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Characterization of cell wall polysaccharides from the medicinal plant *Panax notoginseng*

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Abstract

Panax notoginseng is a commonly used medicinal plant in south-western China. Recent studies indicate that wall polysaccharides are responsible for some of the immunostimulatory activity. Fractionation of the *P. notoginseng* root powder alcohol insoluble residue (AIR) and its compositional analysis enabled us to deduce the polysaccharide and protein composition of the root cell walls. *P. notoginseng* walls are composed primarily of polysaccharide (approximately 97% w/w) and some protein. The polysaccharides include pectic polysaccharides (neutral Type I 4-galactan (21%), arabinan (5%), acidic rhamnogalacturonan I (RG I, 2%) and homogalacturonan (HGA, 24%), non-cellulosic polysaccharides (heteroxylan, 3%), xyloglucan (XG, 3%) and heteromannan (1%)) and cellulose (24%). The root AIR also contains Type II AG/AGPs (5% w/w) typically associated with the plasma membrane and extracellular matrix. Thus, *P. notoginseng* roots contain polysaccharides typical of Type I primary cell walls but are distinguished by their very high levels of Type I 4-galactans and low levels of XGs. The major amino acids in the AIR were Leu (14 mol%), Asx (16 mol%), Glx (10 mol%), Ala (9 mol%), Thr (9 mol%) and Val (9 mol%).

Keywords: Index–*Panax notoginseng*; Cell walls; Composition; Polysaccharides; Protein; Medicinal plant

1. Introduction

Panax notoginseng (Burk) F. H. Chen (Araliaceae) is a well-known medicinal plant found in south-western China (Gao et al., 1996; Lam and Ng, 2001). The AIR root fraction is traditionally used as a tonic and haemostatic agent. Extensive studies have been conducted on the low-molecular-weight biologically active components, especially saponins from this herb (Yang et al., 1983). However, some polysaccharide preparations of *P. notoginseng* roots have also been shown to have immunological activity. A Type II AG with a molecular weight of 1500 kDa, capable of activating the reticuloendothelial system, was isolated from roots of *P. notoginseng* (Ohtani et al., 1987). Gao et al. (1996) fractionated and analyzed the water- and weak alkali-soluble high-molecular-weight (HMW) fraction of *P. notoginseng* roots. The fractions were shown to be composed of the sugars Gal, Glc, Man, Ara and Xyl but these were neither quantified nor analyzed for linkage composition (Gao, 1996; Gao et al., 1996). Sasaki et al. (1990) patented an anti-tumor polysaccharide fraction mainly composed of α-(1,4)-glucan (presumably starch) from roots of *P. notoginseng*.

The lack of detailed structural information on the biologically active polysaccharide fraction from the AIR root fraction was the impetus for the current study. Therefore, we performed a sequential extraction of the polysaccharide-enriched cell wall fraction (AIR) from the roots of *P. notoginseng* in order to elucidate their composition. This fraction will contain both cell wall polysaccharides and proteins as well as cytoplasmic proteins and starch granules.

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2. Results and discussion

2.1. Composition of P. notoginseng root AIR

The HMW components of roots collected as the alcohol insoluble residue (AIR) accounted for 88% w/w of *P. notoginseng* root powder on a dry weight basis. AIR had high starch content (51% w/w), which is common for storage tissues such as roots (Fry, 1988). The monosaccharide analysis of AIR (Table 1) showed it was predominantly composed of Glc (75 mol%), which is mainly derived from starch and cellulose (see below). It also had smaller amounts of Gal (11 mol%), GalA (11 mol%) and Ara (3 mol%). The relative proportions of the monosaccharides determined either as their alditol acetates (Table 1) or deduced from their partially methylated alditol acetates are similar (Table 2).

Linkage composition of AIR (Table 2) showed that it was mainly composed of 4-Glc*p* (72 mol%), indicating high amounts of starch and/or cellulose, together with a smaller content of 4-Gal*p*, representative of Type I 4-galactan, and 4-GalA*p*, typical for HGA. Small amounts of terminal- and 5-Ara*f* (2 and 1 mol%, respectively) were also present in AIR, indicating the presence of some arabinan.

AIR had a protein content of about 3% w/w (Table 3), comprising primarily of the amino acids Ala, Val, Leu, Asx (aspartic acid and/or asparagine), Glx (glutamic acid and/or glutamine) and Thr (Table 3). These amino acids are commonly found in various plant cell wall preparations (Kieliszewski et al., 1992). Although the levels of protein are typical of plant cell walls (Bacic et al., 1988) there is likely to be contamination from cytoplasmic proteins.

2.2. Sequential extraction of cell wall polymers

To analyze the composition of the cell wall-derived polysaccharides it was necessary to de-starch the preparation and to perform a sequential extraction of the walls (see Fig. 1) following established procedures (Fry, 1988). Following fractionation, the AIR fractions were analyzed by methylation analysis to determine linkage composition of the polysaccharides, and the protein composition was determined by amino acid analysis. Based on the characteristic linkage patterns established for cell wall polysaccharides (Carpita and Gibeaut, 1993; Shae et al., 1989; Sims and Bacic, 1995), the composition of *P. notoginseng* cell wall fractions was estimated from the mol% of the relevant sugar linkages (see Section 4) and are summarized in Table 4.

2.2.1. Phenol–acetic acid–water (PAW) extracts (Fr_{PAW})

The yield of Fr_{PAW} was 7% w/w. With a protein content of 29% w/w (Table 3), it accounted for the bulk of protein in AIR. The major amino acids were Ala (16 mol%), Asx (13 mol%), Gly (13 mol%) and Leu (12 mol%). Fr_{PAW} had a high proportion of Gal (71 mol%) and Ara (19 mol%), together with a small amount of GalA (6 mol%) and GlcA (4 mol%) (Table 1).

Linkage analysis showed that Fr_{PAW} had a large amount of Ara*f* (terminal) and Gal*p* residues (3-, 6- and 3,6-linked) (Table 2), typical for Type II AG/AGPs (Johnson et al., 2003). The high proportion of 3,6-Gal*p* and terminal Ara*f* residues are indicative of a highly branched core of 3,6-galactan terminating primarily in Ara*f* residues. The high content of Type II AG/AGP (76 mol%, Table 4) in Fr_{PAW} is consistent with the finding of water-extracted Type II AG/AGP fractions in *P. notoginseng* (Ohtani et al., 1987) and closely related species such as *P. ginseng* (Tomoda et al., 1993). Pectic polysaccharides were also found in the Fr_{PAW} fraction. Unbranched Type I 4-galactan (Fry, 1988) was present in Fr_{PAW} as indicated by the presence of 4-Galp residues (18 mol%). There was also a small amount of HGA (4 mol%).

Type II AG/AGPs are the most extensively studied polysaccharides of *P. notoginseng* and other *Panax* species (Gao et al., 1988; Kiyohara et al., 1994; Tomoda et al., 1993). Fr_{PAW} reacted with β-GlcY reagent, a highly sensitive reagent that specifically binds to AGPs (Willats and Knox, 2000; Yariv et al., 1967), indicating the presence of AGPs. Some Type II AGs are also reported to be associated with pectins to form pectic AGs (Yamada, 2000). Two Type II AG-containing fractions from the roots of *P. ginseng* have been found to

Table 1
Monosaccharide composition (mol%)[a] of *P. notoginseng* root AIR and fractions

Monosaccharide	AIR	Fr_{PAW}	Fr_{HW}	Fr_{SC}	Fr_{1MKOH}	Fr_{4MKOH}	Fr_{R}
Rha	tr	–	2	4	tr	2	–
Ara	3	19	5	16	7	11	–
Xyl	tr	–	–	–	38	16	–
Man	tr	–	–	–	–	tr	tr
Gal	11	71	21	51	42	47	5
Glc	75	–	2	–	2	10	88
GalA	11	6	63	26	8	14	2
GlcA	–	4	7	3	3	–	5

[a] Average of duplicate determinations; tr, trace (<0.5 mol%); –, not detected.