

Molecular Structure and Component Integration of Secondary Cell Walls in Plants

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A model of the molecular structure for the arrangement of components in secondary walls of plant cells is presented.

INTRODUCTION

The plant cell wall (CW), a cell organelle (1), provides mechanical strength, maintains cell shape, controls cell expansion, regulates transport, provides protection, functions in signalling processes, and stores food reserves (2). The integration of these functions into the molecular structure of the CW has not been well understood.

Artistic representations of primary CWs have been developed (2-4), but complexity and variability in secondary CW structure have obstructed conceptualization of component arrangement in the secondary wall matrix. The relevance of secondary CW structure to agronomy, animal science, forestry, plant pathology, molecular biology, and other sciences has impelled many investigators to itemize, quantify, and structurally analyze components of the secondary CW structure. These investigators have accumulated enough knowledge to construct preliminary models of secondary CW structure. This investigation pieces together that knowledge for a preliminary artistic representation of a plant secondary CW.

The secondary CW description and drawing presented in this paper were generated from knowledge of generalized plant primary CW structure, grass secondary CW structure, and wood lignin structure. Information was compiled with a focus on secondary CWs and the arrangement of lignin with other CW components. The diagram constructed from this information will enable teachers and researchers to better understand, criticize, and modify current views of plant secondary CW structure. The accompanying text reviews primary CW structure, summarizes current understanding of secondary structure, and integrates this knowledge to present a hypothetical model of a "representative" secondary plant CW.

PRIMARY CELL-WALL STRUCTURE IN PLANTS

Typical primary plant CWs are composed of cellulose microfibrils (9-25%) and an interpenetrating matrix of hemicelluloses (25-50%), pectins (10-35%) and proteins (10%) (5-7). Albersheim (3) and coworkers (8-10) describe the primary CW composition as cellulose fibers bound together by molecules made of sugar units. Approximately 90% of the CW consists of carbohydrates (mostly pentose and hexose units) and the remaining 10% is protein (3). Cellulose forms the framework of the CW (5) while hemicelluloses cross-link non-cellulosic and cellulosic polymers (9). Pectins provide cross-links and structural support to the CW (11,12) whereas proteins can function either structurally (extensin) or enzymatically (6).

Cellulose is composed of approximately 8×10^3 D-glucopyranose residues linked by $\beta 1 \rightarrow 4$ glycosidic bonds (6). Hydrogen bonds hold about 40 of these glycan chains together to form a cellulose microfibril (3). Cellulose microfibril arrangement in the primary wall is random (5). Cellulose microfibrils are linked to hemicellulosic polysaccharide composed of xylans, mannans, galactans, or combinations thereof. In one example, xyloglucans attach to cellulose microfibrils through hydrogen bonds and glycosidically bond to arabinogalactans which, in turn, link to rhamnogalacturans that bond back to cellulose via arabinan-galactan junctions (3). Cross links made of pectin (based on $\alpha 1 \rightarrow 4$ -linked-polygalacturonan) and extensin (a glycoprotein rich in hydroxyproline) enable support and extension, respectively, of the CW (6,11,12). Proteins other than extensin found in the CW include enzymes such as the cellulose synthases (13,14), hydrolases (15), and oxidases (6) needed for CW thickening, modification,

and lignification, respectively, during secondary growth.

Reorganization, *de novo* synthesis, and insertion of new wall polymers lead to rearrangement of the CW during cell growth (16). This enables inclusion of lignin into the wall and strengthening of the CW matrix. In forage legumes and especially grasses, the order of maximum CW component deposition is hemicellulose, followed by that of cellulose (1 to 6 days later), and then lignin (up to 14 days after maximum hemicellulose deposition) (17). As a definition, secondary walls are derived from primary walls by thickening and inclusion of lignin into the CW matrix (18) and occur inside the primary wall.

SECONDARY CELL-WALL STRUCTURE IN PLANTS

Secondary CWs of plants contain cellulose (40-80%), hemicellulose (10-40%) and lignin (5-25%), (7,19). The arrangement of these components allows cellulose microfibrils to be embedded in lignin, much as steel rods are embedded in concrete to form prestressed concrete (7). In wood, three layers of secondary CW, referred to as the S₁, S₂, and S₃ lamellae, result from different arrangement of the microfibrils (6). The first-formed (outermost) S₁ lamella has both left- and right-handed microfibril helices; the S₂ and S₃ (innermost) lamellae each have one helix of microfibrils; the S₂ and S₃ helices have opposite handedness. During secondary CW formation, lignification occurs in the S₁ and S₂ lamellae as well as in the primary wall and middle lamella. Lignification rarely occurs in the S₃ lamella (6).

Cellulose and hemicellulose appear to be more structurally organized in the secondary CW than in the primary CW (16). Primary wall changes that occur during maturation of the plant cell include loss of water from the CW matrix (20) and alterations /replacements that decrease polysaccharide branching in primary CWs (11,21,22). Hydrolysis of load-bearing polysaccharide may occur in the CW to enable expansion of the cell (23), sometimes resulting in transient loss of specific polysaccharides (16). Enzymes such as β -glucanases can be secreted to selectively degrade polysaccharides and thereby decrease their concentrations in the CW matrix (21). These modification, which set the stage for secondary CW formation, result in a tighter and more rigid structure. Inclusion of lignin adds further rigidity to the matrix.

Lignin monomers originate from the action of phenylalanine ammonia lyase (PAL, in legumes and grasses), tyrosine ammonia lyase (TAL, in grasses only) and other phenylpropanoid-related enzymes directing metabolites to, among other things, lignin biosynthesis (6,15,19,24,25). The shikimic acid pathway and phenylpropanoid metabolism lead to synthesis of the following lignin monomers: *p*-coumaric acid (*p*CA), ferulic acid (FA), diferulic acid (DFA), sinapic acid (SA), cinnamic acid (CA), and *p*-hydroxybenzoic acid (BA). Enzymes subsequently catalyze the formation of three alcohols — *p*-coumaryl, coniferyl, and sinapyl — which interact and polymerize to form lignin in the secondary CW.

Control and development of the CW during plant growth is not completely understood. Current knowledge suggests that cellulose is formed by plasmalemma enzymes, matrix polysaccharides and proteins in the cytomembrane system, and lignin within the CW (26,27). Lignification, which accompanies secondary CW formation, arises from generation of free radicals that react spontaneously to form lignin and even some linkages to wall polysaccharides (2). Free radical linkages between lignin monomers and polysaccharides may produce what is referred to as non-core lignin while polymerization of monomeric free radicals results in highly condensed core lignin.

Cellulose is hydrogen-bonded to hemicellulose (3) whereas ester and ether bonds connect hemicellulose to non-core lignin in secondary CWs (28). The diverse and complex nature of lignin monomers and hemicellulosic moieties in ligno-hemicellulosic bonds make stereotypic conceptualizations of secondary wall structures for all plants extremely difficult. For this reason, discussion of secondary CW Structure is hereafter confined to grasses (family Gramineae).

Research, directed mostly towards the understanding of ligno-hemicellulosic linkages in grasses, has been performed on Italian ryegrass (*Lolium multiflorum* Lam.)

(29), perennial ryegrass (*Lolium perenne* L.) (29,30), sugar cane (*Saccharum officinarum* L.) (31), wheat (*Triticum aestivum* L.) (32), and barley (*Hordeum vulgare* L.) (33). These and other studies (34) have shown that an ester bond connecting arabinose to (non-core) lignin is the major ligno-hemicellulosic linkage in plant secondary CWs. Ferulic and ρ -coumaric acids are major non-core lignin monomers that link hemicellulose and core lignin (28) although diferulic, sinapic, cinnamic, and ρ -hydroxybenzoic acid constituents can also be found. Each of these "cinnamic acids," named as such because they were derived from *trans*-cinnamic acid in phenylpropanoid metabolism, is uniquely bound between hemicellulose and core lignin in the secondary CW matrix.

MODEL OF SECONDARY CELL WALL IN GRASSES

Representative molecular arrangement and bonding among secondary CW components in grasses are depicted in Figure 1. Relative quantities of secondary CW components in this representative grass are 45-60% cellulose, 20-40% hemicellulose, and 5-10% lignin (19). Glucan chains of cellulose, shown as long, ribbon-like fibers, are held together by hydrogen bonds. Cellulose microfibrils consist of approximately 40 of these bonded chains. Three or four glucan chains occur for each β 1 \rightarrow 4-D-xylopyranose chain of hemicellulose with an occasional L-arabinofuranose molecule linked α 1 \rightarrow 3 to the xylopyranose chain. The hemicellulosic xylans and arabinoxylans, represented by the actual molecules or as long, triangular rods, are hydrogen-bonded to cellulose and ester- or ether-bonded to non-core lignin.

In the figure, lignin is shown as an embedding matrix of polymerized lignin monomers. Two types of lignin, namely core and non-core, are encountered. The non-core portion of lignin binds to the hemicellulosic fraction of the secondary CW and the core lignin forms an amorphous matrix. A representative portion of core lignin is presented in the upper right-hand corner of the figure and individual non-core components are shown in molecular form bound to hemicellulose.

Ester bonds between hemicellulose and non-core lignin shown in Figure 1 include: linkage between the O-5 position of arabinose in arabinoxylan and ρ -coumaric, ferulic, and diferulic acids (32,33), as well as a hypothetical linkage between the O-3 position of xylose and cinnamic acid. Some of these lignin monomers, such as ferulic acid (38,33), may be so intimately associated with the hemicellulosic fraction that they fail to cross-link to lignin.

Linkages between non-core and core lignin are demonstrated by both ester and ether bonds in the figure. The predominant ester linkage between non-core and core lignin in secondary CWs is encountered with ρ -coumaric acid, which bonds via its own carboxyl group (33,35) or requires contribution of a carboxyl group from the core (28). Other ester bonds linking core to non-core lignin include those between the core and diferulic (32) and sinapic acids (35,36). Ether linkages between core and non-core lignin are encountered with ferulic, ρ -coumaric, and cinnamic acids (32,36) as well as ρ -hydroxybenzoic acid (36). In the case of ρ -coumaric, sinapic, p -hydroxybenzoic acids (as shown in Figure 1 for BA - and indeed it must be difunctional to take part in polymerization), linkages may exclude involvement of the hemicellulosic fraction.

This figure provides a foundation for a better understanding of secondary CW structure and function.

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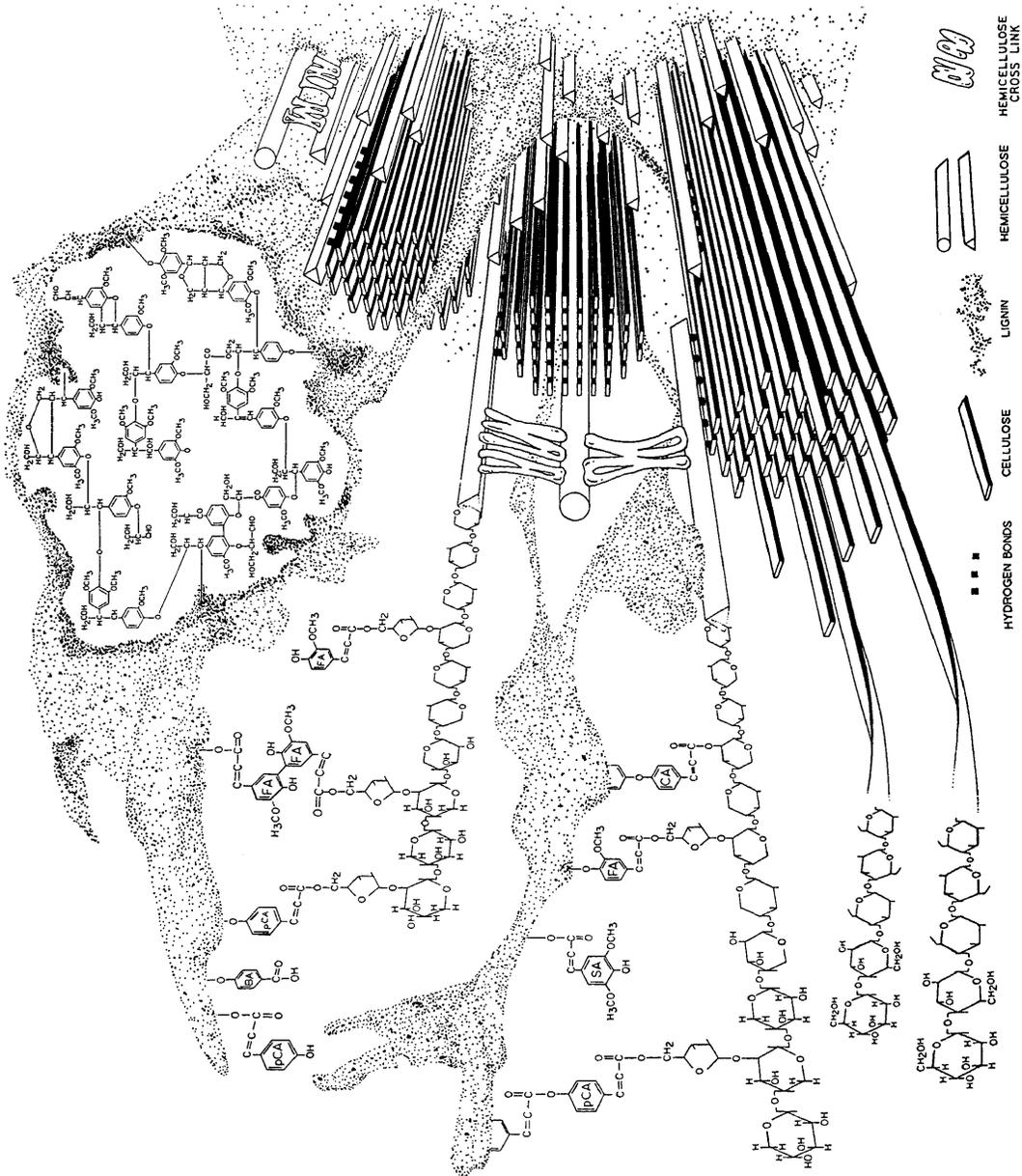


Figure 1. Secondary cell-wall (CW) structure. Components are arranged so that the cellulose microfibrils and hemicellulosic chains are embedded in lignin. Specific linkages and components of non-core lignin are shown for a generalized grass secondary CW. Non-core lignin components include *p*-coumaric (*pCA*), ferulic (*FA*), *p*-hydroxybenzoic (*BA*), sinapic (*SA*), and cinnamic (*CA*) acids.

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