PROFILING METHODS FOR THE ANALYSIS OF CELL WALL POLYSACCHARIDES*

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ABSTRACT

There are techniques are available for the analysis of plant cell wall structures that are fast and require small amounts of material. Some of these approaches include oligosaccharide mass profiling, carbohydrate gel electrophoresis, and capillary electrophoresis.

Keywords: cell walls; carbohydrate analysis; polysaccharides.

INTRODUCTION

The cell wall of plants consists mainly of a variety of different carbohydrate polymers and low amounts of proteins. In some cell types phenolic compounds are present. The carbohydrate polymers of the cell wall are cellulose, different hemicelluloses, and pectins (Cosgrove 2005; Burton *et al.* 2005). The building units of these polymers are neutral or acidic monosaccharides, and in pectins some rare sugars can occur at low levels (Vincken *et al.* 2003). The complexity of carbohydrate polymers is due mainly to the multiple glycosidic linkages of the monosaccharides. Another aspect is that the polysaccharides form networks thus making it difficult or even impossible to isolate specific polysaccharides in their entirety from the cell wall (Carpita & Gibeaut 1993). When covalent bonds are broken it is difficult to reconstruct the native structure with the data obtained by further analysis. Also, the fact that many cell wall polymers show a large variation in size and primary structure contributes to difficulties in interpreting the carbohydrate data (Brett & Waldron 1996). However, an in-depth knowledge of the carbohydrate composition of cell walls is important for understanding the function of the different polymers. Different methods are available to study the chemical structure

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of wall polysaccharides. Unfortunately, classical analytical techniques for describing a polysaccharide in detail are labour-intensive and require relatively large amounts of cell wall material.

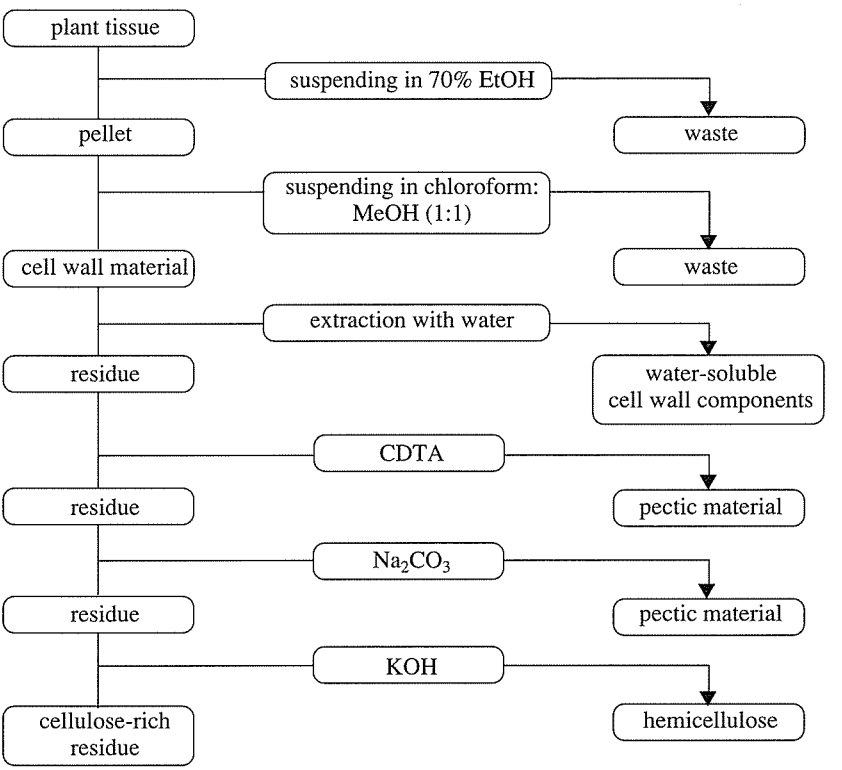
Here, we review some of the more recent wall-profiling techniques that have been developed to reduce analysis time and decrease the amounts of materials needed to assess the structure of wall polysaccharides.

EXTRACTION

The first step in the characterisation of specific cell wall components is the solubilisation, fractionation, and purification of polymers from the cell wall (Coimbra *et al.* 1996). In order to avoid endogenous enzyme activity, cell wall material can be boiled or suspended in a phenol-acetic acid-water (PAW) mixture (Seymour *et al.* 1987). The presence of starch can interfere with glucose measurements of cell wall polymers. However, removal of starch can be accomplished with α-amylase treatment of the cell wall material (Zablackis *et al.* 1995). A drawback of the amylase treatment is the possible loss of buffer-soluble polysaccharides. An easy and quick method to obtain cell wall material is the preparation of alcohol-insoluble residue (AIR) (Coimbra *et al.* 1996). However, these extracts contain much co-precipitated proteins, nucleic acids, and polyphenols. In order to obtain different purified cell wall polymers, sequential extractions with water, chelating agent, buffer, and alkaline solutions are most often used (Redgwell & Selvendran 1986; Coimbra *et al.* 1996; Fry 1988; Southgate 1995). The first extraction is generally done with water to obtain the water-soluble solids. Afterwards a chelating agent such as CDTA that forms a complex with calcium (Mort *et al.* 1991; de Vries *et al.* 1981) or weak alkaline solutions (Na_2CO_3) are used, thereby releasing a substantial part of the pectins from the cell wall (Jarvis 1982). Hemicelluloses can be obtained by treating the cell wall with alkaline solutions that increase in strength (Edelmann & Fry 1992). The part of the cell wall remaining after the different extraction steps contains a large amount of cellulose but also some pectin and hemicellulose that could not be extracted during the previous steps (Vierhuis *et al.* 2000). A possible extraction scheme is shown in Fig. 1. The concentrations of the chemicals used can vary depending on the cell wall components that have to be extracted and the origin of the cell wall material. Also, other experimental settings such as the duration of the extraction and the number of repeated extractions can vary.

Besides extraction with chemicals, wall polymers can be selectively extracted with hydrolytic enzymes. Many enzymes are available in their recombinant form and allow, after purification, a selective hydrolysis of cellulose, pectin, and hemicelluloses such as xyloglucan and galactomannan (de Vries 2003). One advantage of enzymatic solubilisation of polysaccharides is that labile substituents such as esters remain

A. Extraction scheme for the preparation of cell wall polysaccharides



B: Extraction scheme for the preparation of carbohydrate oligomers for mass profiling

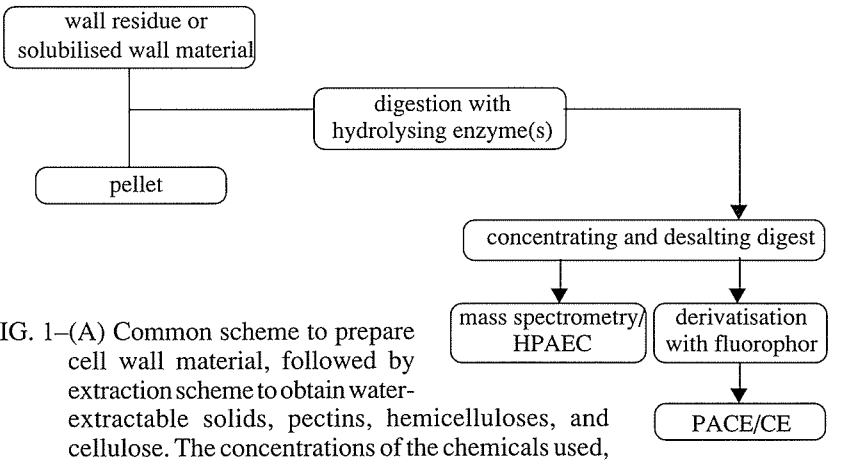


FIG. 1–(A) Common scheme to prepare cell wall material, followed by extraction scheme to obtain water-extractable solids, pectins, hemicelluloses, and cellulose. The concentrations of the chemicals used, the duration, and the temperature during extraction vary according to the requirements of the extracts (derived from Coimbra *et al.* 1996).
(B) Flow chart for oligosaccharide profiling with various methods.

intact on the resulting oligosaccharides. Before characterisation of the polysaccharides can be performed, the enzymatic extracts are generally purified from salts by dialysis or ultra filtration.

CLASSICAL ANALYTICAL TECHNIQUES

After solubilisation of cell wall materials, classical analytical techniques are usually used to give further insights into their composition. Those techniques encompass the determination of the molecular weight distribution, e.g., carried out by chromatography based on size exclusion (White *et al.* 1999). Other techniques include monosaccharide composition analysis, which requires acidic hydrolysis of the polymers. For many cell wall polysaccharides, hydrolysis with trifluoroacetic acid is sufficient (Albersheim *et al.* 1967). However, for cellulose microfibrils including hemicelluloses, hydrolysis in sulphuric acid at high concentration and a second hydrolysis at a lower concentration at high temperature are necessary (Saemann *et al.* 1963). The resulting monosaccharides after hydrolysis can be separated and quantified using high-performance anion exchange chromatography (HPAEC) (Oxenbøll *et al.* 2000) or with GC-MS analysis after derivatisation of the sugar monomers into alditol acetates or TMS derivates (Englyst *et al.* 1982; Sweeley *et al.* 1963). Additional information about the polysaccharide can be obtained by determination of the glycosidic linkage of the different monomers (Lindberg & Lönngren 1978). Non-carbohydrate substituents such as acetyl- or methyl-esters can be determined by colorimetric assays (Müller & Horbach 1981; Klavons & Bennett 1986; Wood & Siddiqui 1971) or by gas or liquid chromatography (Huisman *et al.* 2004; Voragen *et al.* 1986).

Although these methods provide exceptional detail about the structure of a wall-derived polysaccharide, these procedures are generally labour-intensive. Nowadays there are many spectroscopy techniques available that provide data about the structure of carbohydrates. Some of these spectroscopy techniques are fourier transformation infra red (FTIR) (Séné *et al.* 1994; McCann *et al.* 1997) and nuclear magnetic resonance (NMR) (Bootten *et al.* 2004; Renard & Jarvis 1999). Both techniques have the disadvantage that the spectra require substantial statistical conversion for useful interpretation.

OLIGOSACCHARIDE PROFILING TECHNIQUES

The principle underlying oligosaccharide profiling techniques is the use of enzymes with known specificity for the substrate in combination with analytical techniques such as high-performance size exclusion chromatography (HPSEC), high-performance anion exchange chromatography (HPAEC), mass spectrometry (MS), or gel electrophoresis. The disadvantage of using HPAEC is that this method is

rather time-consuming and samples can only be run one at a time. For instance, the separation of an oligomannuronic acid hydrolysis mixture was accomplished in 80 minutes (Campa *et al.* 2004) and in a study on fructooligosaccharides and inulin the total run time per sample was 110 minutes (Corradini *et al.* 2004). A fast technique to get further insights into the structure of enzyme-solubilised oligomers is mass spectrometry (MS), e.g., with matrix-assisted laser desorption ionisation time of flight mass spectrometry (MALDI-TOF MS). This technique can be used to determine the exact molecular weight of oligosaccharides in the range of 400 to 5000 Da but even molecules with a mass up to 30 kDa can be detected. The carbohydrate oligomers are dried on a sample plate together with a light-absorbing matrix-chemical for which dihydroxybenzoic acid (DHB) is often used. The advantage of using MALDI-TOF MS is that low amounts of material are required for analysis (1 μg) and the time for analysis is limited to a few minutes. Taken together with the knowledge of the specificity (cleavage site) of the enzyme used, detailed structural information about the polysaccharide present in the wall material can be obtained. The use of this technique to rapidly assess a large number of wall materials was termed OLIMP (oligosaccharide mass profiling) and allows high throughput analysis (Lerouxel *et al.* 2002). One of the requirements of OLIMP is the availability of specific carbohydrate hydrolysing enzymes to obtain oligomers. Examples of polysaccharides that can be analysed by OLIMP are xyloglucan (Marry *et al.* 2003), xylans (Zhong *et al.* 2005), and pectin structural elements as xylogalacturonan, galacturonic acid oligomers (van der Vlugt-Bergmans *et al.* 2000; Daas *et al.* 1998). Disadvantages of OLIMP are that an expensive mass spectrometer is required, monosaccharides cannot be detected, and the method gives only semi-quantitative information on the relative abundance of oligosaccharides, but not their absolute amounts. Other mass spectrometry ionisation techniques for the analysis of carbohydrate oligosaccharides include electro spray ionisation (ESI) (Matsunaga *et al.* 2004), fast atom bombardment (FAB) (Kolli *et al.* 1998), and pyrolysis mass spectrometry combined with chemical ionisation (CI) (Lomax *et al.* 1991).

Polysaccharide analysis using carbohydrate gel electrophoresis (PACE) is able to detect both monosaccharides and oligosaccharides in a quantitative manner and does not require expensive equipment (Goubet *et al.* 2002; Lerouxel *et al.* 2002). Also, this technique facilitates specific hydrolytic enzymes to obtain carbohydrate fragments. After hydrolysis the resulting glycosides are derivatised with a charged or uncharged fluorophore (e.g., ANTS, 8-aminonaphtalene-1,3,6-trisulfonic acid or AMAC, 2-aminoacridone) (Goubet *et al.* 2002). The separation of the derivatives is then carried out by standard polyacrylamide gel-electrophoresis. The detection can be performed with a fluorescence imager. According to Goubet *et al.* (2002) low quantities of material are required for the detection of fragments (500 fmol of

monosaccharides and 100 fmol of oligosaccharides). Another advantage of PACE over OLIMP is that the carbohydrate spots on the gel can be quantified and the structural isomers can be separated (Goubet *et al.* 2006). One severe limitation of PACE is that the compounds (bands on the gels) cannot be identified unless exact standards are available or an additional analytical technique such as a mass spectrometrical analysis of an excised gel-band is performed.

An alternative method for separating and detecting fluorescently labelled monosaccharides, oligosaccharides, and glycoproteins is high-performance capillary electrophoresis (HPCE). Capillary electrophoresis is an aqueous-based separation method that requires small sample amounts, is fast, and has high separation efficiency (Rassi & Mechref 1998). The separation of compounds is performed in a capillary that is in contact with buffer at both ends. A high-voltage power supply is required to drive the separation. There are several approaches to render carbohydrates amenable to separation and detection by HPCE as most carbohydrates are not charged, which is necessary for separation, and lack chromophores in their structure allowing detection at low levels. A common method of charging carbohydrates is to perform the analysis at high pH values (pH 10–12) or by borate complexation (Altria 1999; Rassi & Mechref 1998). Detection can be done by UV detection, conductivity, or laser-induced fluorescence (LIF), which requires fluorophore-labelled oligosaccharides (Guttman 1996). The advantages of HPCE over conventional chromatography methods are the need for small amounts of material (at attomol range), high separation efficiency, and improved speed. Capillary electrophoresis has been successfully applied for the analysis of pectin oligomers (Zhong *et al.* 1998). Williams *et al.* (2002) were able to reduce the analysis time from a pectin digest by a factor of four compared to conventional HPAEC analysis. However, with capillary electrophoresis only the retention time of a compound is obtained. Therefore, standards are necessary to identify the compound unambiguously. As this task can be difficult for complex biomolecules, the coupling of capillary electrophoreses with mass spectrometry (CE-MS) allows a more specific and selective detection (Li *et al.* 1998). Nowadays electrospray ionisation is most often used between CE and MS because ions can be formed from liquids at atmospheric pressure (Anastos *et al.* 2005). Wang *et al.* (2006) could confirm with CE-MS analysis that the oligosaccharide core structure was conserved among different pathogenic lipopolysaccharide isolates.

The attributes of the various profiling techniques are summarised in Table 1.

With the advent of these various profiling techniques particularly, the speed with which wall materials can be analysed has been drastically reduced allowing the rapid assessment of large numbers of potential wall samples. Thus, the identification and characterisation of potential wall mutants will be greatly expedited, significantly enhancing our knowledge of the biology of the plant cell wall.

TABLE 1–Overview of attributes for carbohydrate profiling techniques

Method		Equipment required	Sample amount	Analysis time per sample	Data obtained
HPAEC	High performance anion exchange chromatography	Auto sampler, pump, detector	5–15 μg	40–120 min	Peaks have to be identified with standards, quantitative, structural isomers can be detected
OLIMP	Oligosaccharide mass profiling	Mass spectrometer	1 μg	2 min	Exact mass of the fragments, not quantitative (only relative abundance)
PACE	Polysaccharide analysis using carbohydrate gel electrophoresis	Electrophoresis apparatus, fluorescence imager	100 fmol	45 min multiple samples	Gel bands have to be identified with standards, quantitative, structural isomers can be detected
HPCE	High performance capillary electrophoresis	CE instrument, detector	attomol	20 min	Peaks have to be identified with standards, quantitative

fmol $= 1.10^{-15}$ mol
attomol $= 1.10^{-18}$ mol

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