Mannans in primary and secondary plant cell walls†

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Abstract

A brief overview of the structure of mannans in plant cell walls and other organisms is presented. In particular, mannans, galactomannans and glucomannans in seed endosperm and vegetative tissues such as bulbs and tubers, galactoglucomannans (GGMs) in primary cell walls, and glucomannans and GGMs in secondary walls of hardwoods and softwoods are covered. Possible roles of mannans in primary plant cell walls other than as storage polysaccharides are discussed.

Keywords: galactomannans; galactoglucomannans; mannans; plant cell walls; polysaccharides


Introduction

Mannans are widely distributed among living organisms. They are found as pure mannans (homomannans), galactomannans, glucomannans and galactoglucomannans. Also, cell walls from non-plant sources such as fungi or bacteria contain mannose-based polysaccharides. However, here the mannose residues are alpha-linked, whereas in the plant cell wall, mannose residues in polysaccharides are beta-linked. In yeast cell walls, including bakers’ yeast (Saccharomyces cerevisiae) (Korn & Northcote, 1960), Kluyveromyces lactis (Raschke & Ballou, 1972) and Candida utilis (Ruszova et al., 2008), mixed linkage mannans containing (1→6) and (1→2)-linked α-mannosyl residues have been reported. Galactomannan has been isolated from cell walls of the fungus Lineolate rhizophorae (Giménez-Albian et al., 2007) and from the bacteria Rahnella aquatilis (Zdorovenko et al., 2006) with both containing (1→6) and (1→3)-linked α-mannosyl residues. Mannans from the walls of the Chinese caterpillar fungus (Cordyceps militaris) consist of a (1→4)-α-D-mannose backbone branched at O-3 with side chains of (1→4)-α-D-glucose and (1→6)-β-D-galactose residues with β-D-galactose at the terminal position (Yu et al., 2009). From the edible mushrooms, Auriculana auricula-judae, (Sone et al., 1978) and Tremella fuciformis (Kakuta et al., 1979) heteromannans have been isolated in which the backbone is (1→3)-α-D-mannan. Lichen (Thamnolia vermicularis var. subuliformis) had a galactofuranosyl oligosaccharide linked to an (1→6)-linked α-mannooligosaccharide (Omarsdottir et al., 2006). As the structures of the mannans from such organisms are generally unrelated to those of higher plant cell wall mannans, they will not be discussed further. It is noteworthy that the galactoglucomannan found in moss (Fontinalis antipyretica) has a backbone of (1→4)-linked β-mannose and glucose residues (Geddes & Wilkie,
Storage Mannans, Galactomannans and Glucomannans

Mannans, glucomannans and galactomannans occur as storage polysaccharides in the endosperm cell walls of seeds (mostly mannans and galactomannans) or in vegetative tissues such as bulbs or tubers (mostly glucomannans) (Matheson, 1990). Their principal chemical structures are shown in Figure 1.

Mannan (a homopolymer of (1→4)-linked β-D-mannosyl residues) (Figure 1a) has been found as the major component in endosperm cell walls of palm seeds such as ivory nut (Phytelephas macrocarpa), where it can make up as much as 60% of the seed (Stephen, 1982; Matheson, 1990). Ivory nut mannan is highly water-insoluble, whereas the other storage mannans, galactomannan and glucomannan, are water-soluble.

Galactomannans found in seed endosperm such as locust bean (carob) (Ceratonia siliqua) gum and guar (Cyamopsis tetragonolobus) gum are well-known for their use in food products. They are found in the endosperm walls and vacuoles of the seeds (Brennan et al., 1996). They consist of a (1→4)-linked β-mannan backbone with variable amounts of α-galactose residues linked (1→6) to mannose residues with the galactose side chains arranged to give hairy and smooth regions (Dea & Morrison, 1975). The mannose to galactose ratio is 3.5 : 1 in locust bean gum (Daas et al., 2000), while the mannose to galactose ratio in guar gum is 1.5 : 1, with the galactose side chain randomly attached throughout the polymer. Tara (Caesalpinia spinosa) gum has a mannose to galactose ratio of 3 : 1 (BeMiller, 2007) (Figure 1b). Konjac gum from corms of Amorphophallus konjac is a well known glucomannan, and is utilised in the food industry (BeMiller, 2007) (Figure 1c). It has a mannose to glucose ratio of 1.5-2.0 : 1 with the β-D-mannopyranosyl units linked (1→4) in a straight chain. The glucose sugars appear to be randomly distributed along the chain (BeMiller, 2007). For further information on storage mannans, consult Matheson (1990), Buckeridge et al. (2000), and Srivastava and Kapoor (2005).

Mannans in Primary Cell Walls

Apart from being storage polysaccharides, mannans are also structural polysaccharides in the primary plant cell wall. Although they are ubiquitous, they are usually only present in small amounts. Generally, mannose is 2 mol % or less of the dry cell wall, and in some cases it was not detected (Thimm et al., 2002). For example, ripe kiwifruit (Actinidia chinensis) cell walls contain 1.2 to 1.9 mol% depending on the tissue zone (Redgwell et al., 1990) and cabbage (Brassica oleracea) cell walls 2 mol% (Smith et al., 1998). A notable exception
is cell wall material isolated from ripe tomato which had 11.7 mol % mannose (Seymour et al., 1990). The walls of the vegetative tissue of the palms *Rhopalostylis sapida* and *Phoenix canariensis* (Carnachan & Harris, 2000) have been reported to contain small amounts (2 mol % or less) of mannose. Fern walls have also been reported to contain mannans (Bremner & Wilkie, 1971). Popper and Fry (2003) found cell walls of some liverworts and mosses to be rich in mannose while the green algae *Chara corallina* and *Ulva lactuca* contained much lower amounts. Interestingly, the β-D-mannan in green seaweed (*Codium fragile*) is found as crystalline microfibrils similar to cellulose microfibrils, therefore replacing cellulose as the principal skeletal component (Mackie & Preston, 1968).

Little work has been done on elucidating the structures of mannans in primary cell walls. This is likely to be due to a number of factors, the most obvious being the small amount of mannose present in primary cell walls (Thimm et al., 2002) and the difficulty isolating them. A GGM from kiwifruit primary cell walls has been isolated and characterised (Figure 2a) (Schröder et al., 2001). It has a mannose : glucose : galactose ratio of 3 : 3 : 2. More recently, a GGM isolated from tomato (*Lycopersicon esculentum*) peel and outer pericarp has been shown to have a significant amount of xylose, with a mannose to glucose to galactose to xylose ratio of 3 : 3 : 1 : 1 (Schröder et al., 2007).

**Mannans in Secondary Cell Walls**

It has been frequently noted that thickened cell walls contain higher levels of mannose. *Arabidopsis thaliana* stems which contain secondary cell walls were found by one group (Brown et al., 2005) to contain ~8% of the noncellulosic neutral sugars in the cell wall material as mannose, while another group found ~7 mol% of the cell wall material (CWM) excluding cellulose was mannose (Harholt et al., 2006). However, a third group (Zhong et al., 2005) reported 4.8% mannose. Sclerenchyma cell walls in *A. thaliana* contain 5% mannose along with 31% cellulose and 48% xylose (Ha et al., 2002).

![FIGURE 2: Structures of galactoglucomannan from: (a) kiwifruit primary cell walls (Schröder et al., 2001); (b) a typical GGM isolated from gymnosperm softwood, water-soluble through acetylation (Timell, 1967); and (c) a typical water-insoluble GGM isolated from gymnosperm softwood (Timell, 1967).](image-url)
Mannans in Wood

Galactoglucomannans are the major hemicellulose in softwoods (gymnosperms) followed by glucuronoxylan, whereas in hardwoods (angiosperms) glucomannans are a minor component and the major hemicellulose is glucuronoxylan (Timell, 1967). Softwoods have a range of compositions, but generally the mannose to glucose to galactose ratio is 3.5 – 4.5 : 1 : 0.5-1.1 (Willför et al., 2005a; 2008), although older literature favours a mannose to glucose to galactose ratio of 3 : 1 : 1 (Timell, 1967) (Figures 2b & c). In pine and spruce wood, GGM is not uniformly distributed in the cell walls, but is greatest near the lumen and least in the outer layer (Sjöström, 1993). The water-soluble O-acetyl-GGM from Norway spruce (Picea abies) has mannose to glucose to galactose ratio of 4 : 1 : 0.5, degree of acetylation of 0.3, with acetyl groups only at C-2 and C-3 of mannose (Willför et al., 2008). New Zealand-grown radiata pine (Pinus radiata) gave a similar ratio of 4.5 : 1 : 1.3 (Brasch, 1983) and 3.6 : 1 : 0.8 (McDonald et al., 1999) with O-acetyl groups only at the C-3 of mannose. The GGM from radiata pine contains regions of two or three contiguous glucose units as well as regions of manno-oligosaccharides (Tenkanen et al., 1997). The molecular weight of 30 to 60 kDa indicates that they are relatively small compared to other cell wall polysaccharides. The alkali-soluble GGM from the secondary cell walls of Norway spruce has been reported to have few side branches with a mannose to glucose to galactose of 33 : 8 : 1 (Capek et al., 2000) with branch points at O-6, O-3 and O-2 of mannose and O-6 and O-3 of glucose.

The ancient Chinese “fossil” conifer (Metasequoia glyptostroboides) contains distinctly less mannose than other gymnosperms and it is present in two different GGM forms (Wenda et al., 1990). One GGM has a mannose to glucose to galactose ratio of 1.5 : 1 : 1 that makes up 6.3% of the wood. The other has a mannose to glucose to galactose ratio of 5.3 : 3 : 1 that makes up 4.8% of the wood. Antibody labelling of glucomannans was observed only in the secondary walls of the differentiating tracheids of cypress (Chamaecyparis obtusa) wood (Maeda et al., 2000).

Water-soluble glucosumman from hardwoods had a mannose to glucose ratio of 2 : 1, with very small amounts of galactose, xylose and arabinose present for aspen (Populus spp.) and a mannose to glucose ratio of 2.1 – 2.4 : 1 for birch (Betula spp.) (Telemen et al., 2003). Both glucomannans are acetylated at O-2 and O-3 of mannose, with a degree of acetylation of 0.3. In the case of aspen, if it is a GGM and not a glucomannan, then the mannose to glucose to galactose ratio would be ~ 20 : 10 : 1 and in the case of birch, there would be even less galactose. Hornbeam (Carpinus betulus) wood has a glucomannan with a mannose to glucose ratio of 1.5 : 1 (Ebrigerová et al., 1972). Glucomannan makes up 5% of the secondary wall of the poplar species, with a mannose to glucose ratio of 2 : 1 (Mellerowicz et al., 2001). An alkaline-soluble GGM has also been isolated from poplar (Populus monilifera), consisting of 1–4 linked β-O-mannopyranosyl and β-O-glucopyranosyl units in the backbone distributed at random, and (1–6)-β-O-galactopyranosyl units attached to mannose and glucose with a mannose to glucose to galactose ratio of 9.7 : 4.1 : 1 with a trace of xylose (Kubačková et al., 1992). Cottonwood (Populus spp.) had 2% (by dry weight) of mannan (Puls & Schusel, 1993), equivalent to up to 5% GGM. Differences in the mannose to glucose ratio occur within a species depending on whether heartwood or sapwood was analysed, as shown in a survey of hardwoods (Willför et al., 2005b).

Interaction of Mannans with other Wall Polysaccharides

Mannans have long been known to be intimately associated with cellulose microfibrils and extensive treatment with strong alkali or other chaotropic agents is ineffective in removing them entirely from the cellulose microfibrils. They are commonly found in the cellulose residue after sequential extraction of walls. There is other evidence that mannans interact with cellulose. Ivory nut mannan was reported to be “crystallised” on cellulose microfibrils (Chanzy et al., 1978). Moreover, it has been shown the lower the number of galactose sidechains on a gua galactomannan, and the lower the acetyl groups, the higher was their affinity for bleached kraft paper (Hannukseta et al., 2002). Likewise, the lower the acetyl content, the more a glucomannan was adsorbed onto commercial cellulose (Laffend & Swenson, 1968). Neither the galactomannan locust bean gum nor xanthan gum (from the microorganism Xanthomonas campestris) will form a gel, but together they gel. Locust bean gum has been shown to adhere to microcrystalline cellulose (Mishima et al., 1998) and solid-state 13C NMR spectroscopy has confirmed that 70% of the mannosyl residues (but not the galactosyl residues) are bound to cellulose from softwood kraft pulp (Newman & Hemmingson, 1998). Schröder et al. (2004; 2006) and field reported that galactoglucomannan isolated from kiwifruit adhered to cellulose of filter paper, whereas storage glucomannans and galactomannans from seeds did not. Whitney et al. (1998) has also shown that the unsubstituted mannan portions of konjac glucosinamman and the galactomannans (locust bean gum, guar and fenugreek (Trigonella foenum-graecum) with low levels of galactose side chains interact with bacterial cellulose, as does glucomannan (mannose to glucose ratio 1.8 : 1) from beech (Fagus crenata) wood and other hemicelluloses, including xyloglucan. However, of all the hemicelluloses trialled, beech glucomannans showed the strongest affinity for bacterial cellulose, followed by xyloglucan and xylans, and with arabinoalactan having the least (Iwata et al., 1998).
Glucomannan-lignin-xylan complexes are thought to exist in spruce wood (Lawoko et al., 2005). Alternatively, mannan might associate with another hemicellulose such as xylan (Kerr & Fry, 2004; Rizk et al., 2000) and in lignified cell walls including wood, xylan interacts with lignin (Barakat et al., 2007).

This, along with other evidence, has lead to the hypothesis that like xyloglucan and xylans, the glucomannans and the GGMs can act as cross-linking tethers between cellulose microfibrils and so contribute to the three-dimensional structure of the plant cell wall. This idea received a considerable boost from recent work in which an endo-β-mannanase from tomato fruit (Schröder et al., 2004; 2006) was shown to act not only as a hydrolase but also as a transglycosylase. Here, mannans such as GGM from kiwifruit as well as glucomannans or galactomannans were cleaved and attached to tritiated mannan-derived oligosaccharides in a transglycosylase reaction analogous to the action of the transglycosylase reaction xyloglucan transglucosylase/ hydrolase (XTH) (Nishitani & Tominaga, 1992; Fry et al., 1992; Farkas et al., 1992). The role of XTH is to continuously modify the xyloglucan-cellulose network during different stages of plant development by breaking and rejoining existing or newly synthesised xyloglucan molecules to existing xyloglucan (Thompson & Fry, 2001), thereby probably restoring and refining the xyloglucan-cellulose network during developmental processes. In this manner, endo-β-mannanase could cleave and lengthen glucomannan or GGM cross-links to allow cell expansion, or simply cleave the cross-links to contribute to softening of texture that occurs on ripening. Beyond the enzyme work, the evidence to support this hypothesis is sparse.

In wood, GGMs could strengthen cell walls by acting as cross-links from cellulose microfibrils to a hemicellulose-lignin complex.

The relatively small amount of glucomannan or GGM in primary cell walls does not negate their importance as cross-linking molecules. We have calculated with mung bean (Vigna radiata) hypocotyl cell walls that only 8% of the surface of the cellulose microfibrils is coated with adhering xyloglucan (Bootten et al., 2004). Nonetheless, the walls are perfectly functional and indeed a large number of cross-links are unnecessary. The fewer cross-links, the easier it would be to modify the walls during cell expansion and fruit ripening.

Conclusions

Mannans have been studied extensively in wood and considerably less in other species but their various roles have not been fully elucidated.

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