 % of the dry weight of the monocot PCW (Fry, 1989a; Hayashi, 1989). It is thought to function in cell enlargement by serving as a substrate for xyloglucan endotransglycosylase (XET) activity, which is able to cut and rejoin intermicrofibrillar xyloglucan chains (Fry, 1989a). Xyloglucan contains sugar residues thought to be characteristic of xyloglucan and pectins (Bauer et al., 1973). In the 1973 model of Albersheim and co-workers (Keegstra et al., 1973), the reducing terminus of xyloglucan is glycosidically linked to what would today be termed an RG-I side-chain. Evidence for these linkages was centred on the products released after EPG digestion of Acer PCWs. EPG solubilized approx. 50 % of the total PCW 'pectin' (as defined by the presence of uronic acid residues) and approx. 10 % of the 'xyloglucan' [as defined by the presence of xylose (Xyl) residues]; a large proportion (approx. 70 %) of this Xyl-containing polysaccharide bound to the anion-exchanger, DEAE-Sephadex, and co-eluted with 'pectin', suggesting a bond between xyloglucan (which is neutral) and an acidic pectin (Talmadge et al., 1973). EPG-digested PCWs released a further proportion of the wall (approx. 15 %) on extraction with 8 M urea or with 0.5 M NaOH containing 100 mM NaBH₄ or on treatment with endo-β-glucanase. The solubilized wall material was shown to contain sugar residues thought to be characteristic of xyloglucan and pectins (Bauer et al., 1973). In the 1973 model (Keegstra et al., 1973) Xyl residues were not distinguished from 2-O-methylxylose and it was assumed that all Xyl was in xyloglucan and all uronic acid residues were in pectin. Later research showed that Xyl (and/or 2-O-methylxylose) and uronic acid residues could be derived from a single species of polymer [such as glucuronoarabinoxylan (GAX) or RG-II] without necessarily implying the existence of a xyloglucan–RG-I complex (Spellman et al., 1983;

However, recent evidence supports the existence of xyloglucan–RG-I conjugates. Thompson and Fry (2000) found approx. 30 % of xyloglucan (extracted from non-lignified suspension-cultured rose cells by 6 M NaOH) to be covalently bonded to anionic material, as judged by high-voltage electrophoresis and by anion-exchange chromatography. The anion-associated xyloglucan did not lose its charge after treatment with 8 M urea, 6 M NaOH or proteinase. Femenia et al. (1999) reported the presence of ‘pectic-xylog–xyloglucan’ complexes in alkali extracts from lignified cauliflower stems. This is also compatible with xyloglucan–pectin covalent complexes, although the possibility that lignin was responsible for the cross-linking of these two polysaccharides cannot be excluded. Enzymic digestion of the complex gave evidence consistent with hemicellulose–pectin complexes. Endo-xylanase and EPG treatments each (separately) caused a decrease in the apparent M₉ of the pectin, xyloglucan and xylan components.

Abdel-Massih et al. (2003) extracted nascent pectin from particulate enzyme preparations (from etiolated pea shoots) incubated with UDP-[U-¹⁴C]galactose. The nascent [¹⁴C]pectin had a strong affinity for paper (a characteristic of xyloglucan; Fry et al., 1992). The paper-binding ability of the [¹⁴C]pectin was greatly reduced by treatment with endo-1,4-β-glucanase, an observation which is consistent with the presence of xyloglucan in the radioactive paper-binding complex. Further support for a linkage between xyloglucan and RG-I has recently been presented by Vidal et al. (2003) who, similarly to Thompson and Fry (2000), observed strong binding of xyloglucans to an anion-exchange resin and subsequent co-elution with highly acidic RGs under high salt conditions.

In the present paper we report evidence for the presence of alkali-stable xyloglucan–pectin linkages in non-lignified suspension-cultured cells of all angiosperm cultures tested, both dicots and monocots

**MATERIALS AND METHODS**

**Cell cultures**

Cell-suspension cultures of rose (Rosa sp., ‘Paul’s Scarlet’), maize (Zea mays L., ‘Black Mexican Sweetcorn’), barley (Hordeum vulgare L.), tomato (Lycopersicon esculentum Mill.) and sycamore (Acer pseudoplatanus L.) were grown under constant dim illumination (10 μmol m⁻² s⁻¹) on an orbital shaker at 25 °C. Arabidopsis thaliana (L.) Heynh. ‘Erecta’ and spinach (Spinacia oleracea L. ‘Monstrous Viroflay’) cells were grown under constant moderate illumination (25 μmol m⁻² s⁻¹) on an orbital shaker at 135 r.p.m.

Rose cells were sub-cultured every two weeks by 10-fold dilution into fresh medium as described by Fry and Street (1980). Barley and Arabidopsis cells were sub-cultured weekly by 10-fold dilution into fresh medium. Arabidopsis medium was adapted from May and Leaver (1993) with the modification that the sole carbon source used was 2 % (w/v) glycerol instead of 3 % (w/v) glucose (Glc). The medium used for barley and maize was as described by Kerr and Fry (2003). Acer was maintained as described by Talmadge et al. (1973) and the spinach culture was maintained in Murashige and Skoog medium (Sigma, M5524) as described by Fry (1982). The tomato culture was maintained as described by Aldington and Fry (1994). Rose, tomato and sycamore were maintained as 55-mL cultures, and Arabidopsis, spinach, maize and barley as 220-mL cultures.

**Radiolabelling of hemicelluloses in cell cultures**

Radioactive arabinose (t-[¹³H]Ara; 148 MBq μmol⁻¹; synthesized by the method of Evans et al., 1974; filter sterilized) was added at 3·7 MBq per 55 mL cell suspension culture 7 d after sub-culturing, when the cultures were in the logarithmic growth stage (Thompson and Fry, 1997). t-[¹³H]Ara labels Ara and Xyl residues within PCW polysaccharides and therefore labels the Xyl moiety of isopimemoverose units in xyloglucan. The culture was incubated for 8 h then filtered through a polypropylene frit in an empty PolyPrep column (BioRad Inc.) under vacuum, and washed with 4 × 5 mL deionised water. The washed cells were used immediately for hemicellulose extraction.

**Radiolabelling of uronic acid residues in cell cultures**

Radioactive glucuronic acid (p-[⁶-¹⁴C]GlcA; 20 MBq μmol⁻¹; synthesized by the method of Sowden, 1952) was added at 1 MBq per 5 mL of Arabidopsis cell-suspension culture. The cells had been transferred 3 d previously from a medium containing 2 % (w/v) Glc as sole carbon source to a fresh medium containing 2 % (w/v) glycerol as sole carbon source; this pre-treatment appeared to promote GlcA uptake. The cells took up approx. 40 % of the ¹⁴C over 8 h. Feeding of p-[⁶-¹⁴C]GlcA labels GalA residues (and therefore pectins) and also GlcA residues within PCW polysaccharides, but not the neutral hexose or pentose residues (Feingold and Avigad, 1980; Brown and Fry, 1993). The cells were collected and washed as described above.

**Buffers**

Unless otherwise stated, all buffer solutions were pyridinium acetate (PyOAc), chosen for its volatility. Concentrations quoted refer to the sum of acetate + acetic acid; for example ‘11 mM PyOAc buffer, pH 4.7’ was 11 mM acetic acid adjusted to pH 4.7 by the addition of pyridine.

**Dialysis**

Polysaccharide samples were dialysed against running tap water for 2 d, then against 11 mM PyOAc, pH 4.7, containing 0·5 % w/v chlorobutanol.

**Polysaccharide analysis**

Driselase digestion was performed as described by Fry (2000). Driselase digests xyloglucan to isopimemoverose,
glucose, galactose and fucose. Radioactive digestion products were separated by paper chromatography in solvent systems 1, 2 and 3 and assayed for radioactivity.

**Paper chromatography and electrophoresis**

Whatman No.1 chromatography paper was used for analytical paper chromatography and electrophoresis. Solvent systems used were: (1) ethyl acetate/pyridine/water (9:3:2, v/v/v) for 20 h (Thompson and Fry, 1997); (2) butan-1-ol/acetic acid/water (12:3:5, v/v/v) for 16 h; and (3) 80 % (w/w) phenol for 48 h. 3H-labelled Driselase-digestion products had non-radioactive internal markers of isoprimeverose, Xyl, xylobiose (Xyl2) and Ara. After separation in solvent system 1, which gives the best separation of isoprimeverose, Xyl, Xyl2, Ara and Driselase-indigestible material (which remained at the origin), internal markers were stained with dilute aniline hydrogen-phthalate, which does not appreciably affect the efficiency of scintillation counting (Kerr and Fry, 2003). The appropriate portion of the chromatogram was then cut and assayed for 3H.

Paper electrophoresis (3 kV for 70 min) was carried out in a white-spirit-cooled apparatus as described by Fry (2000). The electrode-buffer was acetic acid/pyridine/water, (10:1:189, by volume, pH 3-5) and the paper was wetted with half-strength electrode-buffer. This electrophoretic method resolves GlcA, GaLa and aldobiouronic acids (Fry et al., 2001); 4-O-methyl-glucuronate (MeGlcA) migrates close to GlcA but well resolved from GaLa (Popper and Fry, 2003).

**Extraction of 3H-labelled hemicelluloses**

3H-labelled cells (7 g fresh weight) were suspended in 70 mL 6 M NaOH containing 1 % (w/v) NaBH4 and left shaking at 37 °C for 24 h. This treatment was shown by Edelmann and Fry (1992) to extract essentially all the xyloglucan from the walls of cell-suspension cultures. The alkali extract was filtered through a polypropylene frit (in an empty ‘PolyPrep’ column; BioRad), then acidified by the addition of acetic acid (0.5 volumes, added slowly with stirring on ice), dialysed, freeze-dried, and re-dissolved in 60 mL of 11 mM acetate buffer, pH 4-7. This preparation is referred to as 3H-hemicellulose.

**Fractionation of neutral and anionic xyloglucan**

The 3H-hemicellulose solution was enriched in xyloglucan content by precipitation in 50 % ethanol and the pellet was re-dissolved in 11 mM PyOAc containing 8 mM urea (pH 5-3) and fractionated on the basis of charge on Q-Sepharose FastFlow [10 mL bed volume; pre-treated with 2 M sodium acetate (adjusted to pH 7-0 with acetic acid) and equilibrated with 11 mM acetate buffer, pH 5-3, containing 8 mM urea]. Neutral polysaccharides were eluted with 8 mM urea in 11 mM buffer. Acidic polysaccharides were then eluted with 8 mM urea in a step-gradient of buffer (25 mL each of 11, 22, 44, 88, 175, 350, 525, 700, 875, 1050, 1225, 1400 mM acetate, pH 5-3, followed by 2 M sodium acetate, pH 7-0 and finally 1 M NaOH). Each fraction was dialysed, a portion of it was assayed for urea (Coulombe and Favreau, 1963) to confirm that this had been removed by dialysis, and the remainder was Driselase-digested (Thompson and Fry, 1997) to find its 3H-polymer composition.

**Extraction of 14C-labelled pectins**

14C-labelled cells were washed in 6 x 20 mL 0.5 % chlorobutanol (each wash was for 1 h at room temperature with gentle mixing) before extraction in 0-18 M ammonium oxalate (pH 3-7 at 80 °C, for 2 h). The extract (referred to as 14C-pectin) was neutralized, dialysed against running tap water, dialysed against pyridine(acetic acid)/water (1:1:98 by volume, approx. pH 4-7) containing 0.5 % chlorobutanol.

**Cellulose-binding assay**

Solutions (1-25 mL) of the 14C-pectin were incubated for 2 d with gentle mixing in a tube containing a 5 x 25-mm piece of Whatman 3MM paper (=cellulose). Before and 2 d after the addition of the paper, 50 μL of the solution was assayed for radioactivity. A further 400 μL of 14C-polymer solution was dried, hydrolysed (in 2 M TFA, 120 °C, 1 h), and electrophoresed: the radioactive spot co-migrating with external marker GaLA was assayed for 14C.

**Scintillation counting**

Aqueous solutions of 3H-sugars (eluted from the chromatography paper) and of 3H-polysaccharides were mixed with 10 volumes of water-miscible scintillation fluid (‘OptiPhase HighSafe 3’; Wallac Oy, Turku, Finland). Strips of chromatography paper were assayed for 14C (without elution) after addition of 2 mL of water-immiscible scintillation fluid (‘OptiScint’; Wallac).

**RESULTS**

**Radio-labelling of PCW polysaccharides**

To facilitate sensitive detection of xyloglucan, we radiolabelled the wall polysaccharides of cell cultures in the pentose residues by feeding L-[1-3H]Ara in vivo. All rapidly growing 7-d cell-suspension cultures tested (spinach, Arabidopsis, tomato, rose, sycamore, maize and barley) took up >80 % of the exogenous [3H]Ara within 1 h. This is comparable to the reported behaviour of rose and maize cultures (Edelmann and Fry, 1992; Thompson and Fry, 1997; Kerr and Fry, 2003). The cells incorporated the 3H into both [3H]Ara and [3H]Xyl residues of polymers, indicating that 4-epimerisation readily occurred, as reported before (Feingold and Avigad, 1980). Thus, the Xyl residue of isoprimeverose [Xyl-α-(1→6)-Glc] became radio-labelled. Isoprimeverose is the Driselase digestion-product indicative of the presence of xyloglucan. Other radio-labelled Driselase-digestion products generated included Xyl and xylobiose [Xyl-β-(1→4)-Xyl], both of which are largely derived from xylans; and Ara itself, which is derived from several diverse polysaccharides and glycoproteins. Ara residues are labelled to a higher specific activity than Xyl (Wende and Fry, 1997).
Evidence for anionic $^{3}$H-hemicelluloses

The 50% ethanol-precipitated material (xyloglucan-enriched hemicellulose) was subjected to anion-exchange chromatography in the presence of 8 M urea. This led to the elution of only 7–16% of the $^{3}$H in the neutral pool (fractions 1 and 2; eluted with 11 mM PyOAc, which is the same concentration as the loading buffer); in all species, the majority of the $^{3}$H was eluted as acidic material (fractions 6–13 (175–1400 mM PyOAc), 14 (2 M sodium acetate, pH 7.0) and 15 (1 M NaOH)) (Fig. 1). Less than 1% of the total radioactivity was eluted in fractions 3–5.

The anionic $^{3}$H-hemicellulose from spinach and tomato cultures was found to be eluted in two peaks (Fig. 1): fraction 11 (1050 mM PyOAc) and fraction 14 (2 M sodium acetate, pH 7.0). However, anionic $^{3}$H-hemicellulose extracted from Arabidopsis differed in its elution pattern with a greater proportion being eluted in the more anionic fraction 14. Anionic $^{3}$H-hemicellulose from maize was eluted in two major peaks, both of which were less anionic than in spinach, Arabidopsis and tomato. That from rose was eluted in one anionic peak. The $^{3}$H-elution patterns of xyloglucan-enriched hemicellulose were similar to those observed in a replicate experiment in which the cells were fed one-quarter of the amount of radioactivity (data not shown).

Evidence for anionic $^{3}$H-xyloglucans

The 50% ethanol-precipitated material (xyloglucan-enriched hemicellulose) was subjected to anion-exchange chromatography in the presence of 8 M urea. This led to the elution of only 7–16% of the $^{3}$H in the neutral pool (fractions 1 and 2; eluted with 11 mM PyOAc, which is the same concentration as the loading buffer); in all species, the majority of the $^{3}$H was eluted as acidic material (fractions 6–13 (175–1400 mM PyOAc), 14 (2 M sodium acetate, pH 7.0) and 15 (1 M NaOH)) (Fig. 1). Less than 1% of the total radioactivity was eluted in fractions 3–5.

The anionic $^{3}$H-hemicellulose from spinach and tomato cultures was found to be eluted in two peaks (Fig. 1): fraction 11 (1050 mM PyOAc) and fraction 14 (2 M sodium acetate, pH 7.0). However, anionic $^{3}$H-hemicellulose extracted from Arabidopsis differed in its elution pattern, with a greater proportion being eluted in the more anionic fraction 14. Anionic $^{3}$H-hemicellulose from maize was eluted in two major peaks, both of which were less anionic than in spinach, Arabidopsis and tomato. That from rose was eluted in one anionic peak. The $^{3}$H-elution patterns of xyloglucan-enriched hemicellulose were similar to those observed in a replicate experiment in which the cells were fed one-quarter of the amount of radioactivity (data not shown).

The data in Fig. 2 and many related experiments (not shown) were quantified so that the elution profile of $^{3}$H-xyloglucan could be determined (Fig. 3). Between 30% (in Arabidopsis) and 55% (in rose and spinach) of the $^{3}$H-isoprimeverose-yielding polymer (= $^{3}$H-xyloglucan) was eluted in the neutral fraction (Fig. 3). In a subsequent experiment, a value of 70% was obtained for barley (data not shown).

The remaining 30–70% of the $^{3}$H-xyloglucan was eluted in anionic fractions. The distribution of $^{3}$H-xyloglucan between the various anionic fractions differed between species. Arabidopsis $^{3}$H-xyloglucan being particularly highly anionic.

Elution of pectins and xylans

The following observations permitted conclusions to be drawn about the acidic polysaccharides that were associated with the $^{3}$H-xyloglucans in the anionic fractions.

Driselase-generated $^{3}$H-Xyl and $^{3}$H-Xyl$_{2}$, which are indicative of $^{3}$H-xylopolysaccharides, were eluted mainly in the moderately anionic fractions (Fig. 3). Elution of $^{3}$H-Xyl- and $^{3}$H-Xyl$_{2}$-yielding polymers approximately mirrored each other, as expected if they arise from the same polysaccharide, but their elution did not closely mirror that of $^{3}$H-isoprimeverose, supporting the conclusion that xylans and xyloglucans were not tightly associated with each other.

$^{3}$H-Ara is generated by Driselase from both RG-I and arabinoxylans, and was thus expected to be eluted mainly in the anionic fractions, as observed (Fig. 3). In tomato, approx. 10% of the $^{3}$H-Ara-yielding polymer was eluted in the neutral fraction, probably representing the Ara-rich xyloglucans that are characteristic of the Solanaceae (Eda and Kato, 1978; Akiyama and Kato, 1982; York et al., 1996). In most species the highest amount of $^{3}$H-Ara-yielding polymer was eluted with 325–1225 mM PyOAc in fractions 8–12 (Fig. 2). However, in spinach and Arabidopsis $^{3}$H-Ara-yielding polymers were more abundant in later fractions, suggesting that they were more acidic. Staining of paper chromatograms with aniline hydrogenphthalate showed that Driselase had also released non-radioactive GaA (indicating the presence of pectins) from the polymers in fractions 11–14.

Driselase-resistant $^{3}$H-polymers (Fig. 3) cannot be classified precisely. On acid hydrolysis they yield mainly $^{3}$H-Ara, and they probably include AGPs (which largely resist Driselase). Between 5 and 18% of the Driselase-indigestible material was eluted in the neutral fractions. However, the greatest proportion of Driselase-indigestible material was eluted in acidic fractions; in particular, in Arabidopsis, 45% of it was eluted in fraction 14 (1400 mM PyOAc; Fig. 3).

Cellulose-binding of $^{14}$C-labelled pectins

$^{14}$C-Pectins were extracted from Arabidopsis cultures after 8 h in the presence of d-[6-$^{14}$C]GlcA, which radio-labels only the uronate residues (Brown and Fry, 1993). Hot ammonium oxalate was used to extract, relatively
specifically, the pectins. Xyloglucans and other hemicelluloses are not efficiently extracted until 6 M NaOH at 37 °C is used (Edelmann and Fry, 1992). The extracted (uronate-14C)-labelled pectins were assayed for their ability to bind to cellulose (paper) from aqueous solution, a characteristic of hemicelluloses. No drying step was included, as drying appears to promote non-specific adsorption of many polysaccharides to paper.

A substantial proportion (21%) of the total 14C in the oxalate extract was able to bind to paper, suggesting that the pectins were attached to another wall component that has strong paper-binding ability. Acid hydrolysis of the 14C-pectins, before and after paper binding, showed that 20% of the [14C]GalA-yielding polymer (i.e. [14C]pectin) in this extract had bound to paper. Additionally, approx. 10% of the [14C]aldobiouronic acid-yielding material in the extract had bound to paper.

**DISCUSSION**

Xyloglucan is a neutral polysaccharide (Fry, 1989a; Hayashi, 1989), composed of a β-(1→4)-d-glucan backbone...
substituted with α-D-Xyl, β-D-Gal, and in some cases α-L-Fuc and/or α-L-Ara. Widely accepted PCW models propose at least two independent polymer networks: a cellulose–xyloglucan network held together by hydrogen-bonds, and a pectic network held together by Ca^{2+} bridges. In onion epidermal cell walls, both the pectic and the cellulose–xyloglucan networks have a preferred orientation (Chen et al., 1997), which is suggestive of communication between the two networks. A high-M_r complex of xyloglucan linked to another polysaccharide, in particular another abundant PCW polymer such as pectin, would be expected to play a more effective tethering role in PCW architecture (Fry, 1989b; Kerr and Fry, 2003) than lower-M_r, free xyloglucans. The widespread taxonomic distribution of the xyloglucan–pectin linkage, reported here, suggests its importance to angiosperm PCW structure and function. Elucidation of the chemical structure of the linkage and its mode of synthesis are likely to promote our understanding of, and in future enable manipulation of, important physical properties of PCWs.

The extracted anionic [3H]xyloglucan is unlikely to be ester-bonded to pectin. Very mild conditions of NaOH hydrolysis (1 M NaOH at 20 °C) break all detectable ester bonds within 24 h, whereas we extracted anionic [3H]xyloglucan with 6 M NaOH at 37 °C. This suggests the existence of a highly alkali-stable bond between xyloglucan and an acidic PCW polymer.

Kerr and Fry (2003) found that in cultured maize cells, the average M_r of pulse-labelled intraprototoplasmic [3H]xyloglucans increased up to 30 min after labelling but prior to secretion. The increase in average M_r of the [3H]-polymers was too large to be entirely ascribed to on-going (NDP-sugar-dependent) chain elongation within the Golgi cisternae (which could theoretically account for at most a doubling of the average M_r of the [3H]xyloglucans; see fig. 10 of Kerr and Fry, 2003), so it is likely that the increase was due to the post-synthetic bonding of [3H]xyloglucans to each other or to other (non-radioactive) polymers. Their bonding to RGs could account for the widespread formation of ‘anionic xyloglucan’, reported here.
It has also been reported that nascent pectin extracted from pea stems is complexed with xyloglucan (Abdel-Massih et al., 2003), supporting this conclusion. In agreement with the observations of Abdel-Massih et al. (2003), the cellulose- (paper-) binding ability of hot-oxalate-extracted [GalA-14C]pectin, noted in the present work on Arabidopsis cells, indicates an unexpected ability of some pectins to bind to cellulose. Paper-binding is not a characteristic of pectins (particularly if the sample is not dried on to the paper). It is therefore more likely that the [GalA-14C]-pectin, solubilized by hot oxalate, was attached to, and able to bind to paper via, a hemicellulose (such as xyloglucan) that does have strong paper-binding ability. We probably underestimated the proportion of pectin that was hemicellulose-linked in this way, since the extractant used (hot oxalate) would not efficiently break the hemicellulose–cellulose hydrogen-bonds that may have been present in muro. Some hemicelluloses (especially xylans and glucuronomannans) contain GlcA and/or MeGlcA residues, which would be radioactive in the [14C]GlcA-fed cells; such hemicelluloses would also show up as paper-binding 14C-polymers. However, it is unlikely that these hemicelluloses would have been extracted from the cell walls by hot oxalate. We verified that the 14C-residues present in the paper-binding polymers reported here were indeed GalA (diagnostic of pectins), not GlcA or MeGlcA. Thus, we

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Spinach</th>
<th>Isopimemerose</th>
<th>Xylose</th>
<th>Arabinose</th>
<th>Driselase-indigestible</th>
<th>Xylobiose</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 3.** Anion-exchange chromatography profiles of polymers containing individual (pentosyl-3H)-labelled residues. The Q-Sepharose fractions reported in Fig. 1 were Driselase-digested and the radioactive products (isopimemerose, xylobiose, Xyl, Ara and Driselase-indigestible material) were separated by paper chromatography in solvent system 1 and assayed by scintillation-counting. Fractions 2–5 contained little radioactivity and were therefore not analysed.
conclude that some of the pectin in Arabidopsis PCWs was strongly (probably covalently) linked to a cellulose-binding hemicellulose (e.g. xyloglucan).

We investigated the existence of a xyloglucan–pectin linkage in a wide variety of angiosperm cell-suspension cultures chosen to represent the diversity within angiosperm PCW structure. The major hemicellulose in Poales (gramineous monocots) is GAX, not xyloglucan, and the PCWs of Poales (represented here by maize and barley) often contain only about 20 % of the xyloglucan present in non-gramineous monocot and dicot PCWs (Fry, 1989a; Hayashi, 1989). They also contain high concentrations of feruloylated GAXs (present but not feruloylated in dicots) and have lower galacturonan contents than dicot PCWs (Shibuya et al., 1983; Jarvis et al., 1988). Additionally, PCWs of plants within the Poales contain mixed-linkage glucan, a hemicellulose absent from all other angiosperm PCWs (Nevins et al., 1978; Smith and Harris, 1999; Popper and Fry, 2003). PCWs of the Centrospermae (represented by spinach) are rich in ferulic acid, which in these dicots forms esters with Ara and Gal residues of pectic polysaccharides (Fry, 1982; Micard et al., 1997). It is therefore differently linked from the ferulic acid found in gramineous monocots. Xyloglucans so far extracted from different sources show some differences. The majority of xyloglucans extracted from both gymnosperms and angiosperms are reported to have a backbone consisting of repeat units of XXXG, whereas xyloglucans extracted from solanaceous plants (represented by tomato) have two, instead of three, consecutive branched Glc residues, which alternate with two unsubstituted Glc residues (XXGG; Vincken et al., 1997). [For an explanation of the abbreviated nomenclature of xyloglucan oligosaccharides (XXXG etc.), see Fry et al. (1993).] Additionally, xyloglucans isolated from the Solanaceae differ from those of other dicots in the branching of their xylosyl substituents; XXXG and XXFG are absent (Vincken et al., 1997) and Xyl residues are 2-O-substituted predominantly with α-L-Ara and β-D-Gal (York et al., 1996). Xyloglucan extracted from Poales also differs in composition, containing less Xyl, Gal and Fuc than typical dicot xyloglucan (Hayashi, 1989; McDougall and Fry, 1994; Vincken et al., 1997).

It is interesting that despite the wide variability in xyloglucan structure within angiosperms we found that in all angiosperms tested a substantial proportion of the xyloglucan was linked to an acidic polymer. It is possible that the structure of the acidic polymer is highly variable. RG-I is known to vary in methylation and/or acetylation of the GaLA residues in its backbone (Perrone et al., 2002) as well as in the degree of substitution with arabinan and galactan side-chains (McNeil et al., 1982; Lerouge et al., 1993). It seems remarkable that a linkage between xyloglucans and an acidic polysaccharide, probably RG-I, appears to be conserved among angiosperms. This suggests that the linkage is important to PCW integrity and function.

Xyloglucan is likely to have a major functional role in gramineous monocot PCWs since the xyloglucan-modifying enzyme XTH extracted from grass tissues has a high XET specific activity (Fry et al., 1992). This is especially significant since gramineous monocot PCWs are widely reported to contain very much less xyloglucan than do dicot PCWs (Bacic et al., 1988; Smith and Harris, 1995). However, maize xyloglucan has a much higher molecular weight than that of dicot xyloglucan (Kerr and Fry, 2003). This may partially account for the survival of gramineous monocots despite their low xyloglucan content. Anionic xyloglucan was readily detected in suspension-cultured cells of gramineous monocots (maize and barley) as well as in those of dicots.

In conclusion, we obtained evidence for anionic xyloglucan in all angiosperm PCWs investigated despite wide variation in their xyloglucan structures as well as differing overall PCW compositions. This indicates that the xyloglucan–pectin linkage is evolutionarily conserved and may be required for effective PCW structure and function.

ACKNOWLEDGEMENTS

We thank Professor Peter Dominy (University of Glasgow) and Dr Antonio Encina (University of León) for the kind provision of Arabidopsis and barley cell suspension cultures, respectively. We thank the UK BBSRC for funding this project.

LITERATURE CITED


