Biotechnology and Molecular Biology Review Vol. 1 (3), pp. 76-86, September 2006
Available online at http://www.academicjournals.org/BMBR
ISSN 1538-2273 © 2006 Academic Journals

Standard Review

Cellulose-hemicellulose networks as target for *in planta* modification of the properties of natural fibres

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Accepted 7 September, 2006

Plant cell wall polysaccharides are predominant components of fibres. Natural fibres have a wide range of industrial applications, such as in paper and textile industries. Furthermore, their demand for use as bio-composites in building and automotive applications is also increasing. For the various applications, a gain of control over fibre characteristics is important. Inherent fibre characteristics are largely determined by the ratio and interactions of cellulose and hemicelluloses. Two main strategies for bioengineering fibre properties are reviewed: (i) modifying the cellulose/hemicellulose ratio (by biosynthesis or biodegradation of specific polysaccharides), and (ii) interference with cellulose-hemicellulose interactions using carbohydrate-binding modules. These *in planta* approaches may have the potential of complementing the currently used surface modification approaches for modifying fibre characteristics.

Key words: natural fibre, cellulose, hemicellulose, interactions, cell wall modification, carbohydrate binding module, cellulose synthase

Table of contents

1. Introduction

The plant cell wall is a dynamic, highly organised extracytoplasmic matrix consisting of various polysaccharides, structural proteins, and aromatic substances, which are constantly remodelled and restructured during growth and development. These complex matrices define the shape and size of individual cells within the plant body and ultimately plant

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morphology (O'Neill and York, 2003). Other divergent and vital functions provided by the cell wall include tensile strength, defending the plant against pathogens and mediating the communication with symbionts and neighbouring cells (Carpita and Gibeaut, 1993; John et al., 1997; Dhugga, 2001).

Plant cell wall polysaccharides have been receiving a lot of attention in recent years, because they constitute an important part of food and feed, and consequently they can be important in food processing. Cell wall polysaccharides are used commercially as gums, gelling agents and stabilizers (Morris and Wilde, 1997). Digestibility of feed products depends to a large extent on cell wall composition. Forage maize (*Zea mays*) is excellent roughage for ruminants because of its high energy content. The maize forage digestibility is, however, lowered by the presence of a considerable level of lignin and hemicelluloses, which create a physical and chemical barrier to digestion (Fontaine et al., 2003). Moreover, plant cell wall polysaccharides are the predominant components of fibres. Natural fibre is a thread-like material from plants, which can be used for making products such as cloth, paper, and rope. Fibre crops include cotton, hemp, flax, agave and tree species. Natural fibres have a wide range of industrial applications. Wood fibres have found application in pulp and paper industries whereas cotton, flax, hemp and agave fibres are used in the textile industries. Environmental considerations are giving room for the use of natural fibres as an alternative for synthetic polymers in industrial composites (Gustavsson et al., 2005). For the various applications, it is important to gain control over fibre characteristics, which in turn are determined by cell wall composition and interactions of wall components.

2. Polymer networks in plant cell walls

Plant cell walls can be divided into three distinct zones, the middle lamella, primary wall, and secondary wall. The primary cell wall is deposited between the plasma membrane and the middle lamella during cell growth and elongation. The secondary cell wall is added at the inner face of the primary wall after cessation of growth (Raven et al., 1992). The secondary cell wall is more important than primary wall with respect to fibre properties in that natural fibres from most fibre crops such as cotton (textile), flax (textile), and forest trees (paper) are derived from the secondary cell walls of specialized cells and tissues (Aspeborg et al. 2005; Gorshkova and Morvan, 2006, Kim and Triplett; 2001).

A number of models have been proposed for the structure and architecture of the primary cell wall (Carpita and Gibeaut, 1993; McCann and Roberts, 1994; Ha et al., 1997). These models emphasise three independent but interacting networks based on cellulose-hemicellulose (>50% dry weight), pectin (25-40% dry weight) and structural glycoproteins (1-10% dry weight). Cellulose is a linear polymer of □-1,4-linked glucose (Figure 1), with each glucose residue oriented 180˚ to its neighbour such that the polymeric repeating unit is cellobiose (Brown et al., 1996). This allows the glucan chain to adopt a flat, ribbon-like structure. Hemicelluloses are wall polysaccharides that are not solubilized by hot water, but are solubilized by aqueous alkali (O'Neill and York, 2003). They are usually branched polysaccharides, which are structurally homologous to cellulose, in that they have a backbone composed of □-1,4-linked pyranosyl residues, such as glucose, mannose, and xylose (Figure. 1). This structural similarity facilitates a strong, non-covalent association of the hemicellulose with cellulose microfibrils. Xyloglucan is the most abundant hemicellulosic polysaccharide in the primary cell walls of non-graminaceous plants (about 20% dry weight). The cellulose-xyloglucan network is thought to be the principal load-bearing element in the primary cell wall.

Xyloglucan has a 'cellulosic' backbone of □-1,4-linked glucosyl (Glc) residues. Unlike the linear cellulose, however, the backbone residues bear α-linked xylopyranose branches attached to the O6 position of glucose, which may be further substituted by galactopyranosyl residues and other monosaccharides (Figure 1). Cross-linking pectic polysaccharides predominate the middle lamella, which is located between two contiguous cells (Raven et al., 1992) and constitute the embedding matrix for the cellulose-hemicellulose network of the primary cell wall (Carpita and Gibeaut, 1993). Extensin, the main structural glycoprotein in the primary cell wall, adds rigidity and strength to the wall by cross-linking with one another (Brady et al., 1996) or with pectins (Brady et al., 1996; MacDougall et al., 2001). The pectic polysaccharides and the extensin will not be discussed further, as they are not so important with respect to fibre characteristics. Xylans are also present in some primary walls. They are the cross-linking polysaccharides in monocots (Carpita and Gibeaut, 1993); their structure will be discussed in the next section.

The secondary wall synthesis is characterised by a marked increase in the synthesis of cellulose, non-cellulosic cross-linking glycans, and lignin (Awano et al., 2002; Raven et al., 1992). It should be noted, however, that unlike the primary walls, there are no good models of the secondary walls as yet. Xylans including arabinoxylans, glucuronoxylans, and glucuronoarabinoxylans are the major hemicellulosic polysaccharide in the secondary cell wall (Ebringerova and Heinze, 2000). They are composed of a β-1,4-D-xylosyl backbone (Figure 1), which is substituted to varying extent at the O2 or O3 position of the xylosyl residues with glucuronyl (GlcA), acetyl and arabinosyl groups (O'Neill and York, 2003). Mannose-containing hemicelluloses, including (galacto)mannans and

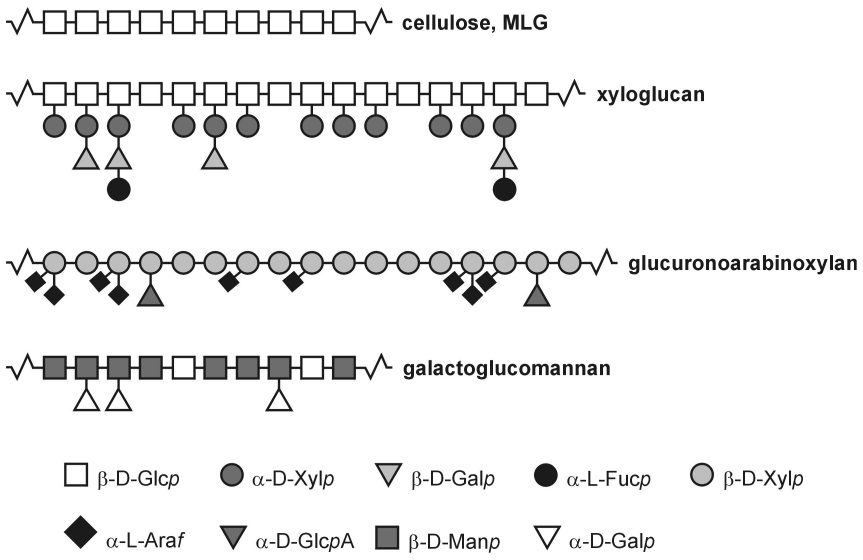

Figure 1. Schematic structures of cellulose and hemicelluloses. Like cellulose, most hemicelluloses have backbones, which are composed of β-1,4-linked-D-pyranosyl residues, such as glucose, mannose and xylose. The mixed-linkage glucan backbone is however, an exception, in that it is composed of β-D-Glc residues of which 30% are 1,3-linked and the remaining 70% are 1,4-linked.

(galacto)glucomannans, are found in considerable amounts in a variety of plant species as carbohydrate reserves. (Galacto)mannans have a β-1,4-linked D-mannosyl (Man) backbone that is substituted at the O6 position of certain Man residues (Stephen, 1982). Glucomannans are abundant in secondary cell walls of woody species; they have a backbone that contains both 1,4-linked Man and 1,4-linked Glc residues. Galactoglucomannans are particularly abundant in the primary cell walls of Solanaceous species (O'Neill and York, 2003). They have a similar backbone as the glucomannans, but some of the backbone Man residues bear single-unit galactosyl side chains at the O6 position (Stephen, 1982) (Figure 1). It should be noted that xyloglucans were also found in the thickened secondary walls of tamarind, where they occur as a reserve polysaccharides (Gidley et al., 1991).

3. Factors that can influence cellulose-hemicellulose networks

Two main approaches have been identified for the modification of the cellulose-hemicellulose networks in the plant: (i) by interfering with the biosynthesis or degradation of polysaccharide, and (ii) by directly interfering with the cellulose-hemicellulose interactions. In order to use the first approach, an understanding of the (hemi)cellulose biosynthesis is imperative. An overview of literature on (hemi)cellulose biosynthesis and its potential for fibre modification is given below, whereas the enzymic *in planta* degradation of cellulose-hemicellulose networks is only briefly discussed. Similarly, an overview of the potential of carbohydrate-binding modules (CBMs) with respect to influencing the interactions between cellulose and hemicellulose is given.

3.1. Modification of the cellulose/hemicellulose ratio

3.1.1. Biosynthesis of wall polysaccharides

3.1.1.1. Cellulose biosynthesis.

The crystalline cellulose microfibril is formed by the spontaneous association of about 36 □-D-glucan chains, which are simultaneously synthesised by a large membrane-localised complex that has been visualised by microscopy (Tsekos and Reiss, 1992). The association of the membrane complex with cellulose microfibrils as revealed by freeze-fracture electron microscopy suggested that the complexes are the sites of cellulose synthesis (Kimura et al., 1999). In vascular plants, these complexes are rosette structures with six-fold symmetry and a diameter of 24-30 nm (Mueller and Brown, 1980). The rosettes have been proposed to consist of six subunits, each of which has six catalytic subunits molded in the rosette structure (Delmer and Amor, 1995; Brown and Saxena, 2000). In addition to freeze fracture evidence, mutation studies also showed positive correlation between cellulose content and numbers of rosettes in the mutants (Kokubo et al., 1991; Arioli et al., 1998), confirming that they are the biosynthetic machinery of cellulose. However, the underlying mechanisms of rosette assembly, the precise nature of cellulose biosynthesis, as well as the full identity of the components of the cellulose synthase complex are still not well understood.

Cellulose synthase (CesA). CesA proteins are thought to catalyse the polymerisation of glucose into glucan chains, using UDP-glucose as the donor substrate. The first two plant genes for cellulose biosynthesis, GhCesA-1 and GhCesA-2, were identified by random sequencing of cDNA libraries from developing cotton fibres (Pear et al., 1996). Antibody labelling of the membrane-bound rosette with an antibody raised against the cotton CesAs demonstrated that CesA proteins are indeed members of the multiple-enzyme cellulose synthesising complex (Kimura et al., 1999; Itoh and Kimura, 2001).

The GhCesA proteins had conserved regions surrounding the D, DxD, D and QxxRW motifs, (x stands for any amino acid), previously identified in *Acetobacter xylinum* CesA (Saxena et al., 1995). This motif is presumably involved in substrate binding and catalysis, and is characteristic of processive glycosyltransferases (GTs).

From hydropathy analysis plots, it was predicted that the deduced GhCesA proteins have two trans-membrane helices in the N-terminal region and six in the C-terminal region (Delmer, 1999). The central region of the protein, comprising the D, DxD, D and QxxRW motifs plus two plant-specific insertions, is predicted to be within the cytoplasm. This is consistent with the notion that these motifs are involved in binding the substrate UDP-glc and carry out catalysis on the cytoplasmic face of the enzyme. A zinc-binding domain at the N-terminal of GhCesA was found as another plant CesA-specific motif. Mutant studies have since demonstrated the involvement of CesA proteins in cellulose biosynthesis. Earliest evidence came from the studies on two cellulose-deficient *Arabidopsis* mutants, *rsw1* (Arioli et al., 1998), and *irx3* (Turner and Somerville, 1997; Taylor et al., 1999). There is a body of evidence that indicates that at least three CesA proteins are involved in cellulose synthesis within the same cell at the same developmental stage. Three *Arabidopsis* CesA isoforms (AtCesA4, 7 and 8) have been shown to interact and constitute the subunits of the same complex that synthesises cellulose during secondary wall deposition in xylem cells (Gardiner et al., 2003; Taylor et al., 2003). Similarly, three CesA isoforms (AtCesA1, 3 and 6) have been reported to be required for cellulose synthesis in primary-walled cells of *Arabidopsis* (Fagard et al., 2000; Scheible et al., 2001; Desprez et al., 2002; Robert et al., 2004).

CesA genes are members of a large multi-gene family. In *Arabidopsis* there are up to 40 genes that bear similarity to the original CesA genes. This super-family has been divided into one family of ten 'true' CesA genes (CesA) and six families of about 30 cellulose synthase-like genes (Csl) that are less closely related (Richmond and Somerville, 2001). A web site maintained by Richmond and Somerville (http://cellwall.stanford.edu) documents sequence data for cellulose synthase and cellulose synthase-like genes in several different plant species.

Other proteins involved in cellulose biosynthesis

Apart from the CesA proteins, other proteins whose functions have been linked to cellulose biosynthesis have been identified. For instance, the role of a membrane-anchored endo-1,4-□-glucanase (KORRIGAN) in deposition of cellulose in the cell walls has been reported (Nicol et al., 1998; Lane et al., 2001). The gene of this glucanase was isolated from the *kor* mutant that causes a decrease in the content of crystalline cellulose and abnormal cell wall formation. It was demonstrated that the catalytic domain of the enzyme is located on the outside of the plasma membrane (cell wall side) (Molhoj et al., 2001).

In addition to *korrigan*, a novel membrane-associated form of sucrose synthase was proposed to be involved in cellulose synthesis, by channelling UDP-glucose to cellulose synthase in a closely coupled reaction (Amor et al., 1995). Immunolocalisation of sucrose synthase at the site of cellulose deposition in tracheary elements was demonstrated by electron microscopy (Salnikov et al., 2001). Furthermore, the involvement of an enzyme, UDP-Glc:sterol glucosyltransferase (SGT), in cellulose biosynthesis was suggested recently (Peng et al., 2002). The authors observed that digestion of noncrystalline cellulose with cellulase released not only CesA proteins, but also small amounts of a sitosterol linked to glucose (Peng et al., 2001; Peng et al., 2002). Further metabolic studies led the authors to propose a biosynthetic pathway for cellulose that starts with a SGT-mediated transfer of a glucosyl residue from the soluble cytoplasmic substrate UDP-glucose onto sitosterol to form sitosterol-□-glucoside (SG) on the inner surface of the plasma membrane. The idea is that SG, being a hydrophobic glucoside, may serve as a primer for glucan chain elongation. A link between sterol biosynthesis and cellulose synthesis has indeed been established recently, through the analysis of sterol biosynthesis mutants (Schrick, 2004).

There were reports, which indicated that modulation of cellulose content, through altered expression of the CesA proteins, could alter the cellulose-hemicellulose networks. Analyses of the various primary and secondary cell wall mutants of *Arabidopsis* revealed that some of them had severe reduction in cellulose synthesis, with consequential abnormal cell wall assembly (Turner and Somerville, 1997; Arioli et al., 1998; Fagard et al., 2000; Taylor et al., 2004). Probably a lower cellulose/hemicellulose ratio yields more amorphous cellulose fibres, whereas a higher cellulose/hemicellulose ratio might favour cellulose crystallinity, and consequently fibre properties might be altered. The impact of modifications should be further established with biophysical, biochemical and X-ray crystallographic analyses.

3.1.1.2. Hemicellulose biosynthesis.

Unlike cellulose, which is synthesized at the plasma membrane by CesA proteins, the non-cellulosic matrix polysaccharides (hemicelluloses) are produced within the Golgi by glycan synthases and glycosyltransferase (Keegstra and Raikhel, 2001). Research on hemicellulose biosynthesis is trailing behind that on cellulose. Efforts to identify enzymes mediating the biosynthesis by using biochemical purification strategies were successful for *Arabidopsis* xyloglucan fucosyltranferase (Perrin et al., 1999), galactomannan galactosyltransferase from fenugreek (Edwards et al., 1999) and xyloglucan xylosyl transferases from *Arabidopsis* (Faik et al., 2002). Mutant screens and reverse genetics strategies have also led to the identification of *Arabidopsis* xyloglucan galactosyltransferase (Madson et al., 2003). The sequence similarity between the CesA genes and the Csl genes, especially with respect to the conserved D, DxD, D and QXXRW motifs, originally suggested that they also encode processive glycosyltransferases (Cutler and Somerville, 1997). Based on this similarity, it was suggested that the backbone of hemicelluloses might be biosynthesized by Golgi-resident Csl proteins (Richmond and Somerville, 2001; Hazen et al., 2002). This hypothesis was later supported by a biochemical evidence that a CslA gene from guar encodes β-mannan synthase, which is involved in the formation of the β-1,4-mannan backbone of galactomannan (Dhugga et al., 2004). This biological function of the CslA gene was confirmed by heterologous expression of the *Arabidopsis* CslA in *Drosophila* (Liepman et al., 2005). Burton et al. (2006) recently provided additional evidence for the involvement of Csl genes in hemicellulose biosynthesis. They used comparative genomics to link a major quantitative trait locus for (1,3;1,4)-β-D-glucan content in barley grain to a cluster of cellulose synthase-like CslF genes in rice. They then expressed the rice CslF genes in *Arabidopsis* and detected β-glucan in the transgenic *Arabidopsis*, thus confirming that the rice CslF proteins are involved in (1,3;1,4)-β-D-glucan biosynthesis.

Interference with hemicellulose biosynthesis might have implications on cellulose-hemicellulose interactions. Evidence for this was provided by the following observations, made with different cellulose-hemicellulose composites produced in the *Acetobacter* model system (Whitney et al., 1995; Whitney et al., 1998; Whitney et al., 2000). It was shown with the *Acetobacter* model system that the interactions of hemicelluloses with cellulose microfibrils during cellulose biosynthesis cause cellulose to lose its crystallinity (Whitney et al., 1995; Whitney et al., 1999). Varying levels of reduction in cellulose crystallinity of the native bacterial cellulose were observed in the composites. For instance in the cellulose-xyloglucan composite, a 29% reduction was observed, whereas in cellulose-glucomannan composite, a 57% reduction was observed (Whitney et al., 1998; Whitney et al., 1999). It was also demonstrated that the composites have lower stiffness, leading to dramatic reduction in mechanical strength, for example 80% reduction in composites with xyloglucan (Whitney et al., 1999; Whitney et al., 2000). From the foregoing, a gain of control over the hemicellulose deposition has great potential to alter cellulose-hemicellulose networks. With the recent breakthroughs of identifying the genes responsible for the biosyntheses of the backbones of two hemicelluloses (mannan and mixed-linkage glucan) and the unflinching efforts to identify the genes encoding other hemicellulose synthases, research activities, which would aim at modulating hemicellulose content, should commence, in earnest.

In addition to modulating hemicellulose content, there were reports, which indicated that fibre properties could be modified by interfering with the side chain substitution of the cross-linking polysaccharide. Studies on *Arabidopsis* mutants with mutations of the MUR2 and MUR3 genes, which encode xyloglucan-specific fucosyl and galactosyl transferases, respectively, revealed that tensile strength of the fibre was enhanced by increased galactosylation of the xyloglucan (Ryden et al., 2003; Pena et al., 2004).

3.1.2. Degradation of wall polysaccharides

Another approach that can be employed to modify the cellulose-hemicellulose network is *in planta* polysaccharide degradation. Cell wall disassembly is a common feature of many developmental processes such as, fruit softening, organ abscission and dehiscence, and seed germination. These processes are characterised by marked irreversible changes in wall structure and wall strength (Rose et al., 2003). Owing to the heterogeneous nature of the plant cell wall, many endogenous wall-degrading proteins act in synergy, where one class of protein enhances the activity of the other. For instance, exo-acting glycoside hydrolases removing polysaccharide side chains might expose the polymer backbone and enhance its rapid depolymerization by endo-acting glycoside hydrolases (Rose et al., 2003). Similarly, the non-hydrolytic disruption of non-covalent polysaccharide interactions by proteins such as expansins, may also facilitate easy accessibility of a range of substrates to their enzymes (Rose et al., 2003). With a repertoire of characterized bacterial glycoside hydrolases (see web site for carbohydrate active enzymes (CAZY), http://afmb.cnrsmrs.fr/~cazy/CAZY/index.html), it might be possible to modify cellulose-hemicellulose ratio by targeting the expression of the genes encoding these enzyme activities to the cell wall. Oomen et al. (2002) reported fragmentation of the backbone of a branched pectic polysaccharide, rhamnogalacturonan (RG) I by expressing rhamnogalacturonan lyase from *Aspergillus aculeatus* in potato cell wall. Moreover, Sørensen et al.

Table 1. Summary of carbohydrate-binding modules with relevance for binding cellulosic- and hemicellulosic polysaccharides. Data on specificity are approximate, and were taken from http://afmb.cnrsmrs.fr/~cazy/CAZY/index.html, and Najmudin et al. (2006).

Module	Approx. size (aa)	Occurrence	Specificity					
			CC	AC	X	XG	M	MLG
CBM1	~40	fungi	X	X				
CBM2a	~100	bacteria	X					
CBM2b	~100	bacteria		X	X			
CBM3	~150	bacteria	X					
CBM4	~150	bacteria		X	X	X		
CBM5	~60	bacteria	X	X				
CBM6	~120	bacteria		X	X			
CBM9	~170	bacteria	X	X	X	X		
CBM10	~50	bacteria	X	X				
CBM11	180-200	bacteria		X		X		
CBM13	~150	plants			X			
CBM15	~150	bacteria			X			
CBM17	~200	bacteria		X		X		
CBM22	~160	plants			X			X
CBM27	~122	bacteria					X	
CBM28	~178	bacteria		X		X		X
CBM29	~124	fungi	X	X	X	X	X[a]	
CBM30	~174	bacteria	X					X
CBM31	~124	bacteria			X			
CBM35	~130	bacteria			X		X	
CBM36	120-130	bacteria			X			
CBM37	~100	bacteria	X	X	X			
CBM43	90-100	plants		X				X
CBM44	~150	bacteria	X	X		X	X[b]	X

Legend: CC = crystalline cellulose; AC = amorphous cellulose; X = xylan; M = mannan; XG = xyloglucan; MLG = mixed linkage □-glucan,
[a]Binds both glucomannan and galactomannan. [b]Binds only glucomannan, not galactomannan.

(2000) had earlier reported the removal of part of galactan side chains that were attached to the RG I, by expressing an endo-galactanase from *Aspergillus aculeatus* in potato.

3.2. Interference with cellulose-hemicellulose interactions

Polysaccharide-binding proteins might also modify cellulose-hemicellulose networks. Of particular interest are proteins or parts thereof, which specifically bind polysaccharides without exerting an activity towards them. In nature, numerous organisms express a range of glycoside hydrolases, esterases, and polysaccharide lyases. Cell wall polysaccharide hydrolases from aerobic micro-organisms are generally modular in structure comprising a catalytic module appended to one or more non-catalytic carbohydrate-binding modules (CBMs) (Boraston et al., 2004). However, in anaerobic bacteria the plant cell wall degradative enzymes assemble into large multi-protein complexes that bind tightly to cellulose (Bayer et al., 1998). The main function of CBMs is to attach the enzyme to the polymeric surface and thereby increase the local concentration of the enzyme, leading to more effective degradation (Bolam et al., 1998; Gill et al., 1999).

In addition to binding, some CBMs may also display functions such as substrate disruption or the sequestering and feeding of single glycan chains into the active site of the adjacent catalytic module (Din et al., 1994; Southall et al., 1999).

3.2.1. Family classification and ligand specificity of CBMs.

CBMs are divided into 45 families based on amino acid sequence similarities, details of which can be found in the regularly updated web site for carbohydrate active enzymes (CAZY), http://afmb.cnrsmrs.fr/~cazy/CAZY/index.html. The classification has predictive value for binding specificity and structure. The ligand specificity CBMs with relevance to cell wall polysaccharide binding is presented in Table 1. The CBMs exist in different sizes, ranging from 40-60 to 200 amino acids. Families 1, 5 and 10 are examples of the small CBMs while families 11 and 17 represent the large CBMs. CBMs can accommodate the heterogeneity of the plant cell wall polysaccharides (Boraston et al., 2004). For example, most CBMs that recognise cellulose, bind to both crystalline and amorphous cellulose but with differing binding affinities. It has been shown that CBMs that bind single cellulose chains can also accommodate xyloglucan side chains, such as, for instance CBM44, which bind with equal affinity to cellulose and xyloglucan (Najmudin et al. 2006). In addition to that, some of them can accommodate backbone heterogeneity through selective flexibility, as exhibited by those of the family 29, which recognises the β-1,4-linked backbone of mannose and glucose and to a lesser extent, those of xylan and xyloglucan (Table 1) (Freelove et al., 2001; Charnock et al., 2002).

3.2.2. Relationship between structure and function of the CBMs.

NMR and X-ray crystal structures have revealed that the CBMs that bind soluble polysaccharides are grooved and that the depth of the clefts varies from very shallow to being able to accommodate the entire width of a pyranose ring (Boraston et al., 2004). Examples of these CBMs include family 29 (Freelove et al., 2001), family 2b (Bolam et al., 2001) and family 22 (Charnock et al., 2000). The 3-D structures of many of these CBMs show a characteristic groove (Figure. 2A-D). Aromatic residues (Trp, Tyr) play a pivotal role in ligand binding and the orientations of these amino acids are key determinants of specificity of these CBMs (Simpson et al., 2000). Alternatively, CBMs binding insoluble crystalline cellulose have a flat surface, which enables them to attach to cellulose. CBM3 is a typical flat-surface-binding CBM (Figure. 2E). Its 3-D structure shows that the residues, which are involved in binding, are oriented in a geometry that is complementary to the flat surface of cellulose. Generally, at least two aromats are required for interaction with the target ligand, the binding of which is often reinforced by hydrogen bonding interactions between the CBM and the carbohydrate. The number of glycosyl residues bound by the CBM can be different, i.e. six for CBM2b (Simpson et al., 2000) and CBM29 (Charnock et al., 2002), and four for CBM22 (Charnock et al., 2000). It has been suggested that short and shallow grooves might better accommodate polysaccharides with side chains (Boraston et al., 2004). This is important with respect to hemicelluloses because they are often heavily branched.

3.2.3. Binding affinity of CBMs.

Some polysaccharide-degrading enzymes may possess more than one CBM, in order to facilitate increased affinity of the enzymes for the polysaccharide. This idea is supported by many binding affinity studies involving one versus two CBMs. For example, an artificial protein construct, consisting of two covalently linked family 1 CBMs, had 6-to-10-fold higher affinity for insoluble cellulose, as compared to the individual modules (Linder et al., 1996). Also, two CBMs of the family 29, CBM29-1 and CBM29-2, exist naturally in tandem as component of the *Piromyces equi* cellulase-hemicellulase complex (Freelove et al., 2001). The tandem CBM29-1-2 was shown to possess much higher binding affinity than the single CBM29-1 and CBM29-2 modules, indicating a synergy between the two single modules. Another possible role for the multiple CBMs is that they increase the diversity of polysaccharides that the parent enzyme can interact with (Gill et al., 1999). Prime example of such enzymes is the *Cellulomonas fimi* xylanase 11A, which contains two family 2b CBMs, CBM2b-1 and CBM2b-2. CBM2b-1 specifically binds to xylan while CBM2b-2 additionally binds to cellulose (Bolam et al., 2001). As for the tandem CBM29-1-2, the two family 2b CBMs were also shown to have higher affinity when incorporated into a single protein species, than when expressed as discrete entities. Another interesting CBM, CBM22 from *Clostridium thermocellum* (Charnock et al., 2000), which has affinity for xylan also exist in *Arabidopsis* xylanases in multiple copies (Henrissat et al., 2001; Suzuki et al., 2002).

3.2.4. CBMs can modify properties of composites.

In *Acetobacter xylinum*, which has long been regarded as the model system for cellulose biosynthesis, polymerisation and crystallization of cellulose are coupled processes. It was observed that interference with crystallization in the model system results in acceleration of polymerisation (Benziman et al., 1980). A number of cellulose-binding, organic substances like carboxymethyl cellulose (CMC) and fluorescent brightening agents (FBAs, e.g. calcofluor white) prevent microfibril crystallization in the *Acetobacter* model system, thereby enhancing polymerization (Haigler, 1991). These molecules bind to the polysaccharide chains immediately

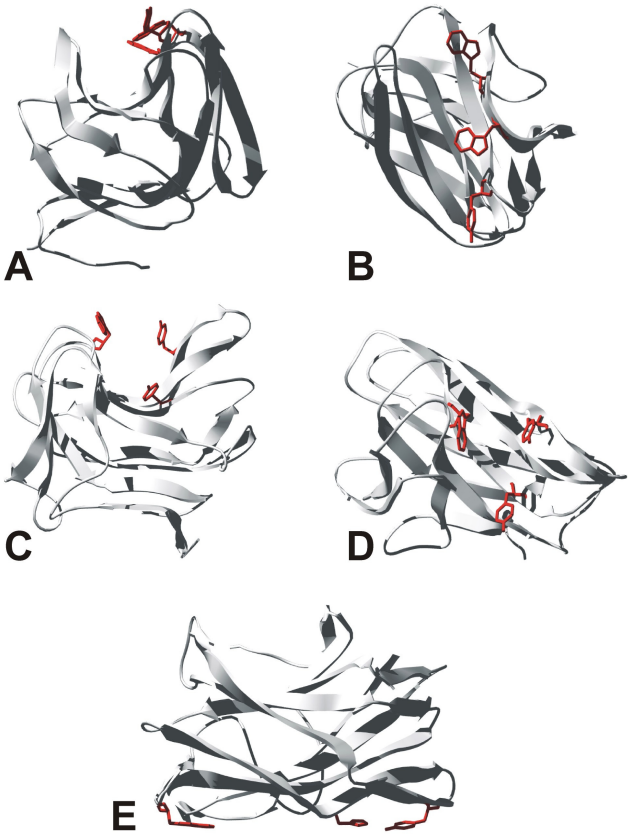

Figure 2. 3-D structures of selected CBMs that bind hemicelluloses (A-D) and crystalline cellulose (E), showing topology and residues involved in binding. Aromatic amino acids residues, which have been implicated in binding glycosyl residues, are indicated in red. The CBM29-2 (1GWK) with aromats Trp24, Trp26 and Tyr46 has a shallow groove for polysaccharide binding (A: front view; B: structure has been rotated 90˚ with the front view facing up) than CBM22-2. CBM22-2 (1DYO) with aromats Trp53, Tyr103 and Tyr134, has a less shallow polysaccharide-binding groove (C: front view; D: structure has been rotated 90˚ with the front view facing up) than CBM29-2. It can be seen that the aromats in CBM29-2 are positioned differently in the groove as compared to those in CBM22. CBM3 (1NBC) with residues His57, Tyr67 and Trp118 (E), reveal the coplanar orientation of the residues involved in binding, which is characteristic of CBMs that bind the flat surface of cellulose. 1DYO, 1GWK, and 1NBC represent the codes for the files containing the atomic coordinates for building the structural models.

after their extrusion from the cell surface, preventing normal assembly of microfibrils and cell walls (Haigler, 1991). It was also demonstrated that microbial CBMs could modulate cellulose biosynthesis, by achieving an up to 5-fold increase in the rate of biosynthesis as compared with the controls (Shpigel et al., 1998). A hypothetical model of the physico-mechanical mechanism of action has been proposed, whereby a flat-surface, cellulose-recognizing CBM slides between cellulose fibers and separates them in a wedge-like action (Levy and Shoseyov, 2002). The authors speculated that when the interaction occurs during the initial stages of crystallization, the result is increased rate of synthesis and splayed fibrils. Post-synthesis interaction results in non-hydolytic fiber disruption (Levy and Shoseyov, 2002).

Furthermore, it was indicated that CBMs could interfere with the attachment of hemicellulose to cellulose, as reported for CBM3, which competed with xyloglucan for binding sites when it was added first to *Acetobacter* cellulose (Shpigel et al., 1998). The competition decreased the amount of the xyloglucan that bound to the cellulose in the absence of CBM by about 12%. This indicates that the CBMs can be used to prevent cellulose-hemicellulose interactions in plants, leading to the production of cellulose fibres with higher crystallinity, as discussed earlier under hemicellulose biosynthesis.

In plant systems, it was shown that the elongation growth of *Arabidopsis* seedlings and peach (*Prunus persica* L.) pollen tubes could be affected *in vitro* by exogenous supply of a recombinant bacterial cellulose-recognising CBM3 (Levy et al., 2002). Furthermore, it has been shown that CBMs can modulate cell wall structure and growth of transgenic plants (Kilburn et al., 2000; Quentin, 2003); Safra-Dassa et al., 2006; Shoseyov, 2001. Introduction of the CBM3 gene from *Clostridium cellulovorans* into potato plants (Safra-Dassa et al., 2006) and poplar tree plants (Shoseyov, 2001) was reported to enhance the growth rate of the transgenic plants. A similar enhancement in plant growth was reported for transgenic plants expressing a mannan-recognising CBM27 (Kilburn et al., 2000). Most of these research activities relating to the potential use of CBMs for plant cell wall modification have been on the cellulose-specific CBMs. Using immunohistochemistry to investigate the specificity of CBMs that recognise xylan polysaccharides in cell walls, McCartney et al. (2006) showed that these CBMs display significant variation in specificity for xylans in both primary and secondary cell walls. This, together with the summary of ligand specificity in Table 1, reveals that, if one were to use CBMs for *in planta* wall modification, the choice of CBM for influencing the interaction between cellulose and hemicellulose is likely to be critical. With the detailed characterization of CBMs known to date, it is now time to investigate them more intensively in plants for modification purposes. The results so far seem very promising. By introducing a grooved CBM, which exhibits promiscuous recognition for different hemicelluloses and for cellulose, more especially in the secondary wall, evidence was obtained that grooved CBMs also can yield transgenic plants with altered wall structures and plant growth (Obembe et al., unpublished results). The ultimate goal is to provide valuable information for modification of the interactions of cellulose with non-cellulosic polysaccharides for various industrial applications, such as for instance the production of cellulose fibre with high tensile strength for textile manufacturing and the production of fibres with less attachment of lignin for paper manufacturing.

4. Concluding Remarks

A number of in-roads have been made into *in planta* modification of cell wall for enhancing cellulose fibre properties. However, at the moment the tools to do this in a deliberate manner are not in our hands as yet, but might be in the future, especially with the increasing understanding of the plant cell wall biosynthesis. It is envisaged that the strategies, when developed, can be adapted for modifying fibre properties in fibre crops like flax and hemp, with a view to achieving higher quality fibre for use in the various applications. Besides chemical derivatization, *in vitro* enzyme-mediated modification of fibres is the trend nowadays for tailoring cellulose fibres with enhanced properties for specific industrial applications (Gustavsson et al., 2004; Gustavsson et al., 2005). This approach has the advantage that plant development is not compromised. However, it has its own limitation as well, in that it cannot modify inherent fibre properties. This is only possible during cell wall polysaccharide biosynthesis, and as such, with an *in planta* approach that ensures minimal effect on plant development. Hence, both approaches might complement each other in future. The in-roads that have been made in the 2 lines of investigations (modification of cellulose/hemicellulose ratio and interference with cellulose-hemicellulose interactions) could be combined to create a sort of synergy to achieve a more specific *in planta* modification of the fibre properties.

5. Acknowledgements

The plant cell wall modification project was supported by the Netherlands Foundation for the Advancement of Tropical Research (WOTRO), the Netherlands.

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