CHAPTER 2

Overview of Lignocellulose: Structure and Chemistry

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2.1 Introduction

In the Earth's ecosystem, autotrophs are organisms that use energy and simple chemicals in the environment to synthesize complex organic compounds (biomass) that can be used by heterotrophs as an energy and carbon source. On land, higher plants are the major producers of biomass through the process of photosynthesis, in which plants capture sunlight, split water atoms, release oxygen, and use hydrogen to reduce carbon dioxide. At this point in the process, energy and carbon are stored as sugars that are often further polymerized in the plant to form polysaccharides, such as starch and cellulose. The plant cell walls contain the major material that plants produce. Within that material, many components—including polysaccharides, lignin, proteins, minerals, and others— inter-connect to form complex matrix structures. Lignocelluloses are dead plant matter which are primarily composed of cell wall material.

Lignocellulose materials have been used as an energy source since humans first learned to burn wood and dead plants for cooking and heating. Currently, lignocelluloses in agricultural and forestry residues, dedicated energy plants, and industrial wastes such as bagasse are considered to be a renewable source of transportation fuel; however, instead of simply burning them, advanced

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technology must be developed that can efficiently process these highly heterogeneous materials into fuels. The concept of biorefinery has been proposed to use sustainable technologies that can produce transportation fuels from lignocelluloses, benefiting the environment by reducing greenhouse gas emissions.[1,2] These technologies can be categorized into two pathways. The first is the biochemical pathway, which includes sequential processes, such as thermochemical pretreatment to precondition biomass feedstock, enzymatic saccharification to hydrolyze polysaccharides into simple sugars, and microbial fermentation to metabolize sugars to ethanol or other alcoholic fuels. The second pathway is the thermochemical pathway, in which heat and chemical catalysts break down the polymeric materials in the plant cell walls and produce synthetic gas or bio-oil. Scientists and engineers are now engaged in optimizing these processes, aiming to reduce operational costs, increase the yields of desirable fuels and chemicals, and achieve 'zero-waste' utilization of biomass. This chapter is intended to summarize the current research into plant-cell-wall structure and chemistry with respect to a greater understanding of the deconstruction mechanism of the biomass-to-biofuels process.

2.2 Cell Wall Biogenesis

Research into the model plant Arabidopsis (*Arabidopsis thaliana*), has suggested that an estimated 10% of the plant genome, approximately 2500 genes, has a putative function that involves cell-wall biosynthesis, modification, and metabolism. Annotation of these genes is largely based on the homologous comparison of prokaryotic sequences in other organisms. Although most of these gene functions are yet to be elucidated experimentally, the biochemical activities include the generation of substrates, polymerization, trafficking control, and cell-wall modification and rearrangement. It can be concluded that cell-wall biosynthesis is regulated in a spatially and temporally complex manner. Further studies with a systems-based approach are needed to fully understand how these chemically and structurally different polymeric materials assemble to form the dynamic structure of cell walls. In this regard, comprehensive reviews are available in the literature.[3,4] Here, I intend to give an update about how cellulose is synthesized and incorporated with other major matrix polymers, *e.g.*, hemicelluloses, pectins, and lignins. The models presented here are somewhat hypothetical and are based primarily on the observation of nano-scale imaging and analysis of the enzymatic digestibility of the plant cell walls.

Figure 2.1 illustrates the major steps of the biosynthesis of cell-wall components and their assembly. In general, cell-wall biogenesis can be described as four major processes: (1) cellulose synthesis that forming macrofibrils; (2) hemicellulose and pectin synthesis and modification; (3) macrofibril splitting when cells elongate and expand; and (4) monolignol synthesis and lignin polymerization. Cellulose (Figure 2.2) is synthesized by multi-enzyme complexes called "rosettes", which are proposed to contain 36 cellulose synthases (CesA) in each rosette that is embedded in the plasma membrane (PM). The

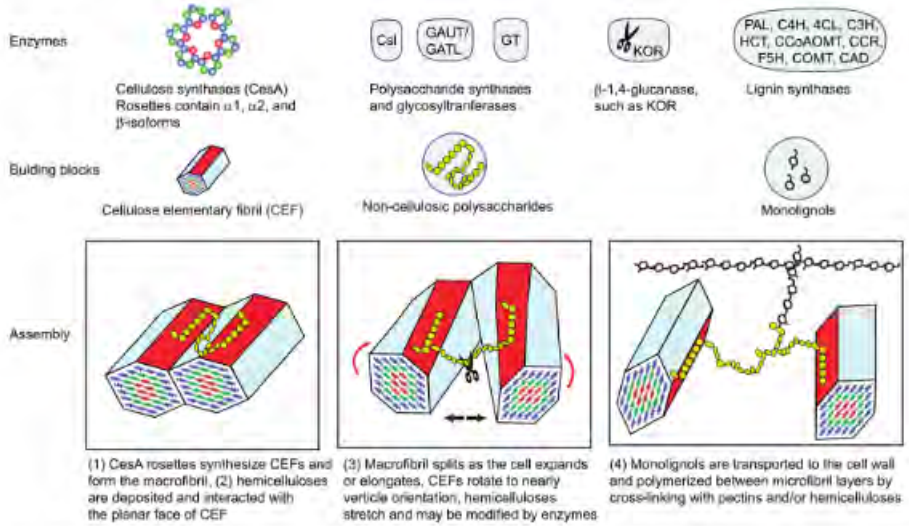

Figure 2.1 The major steps of synthesis and assembly of cell-wall polymers (*i.e.*, cellulose, hemicelluloses/pectins, and lignins). In addition to these synthases, many other proteins may also be involved in cell-wall biogenesis, such as cellulose synthase interactive protein (CSI), tracheary element differentiation-related (TED) proteins, COBRA-like proteins, chitinase-like proteins, and probably many other proteins yet to be discovered. In addition, the cytoskeleton is associated with the cellulose-synthesis process by playing a role in controlled microfibril orientation.[4]

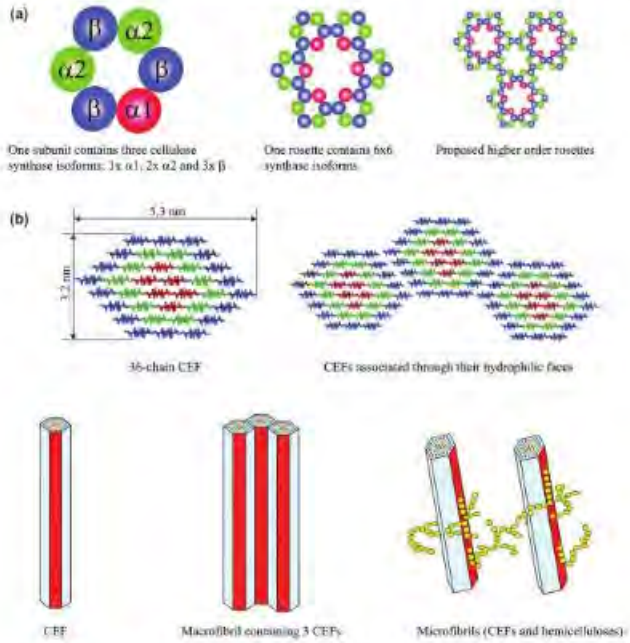

Figure 2.2 Cellulose synthase (CesA) and cellulose fibrils. (a) A 36-mer rosette contains (1) α1, (2) α2, and (3) β-CesA isoforms and a proposed higher order of rosette array. (b) Cellulose elementary fibril (CEF), macrofibril, and microfibril. A 36-chain CEF contains 18 chains (blue) on the surface, 12 chains (green) in the transition, and 6 chains (red) in the core. The chain arrangement is based on known native cellulose I.[9,10] The dimensions of hexagonal CEF are 3.2×5.3 nm.

most-accepted model of the rosette is composed of six identical subunits, in which each subunit contains (1) α1, (2) α2, and (3) β-isoforms. Each CesA enzyme synthesizes 1 linear β-1,4-linked glucan chain and 36 glucan chains form the rigid cellulose elementary fibril (CEF). Cellulose synthesis is believed to be a rapid process, and many rosettes spin CEFs simultaneously in the PM. These CEFs may initially coalesce through the hydrophilic faces to form ribbon-like bundles, called macrofibrils, which have been observed by atomic force microscopy (AFM) in fresh growing cells.[5] During cellulose synthesis, hemicelluloses are synthesized by cellulose-synthase-like (Csl) enzymes and

glycosyltransferases (GTs), and extruded from Golgi vesicles to the wall. The similarity of the backbone structure of hemicelluloses, such as xyloglucan and mixed-linkage β-1,3 or β-1,4-glucan, facilitate the initial binding between hemicelluloses and the planar face of the CEF. As the cell expands or elongates, the macrofibril splits and hemicelluloses stretch, causing the CEF to rotate to nearly vertical orientation.[6] A β-1,4-glucanase, such as KORRIGAN (KOR), which was found to play a critical role in cell elongation, may function to cleave hemicelluloses to allow further splitting of CEFs. Pectins are also synthesized in Golgi by galacturonosyltransferase (GAUT), GAUT-like (GATL), and other GTs. Hemicelluloses and pectins are modified by GTs to form cross-linked matrices that surround the CEFs.

Cells that have secondarily thickened walls are lignified. Many enzymes are involved in the synthesis of the monolignols,[7] such as the following:

- phenylalanine ammonialyase (PAL)
- cinnamate-4-hydroxylase (C4H)
- 4-coumarate : CoA ligase (4CL)
- *p*-coumarate-3-hydroxylase (C3H)
- *p*-hydroxycinnamoyl–CoA : quinate/shikimate
- *p*-hydroxycinnamoyltransferase (HCT)
- caffeoyl-CoA-*O*-methyltransferase (CCoAOMT)
- cinnamoyl-CoA-reductase (CCR)
- ferulate-5-hydroxylase (F5H)
- caffeic acid *O*-methyltransferase (COMT)
- cinnamyl alcohol dehydrogenase (CAD)

The monolignols are incorporated into highly complicated lignin polymers containing three units: guaiacyl (G), syringyl (S), and *p*-hydroxyphenyl (H). A fourth unit is the recently reported caffeyl alcohol (C).[8] Lignification occurs at the later stage of secondary wall thickening and most likely takes place between layers of CEF hemicelluloses.

2.3 The Cell Wall Structure in Biomass

Lignocellulosic biomass is primarily composed of dead plant cell walls. The wall structures are dramatically modified after maturation, senescence, and harvest. Unlike in living plants, most of the cytoplasmic components and cell membrane are degraded during the process of plant senescence. The cell walls in biomass can be generally classified as three types (Table 2.1). The primary cell wall (PW) is the cell that never undergoes secondary thickening in the wall. This type of cell can be easily identified in plant tissues; such cells are relatively small in size (*ca.* 50–100 μm in diameter) and polyhedron shaped with thin walls (~100 nm). The PWs are composed primarily of polysaccharides (~90%, mostly cellulose) and proteins (~10%). The CEFs under high-resolution AFM appear to be macrofibrils that contain a number of CEFs arranged in parallel. These CEFs have been found aligned together through their hydrophilic faces,

Table 2.1 Summary of the structural features of the primary (PW), parenchyma-type secondary (pSW), and sclerenchyma-type secondary (sSW) walls.

Wall types	*PW*	*pSW*	*sSW*
Cell size and shape	*ca.* 50–100 μm	*ca.* 200–300 μm	Varies, fibers can be as long as several mm
Cell shape	Polyhedron	Polyhedron	Fibers or tubes
Wall thickness	~100 nm	*ca.* 2–5 μm	*ca.* 5–10 μm or larger
Cell types	Non-thickened parenchyma, guard cells in stomata, companion cells in the phloem	Thickened parenchyma, collenchyma, sieve elements	Fibers, sclereids, tracheids, vessels
Surface structure	Disordered ribbon-like macrofibrils	Mixed small macrofibrils and microfibrils	Coated by a warty layer
Lignification	Non-lignified	Partially lignified	Fully lignified
Accessibility to cellulases	Fully accessible	Accessible on surface	Not accessible
Digestibility by cellulases	Fully digestible	Partially digestible	Not digestible

so that the macrofibrils appear to be structured like ribbons (Figure 2.3). Hemicelluloses are not major components in the PW, therefore the surface of the macrofibril appears fairly clean and highly accessible to the carbohydrate-binding module (CBM) that specifically recognizes the planar faces of crystalline cellulose.[6,11–15]

The second type of cell wall in biomass is the parenchyma-type secondary wall (pSW). After vegetative growth, especially in grass, the cell walls in parenchyma tissue usually are secondarily thickened to enforce the strength of the plant body. This type of cell is large (*ca.* 200–300 μm in diameter) and polyhedron shaped with thick walls (*ca.* 2–5 μm). Lignifications may also occur during cell expansion and elongation. However, the pSW may not be completely lignified after plant maturation. The surface of the pSW contains mixed small macrofibrils and mostly individual microfibrils that are composed of only one CEF with hemicelluloses associated on the surface. These microfibrils are arranged in parallel and embedded in the matrix polymer networks (*i.e.*, hemicelluloses and pectins). The CEFs appear vertically oriented as planar face-to-planar face with matrix polymer bridges between them (Figure 2.3). The surface of the pSW is partially accessible to the cellulase binding, in which the macrofibrils are fully accessible and the microfibrils are partially available because the matrix polymers block the planar face of the CEF.

The third type of cell wall is the sclerenchyma-type secondary wall (sSW), which includes fibers in the vascular bundle (VB) sheath, inter-fascicular fibers, xylem vessels and tracheids, and hypodermis sclerenchyma. This type of cell provides mechanical support and transports water and minerals in plants.

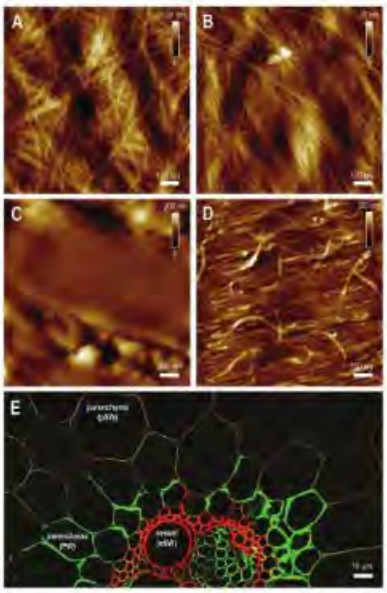

Figure 2.3 Three types of cell walls in mature maize stem. Atomic force micrographs of (a) the primary cell wall (PW) showing macrofibrils, (b) a parenchyma-type secondary cell wall (pSW) showing mixed macrofibrils and microfibrils, (c) a sclerenchyma-type secondary wall (sSW) surface showing no fibril structure, (d) a longitudinal section of the fiber wall showing microfibrils heavily coated by matrix polymers, and (e) green-fluorescence-protein-tagged CBMs showing the accessibility to different wall types, strong binding to PW, weak binding to pSW, and no binding to sSW.

The sSWs are thick (*ca.* 5–10 μm or larger) and extremely elongated—as long as several mm. The dimensions of the sSWs vary between cell types, for instance, the fiber cells are narrow (~10 μm) and the vessel cells are large (*ca.* 50–300 μm). The sSWs are completely lignified after primary growth of plants, and account for the majority of biomass, especially in woody plants. The inner surface of the sSW is further covered by a warty layer, so that the microfibril structure cannot be observed in untreated biomass. The sections of the sSW show a similar microfibril structure and arrangement as the surface of the pSW, except the diameters of the microfibril appear slightly larger, possibly because of the association of more matrix polymers.

2.4 The Cell Wall Chemistry in Biomass

Lignocellulosic biomass is composed of polysaccharides (~70% dry weight) and lignins (~25% dry weight) and a small amount of proteins and minerals. The polysaccharides are cellulose, hemicelluloses, and pectins. Lignins are a group of highly branched phenylpropanoid polymers found in lignified cell walls.

Cellulose is the most abundant biopolymer on Earth, accounting for approximately 50% of the dry weight of lignocellulosic biomass. Cellulose is one of the most important natural polymers with wide applications in our daily life, such in as pulp and paper, textiles, food, and biomedical and many other industrial materials. Recently, cellulose has also been considered to be the renewable source of glucose for the production of biofuels and other by-products.[16] Cellulose is composed of simple, linear chains of glucose residues that are linked by β-1,4-glycosidic bonds. However, the physical properties of cellulose are complex, depending on the degree of polymerization, the number of chains in a single cellulose fibril, and the inter- and intra-chain hydrogen bonding and van der Waals interactions. Based on these physicochemical properties, cellulose can form different allomorphs, such as I_α, I_β, II, III, and IV. Furthermore, the cellulose fibers in nature are associated with hemicelluloses, and are often bundled to form larger macrofibrils or aggregates.

The basic structure of native cellulose in higher plant cell walls is 36-chain CEFs. Although the crystalline structure of the CEF has not been solved, two major allomorphs of native cellulose, *i.e.*, I_α from freshwater alga *Glaucocystis nostochinearum*, and I_β from the tunicate *Halocynthia*, structures have been determined by using synchrotron and neutron diffraction techniques. Many features of fundamental structure are common between cellulose I_α and I_β. The cellulose chains lie parallel and are inter-connected by hydrogen-bond networks. The intra-chain H-bonds between a hydroxyl group and the next ring oxygen (O3–H···O5) and between hydroxyl groups (O2–H···O6) lock each glucose residue to form the flat ribbon structure of the cellulose chain. The inter-chain H-bonds between hydroxyl groups of the neighboring chain (O6–H···O3) facilitate the interaction between chains forming the cellulose sheets. The cellulose crystal is formed by stacking planar cellulose sheets through the van der Waals interaction and weak H-bonds of C–H···O between neighboring sheets. The difference between cellulose I_α and I_β is the relative position of each chain or glucose residue. In I_α, all chains are identical but alternate glucose units in each chain, whereas in I_β, two distinct kinds of chains are arranged in alternating sheets. A mostly accepted hypothesis has been proposed that these two crystalline forms of cellulose co-exist in native cellulose materials. However, other researchers have also observed disorder in plant-cell-wall CEF surface chains, which blurs the distinction between the I_α and I_β allomorphs.[17] In addition, the reported number of cellulose chains in a single CEF is inconsistent, based on the data collected using different techniques, with numbers ranging from 18 to 36 chains.[18] The CEF is normally presented as a hexagonal shape in its cross-section, containing 36 chains. The cellulose chains

arrange as 3 layers, 18 in the surface, 12 in the transition, and 3 in the core. In a mature cell, many other polysaccharides, such as hemicelluloses and pectins, are deposited in the wall and are further rearranged and modified; therefore, it is expected that the 18 surface chains are disordered to some extent.

Hemicelluloses and pectins are commonly called "matrix polysaccharides" because of their highly branched chemistry and amorphous structure. Unlike cellulose, matrix polysaccharides do not form a fibrillar structure because of the mixed linkages of glucose, such as the mixed-linkage β-1,3- and −1,4-glucans, or because of the branched structure with different glycosyl residues. Major hemicelluloses in biomass include xylan, xyloglucan, glucuronoxylan, arabinoxylan, and glucomannan. Approximately 20 different glycosyl residues are involved in hemicellulose structure. Pectins are α-1,4-linked-galacturonic acid polysaccharides, including homogalacturonans, rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II), and substituted galacturonans. Although hundreds of genes have been identified to be putatively involved in the biosynthesis of these matrix polysaccharides, resulting in complex chemical structures in the cell walls, the biodegradation of these polymers is relatively efficient compared with cellulose. Extensive reviews about the complex chemistry and the structure of hemicelluloses and pectins are available in the literature.[19]

Lignins are a group of hydrophobic polymers that contain three major units, called guaiacyl (G), syringyl (S), and *p*-hydroxyphenyl (H). The biological functions of lignin are to provide mechanical support for the cell walls and to restrict water transport through vascular tissue to ensure efficient water conduction in the plant. Lignins may also play a role in fighting off pathogen attack,[20] which is generally considered to be one of the most important limiting factors in the enzymatic cell-wall saccharification process.[6] The primary structure of lignins remains obscure; the three monolignol units (*i.e.*, G, S, and H) are polymerized *via* radical oxidation and coupling during the cell-wall lignification process.[21] The dimerization of the two subunits occurs primarily at their β-positions forming β–β, β–O4, and β–O5 covalent bonds, and the dimer is dehydrogenated and further coupled with another radicalized monomer. The hydrophobic chemistry and heterogeneity of these phenolic compounds are in fact the barriers that hinder the penetration of biocatalysts (enzymes) from accessing their polysaccharide substrates.

2.5 Conclusion and Future Directions

The cell walls in a living plant are dynamic and highly heterogeneous in chemistry, structure, and function. The complexity of such chemical and structural heterogeneity in cell walls is somewhat overstated when translated to the field of lignocellulose research. In fact, compared with the cell-wall biosynthesis that may involve at least hundreds of enzymes and proteins, the enzymes that are required to effectively degrade (solubilize) the cell walls could be as few as three. Such an example would be an endo-glucanase, an exo-glucanase, a β-glycosidase, and a limited number of hemicellulases. The synthases are highly specific in their

catalytic activities on glycosyl bonds, whereas the hydrolases are much less specific. For instance, a β-1,4-glucanase could effectively hydrolyze a β-1,4-linked glycosidic bond in any polysaccharide.

The digestibility of the cell walls in biomass by cellulolytic enzymes is strongly correlated with the cell-wall structure. In untreated biomass, the PWs are completely degradable, and there is partial and no digestibility in pSWs and sSWs, respectively. The most important difference between these three types of cell walls is lignin content; the PWs are not lignified, and the pSWs and the sSWs are partially and completely lignified, respectively. A chemical treatment that removes lignins without changing polysaccharides (*e.g.*, acid chlorite treatment at room temperature) enables complete digestion of all types of cell walls, suggesting that lignins are the important factor that hinders the enzyme accessibility to structural polysaccharides.[6]

It is now believed that the architecture of the cell-wall network plays a much more important role than the primary chemistry of these polymers with respect to biomass digestibility. A deeper understanding of primary polymer chemistry in the cell walls is important to adding industrial value to end-products produced by biomass. However, such understanding is limited in providing the knowledge needed to improve the enzymatic process of saccharification. The nano-scale microfibril networks formed by CEFs and matrix polysaccharides are highly accessible to cellulolytic enzymes, especially to the fungal-free enzymes;[6] the small physical size of these enzymes (5–10 nm) matches the porosity of the CEF network, which permits enzyme penetration inside the microfibril network and efficient digestibility. However, the enzyme accessibility to polysaccharides is hindered by the lignin layers formed between the CEF-hemicellulose lamellae in untreated biomass. Current state-of-the-art technologies for bioprocessing biomass to biofuels require a thermochemical pretreatment step. Many pretreatment approaches have been developed that aim to enhance biomass digestibility by enzymes; these approaches may affect cell-wall accessibility by directly modifying (*i.e.*, oxidizing) or delocalizing lignin. That is, the dilute acid pretreatment hydrolyzes hemicelluloses, resulting in lignin migration and aggregation at elevated temperature. Nevertheless, an optimal pretreatment method should: (1) maximize polysaccharide accessibility to enzymes; and (2) minimize modification of cell-wall architecture or sugars. Therefore, efforts to improve pretreatment should be focused on the development of chemical specificity to lignin modification at low temperature (sugars may be degraded at elevated temperature, which produces compounds that inhibit saccharification and fermentation in later steps). Effectively delocalizing lignin with chemical catalysts remains a big challenge because of the structural barriers involved in plant tissues and cell-wall architecture. One promising approach is the genetic modification of lignin biosynthesis; engineering energy plants with desirable lignin content and composition that do not affect plant growth and can be degraded with a designed chemical pretreatment upon harvest.[22–24] Finally, a systematic effort must be considered that coordinates improvement of the cocktail of cellulolytic enzymes, fermentation strain development, and product marketing.

Acknowledgements

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