Chapter 13

Cellulose and the Hemicelluloses: Patterns for the Assembly of Lignin

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The results of our most recent studies point to significant coupling between the tertiray structures of all three major constituents of the cell wall in native woody tissue. It is clear that the hemicelluloses are intitmately involved in the aggregation of cellulose, and the hemicelluloses, together with cellulose, provide the pattern for assembly of the lignin. Our studies of the organization of lignin in native woody tissue, including Raman microprobe spectral analyses and measurements of photoconductivity and fluorescence, leave little question that the level of order in lignin is well beyond that expected from random free radical coupling in the absence of a stronger organizing influence We believe that a dominant organizing influence is provided by strong associative interactions between lignin precursors and the polysaccharide matrix and that the hemicelluloses in particular play a central role in the selective binding of specific lignin precursors. In this context the hemicelluloses are viewed as carriers of an important component of the information needed for the organization of lignin. The binding of the lignin precursors prior to their polymerization is expected to be one of the fundamental organizing influences that determines the primary structure of lignin. The precursors that are expected to participate in this include, in addition to the monolignols, a number of oligomers of lignin which are formed by membrane bound enzymes. Our proposal concerning the role of the hemicelluloses in the assembly of the cell wail suggests, for the first time, direct pathways for genotypic control of the assembly of lignin. Thus, variations in the structures of the hemicelluloses, the biosynthesis of which is controlled within the Golgi apparatus, are expected to result in corresponding systematic variations in the structure of the associated lignin. In consequent, it would appear that any effort to modify the assembly of lignin requires an orchestrated modificationof the pathways for biosynthesis of the hemicelluloses, together with any programmed changes in the phenylpropartoid pathways for synthesis of mono- and oligolignols. Finally, our emerging view of the complexity and dynamic character of signification in the developing cell wall matrix

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leads us to the view that the wall of the developing cell is a very active participant in both intracellular and extracellular processes.

The results of our studies of molecular organization in wood cell walls point to significant coupling between the tertiary structures of all three major constituents of the wall in native tissues. It is clear that the hemicelluloses are intimately involved in the aggregation of cellulose, and the hemicelluloses, together with cellulose, have a significant influence on the structure of lignin. Yet these patterns of correlation in organization at the nanoscale level are not addressed within the framework of the paradigms associated with the biogenesis of plant cell walls; the nanoscalc level here is taken to encompass domaim in the range of 1 to 10 nm. The purpose of this chapter is to examine those aspects of structure that cannot be rationalized within the framework of established paradigms, paying particular attention to key observations concerning the organization of lignin and the regulation of its assembly at the nanoscale level Some proposals concerning relationships between the structure of lignin and the organization of the polysaccharide matrix are presented which can account for the diversity of molecular organization observed in cell walls, and allow for the regulation of details of structure by the organism beyond any heretofore envisioned. Such regulation of these structural details is more consistent with the general patterns prevailing in biological systems and our hypotheses also provides a more appropiate framework for any current or future efforts to accomplish genetic modifications of woody species

Historical Context

The prevailing paradigms with respect to assembly and molecular organization of the cell wall were established well over three decades ago, before many of the studies addressing both the structure and processes of assembly of cell wall constituents were even undertaken. The paradigms are based on a two phase model with the underlying premise that cellulose is the key structural component, and that lignin and the hemicelluloses are the matrix in which the cellulose is embedded *(1, 2).* In this context, lignin is usually regarded as the binding component, and the hemicelluloses are frequently seen as coupling agents between the lignin and the cellulose. This view has evolved from (i) early assimilation into the models of the cell wall, and the biosynthetic processes leading to its formation, as well as (ii) of many notions that have their origins within the branch of materials science dealing with artificial fiber/matrix composites. It also reflects the premise that each of the constituents is synthesized separately at a different location within the cell, with the polymerization of lignin during the final Consolidation of the cell Wail structure being viewed as the analog of the curing of the matrix polymer in artificial composites.

While for a number of decades the accepted model has been a useful one, it has serious shortcomings from both physical and biological perspectives Perhaps the most serious flaw, from a biological perspective, is that the model has no provision for coordination of the deposition of the three different major constituents of the wall, nor does it afford plausible approaches for genetic control of cell wall organization to the degree suggested by phenotypic expression.

A number of recent developments have raised additional questions about the validity of the, model of the cell wall from a physical perspective as well. These questions arise because the components of the cell wall are intimately integreated at a finer scale than is the case with synthetic composites. They are, in fact, integrated at a level approaching that of individual macromolecules, both in terms of the final structure and during its assembly. In addition, there is evidence of couping in the assembly processes at intermediate stages preceding final consolidation.

Need for a New Model

From a physical perspective, the considerations which point to the need for re-examination of the accepted model are associated with scale. In most synthetic composites the amount of material at the surface is small compared to that within the boundary of an individual domain. The properties of the composites are then dominated by bulk properties of the components, with any modiftcatiom arising from the relatively weak (in terms of its contribution to the overal structure) coupling at the interfaces between the domains. In higher plant cell walls, in contrast, the cellulosic domains usually arc such that more of the material is at the surface than in the bulk. For all other constituents, the domains are even smaller than they arc for cellulose. Thus, in these nanoscale composites, the coupling interactions at the interfaces between the domains may well be the dominant determinants of the properties of the aggregates. Or, indeed, the integration of the constituents at the nanoscale level may not even admit an explicit definition of an interface. That this is not simply an abstract matter is well demonstrated by recent observations of the effects of hemicelluloses on the aggregation of cellulose, as well as the long established effect of polysaccharide matrices on the structures of dehydrogenation polymers (DHPs) of lignin precursors. In the studies of the effects of hemicelluloses, it was shown that, when nascent cellulose produced by *Acetobacter xylinum* aggregates in the presence of hemicelluloses, its structure is very similar to higher plant celluloses, suggesting that one of the functions of the hemicelluloses is the regulation of cellulose aggregation *(3, 4)*. The effects of polysaccharide matrices on the patterns of assembly of monolignols in dehydrogenation polymers also point to a clear influence of microenvironment and association on the primary structures and interunit linkages of the resulting polylignols*(5-7)*.

The considerations arising from a biological perspective arc perhaps even more compelling because they arc associated with regulation of molecular organization in the cell wall matrix. Current models of biosynthesis implicitly incorporate the premise that the primary structures of the constituents of cell wall polysaccharides are determined by intracellular or membrane bound biosynthetic activities *(8, 9)*, while the primary structure of lignin is defined by a random free radical coupling reaction that occurs extracellularly, *i.e.* outside of the plasma membrane. Thus, the level of control exercised over the primary structure of the polysaccharides seems to far exceed the level exercised over the structure of lignin. Under these circumstances, lignin would seem to be exceptional among constituents of biological tissues. The final structure of lignin, it would follow, would be determined by the kinetics of the various possible reactions between the different free radicals, with the regulation of the assembly process occurring only through the influence of microenvironment on the kinetics. When one considers the degree to which the details of biological structures much more complex than lignin are under precise program control of the cell through a hierarchy of biochemical processes *(10)*, free radical polymerization with its susceptibility perturbation of kinetics and hence, structure by the microenvironment seems quite primitive in contrast An alternative model for the assembly of the lignin, one wherein the specific details of structure are under more direct control of the cell, would seem to be more consistent with the pattern of assembly of structural tissues in biological systems in general. It would also be in order to incorporate into any new model a possible degree of distribution in the processes of primary structure formation for the different constituents, rather than confining consideration to mechanisms whereby each of the macromolecular components is assembled in final form at one single location. Such a model could provide a more effective basis for organizing the information concerning the diversity of structures known to occur in native lignins.

A Comprehensive Model for the Assembly of Lignin

The more comprehensive model proposal here has grown from the realization that for many cell wall constituents, the control and regulation of structure must be hierarchic so that it occurs at multiple levels, in different stages and at different locations. Thus,

while the major component steps in the formation of the primary structures are carried out within the cell by membrane-bound enzymes in different biosynthetic apparatuses, at the cell surface or in its interior, further modification appears to be necessary prior to assembly into the cell wall matrix. At the first level, the primary structures are formed and optimized for volubility and freedom of movement within the cytoplasm to prevent premature aggregation or irreversible associations. Upon diffusion out through the cell membrane, or deposition by exo-cytosis, some further modification by extracellular or cell membrane-bound enzymes would then be necessary to impart to the constituents the characteristics prerequisite for participation in the processes of the assembly, integration and consolidation of the matrix. For some constituents, particularly the polysaccharides, the assembly and consolidation involve entry into tightly bound insoluble structural aggregates that are the key to the mechanical stability of the polysaccharide matrix. For other constituents such as the precursors of lignin, integration into the matrix requires participation in additional coupling reactions.

Among the polysaccharides the instances that are most clearly illustrative and well established are those of mannans and glucomannans in the secondary walls of gymnosperms and of xylans in the secondary walls of angiosperms. These are polysaccharides that are just as insoluble as cellulose even in mildly alkaline aqueous media. Their volubility within the cytoplasm must, therefore, be attributed to initial biosynthesis with limited branching to prevent aggregation. The complementary steps prior to assembly into the extracellular polysaccharide matrix would be enzymatic removal of the branching after release outside the cell membrane. The limited branching is seen as essential to prevent crystallization within the cell boundaries, while the de-branching is seen as prerequisite for tight aggregation with cellulose in the cell wall. The availability of a wide array of glycosidases capable of shortening or removing the branching in the extracellular region adjacent to the plasma membrane is well established.

Though this chapter is not perhaps the best context for presenting it in detail, a similar argument can be made for synthesis of cellulose in higher plants within an internally located biosynthetic apparatus, followed by limited debranching at the sites of deposition from the cell membrane with concomitant organization of the molecules into fibrillar bundles. For such a working hypothesis, the membrane bound complexes which have been visualized as sites of cellulose biosynthesis would instead be viewed as the loci of an enzyme system that coordinates aggregation of the fibrillar bundles simultaneously with the debranching. Two decades ago, the isolation of a "soluble cellulose" was reported It was later found to have a glucose unit attached as a branch residue at the 2 carbon on every third anhydroglucose unit *(11);* most traditional tests for the presence of cellulose cannot discern the presence of these soluble gluco-glycans. A more comprehensive discussion of the issues of assembly of cellulose will be presented elsewhere.

The assembly of lignin represents an even more compelling example of distributed processes or stages, separated in time and space, that result in the biogenesis of a key cell wall constituent. Thus, many of the monolignol precursors of lignin are produced within the cytoplasm as the glucosides, which, upon transmission to thle exterior of the membrane, are hydrolyzed by glucosidases to release the primary precursor *(12, 13).* This view is further reinforced by recent direct evidence for the occurrence, in Dignifying tissues, of glucosidases that are specific to the monolignol glucosides *(14, 15).* The interior biosynthetic apparatus would be viewed as producing an intermediate structure optimized for the environment defined by the cytoplasm, the monolignol glucosides have a high degree of volubility in aqueous media and should thus be well solvated in the cytosol. Upon secretion into the extracellular environment, the hydrophilicity of the precursors is altered by removal of the glucose to enhance the affinity of the monolignol for the polysaccharide aggregates. In addition to facilitating diffusion into, and association with, the polysaccharide matrix, the monolignol is now more susceptible to the free radical coupling or propagation reactions leading to the formation of lignin.

Another clear instance of distributed assembly is the formation of different dilignols by extracellular or membrane bound enzymes, as recently established by Lewis and coworkers *(16-19)*. Though it will be further suggested below that this may also be an essential intermediate step in the biosynthesis of lignin, it is presented here as another example of the distribution of the formation of primary structure between interior biosynthetic apparatus and enzyme systems bound to the cell membrane or released in its immediate vicinity. Thus, here again, the interior apparatus would be viewed as producing an intermediate structure optimized for the environment defined by the cytoplasm, while the further modification would be regarded as the second phase of control of the biosynthetic process in which structures are further elaborated in response to the activity of the membrane bound enzymes.

This proposal concerning stages in the assembly of lignin exemplifies the notion of distributed assembly, which is so often characteristic of the formation of structure in biological systems. Such assembly systems afford the possibility of sequential control of the stages of synthesis and the level of coupling of the synthetic processes for polysaccharides and lignin that is reflected in cell wall matrix organization. Coupling of the processes would admit much more precise orchestration of the assembly of the cell wall than allowed in current models

Before discussion of the mechanisms of such a coupling, however, it is important to review evidence of association between the polysaccharides and lignin. The reports *(5-7)* noted above, which indicate an influence of polysaccharide matrices on the primary structures of DHPs formed within them, point to a strong associative interaction between the polysaccharides and the lignin precursors. At the least, the interaction appears to organize the monolignols in a manner which results in a DHP that is different from the DHP arising under otherwise identical conditions but in the absence of the pdysaccharides. That subtle differences in the polysaccharides or in the conditions of polymerization can result in significant differences in the DHPs argues for a strong associative interaction *(20)*. More recently the work of Grabber *et al.* provided additional support for this view *(21, 22)*.

Two other studies undertaken in our laboratory also argue for strong associative interactions. Theoretical modeling analyses of the interactions of mono-, di- and trilignols with the surface of a cellulosic aggregate point to rapid adsorption of the lignol from an aqueous medium onto the surface of the cellulosic *(23)*. These results leave little question that at the nanoscale level, the dominant interactions between oligolignols and polysaccharides are associative. Perhaps more compelling, because they suggest coherence on an even larger scale, are the observations of photoconductivity in woody tissues *(24)*. The measurements of photoconductivity, together with complementary analyses of the fluorescence spectra of the same tissues, point to a high degree of spatial organization of lignin in the native state. The photoconductivity also points to the existence of pathways for electron transport that have not heretofore been recognized as an inherent characteristic of the structures of native lignins. The level of organization suggested by these observations is not at all consistent with the type of structure anticipated from random free radical coupling reactions. Additional evidence pointing to the organizing influence of the cell wall matrix on the assembly of lignin may be derived from the work of Sarkanen and coworkers who observed that the presence of lignin during the polymerization of monolignols modified the course of the polymerization reaction *(25)*.

The final piece of information pointing to the probability of a high degree of coupling in the assembly of cell wall constituents, is the observation that branches in hemicelluloses are specific in type, and are rarely more than three sugar units in length, and more commonly one or two units in length. This needs to be considered in light of the observatiom mentioned earlier that the hemmicelluloses are integrated into the structure of cellulose fibrils during aggregation *(3, 4)*.

When taken together, the different groups of observations suggest the plausibility of a paradigm wherein one of the key roles of the polysaccharides is to organize the structure of the lignin. Cellulose would provide the primary framework, and the hemicelluloses, integrated into its structure or adsorbed onto its surface, would have the

various branches available to associate with specific lignin precursors. In this scheme it would be anticipated that the polysaccharide backbones and specific monosaccharide branches are designed to organize the monolignols, while disaccharide and trisaccharide branches are designed to selectively bind specific dilignols or trilignols. The attribution of such selectivity to sites for association on the polysaccharides and their oligosaccharide branches is quite consistent with their well established role in many biological recognition mechanisms. It is suggested that these associations may well be as specific as the nucleotide base pairings that play such an essential role in the ribosomal assembly of proteins. Completion of the assembly process would then be expected to proceed by a polymerization process governed by free radical propagation in addition to free radical coupling.

What is proposed here is a role for the hemicelluloses well byond that of regulating aggregation of cellulose, as was proposed earlier *(3, 4)*. In the present context the hemicelluloses are viewed as performing an even more essential function with respect to lignin. This is an organizing function not unlike that of the messenger RNA in the assembly of enzymes or other proteins *(10)*, though the same absolute precision in control of the assembly process may not be required. Such an assembly process for lignin would, however, provide for control of the primary structure of lignin by a process not as susceptible to pertubation by macroscale variations in thermal and environmental conditions as would be expected in a polymerization reaction wherein the coupling mode kinetics and the microenvironment are the dominant determinants of primary structure.

Implications and Conclusions

Though the model presented here represents a significant departure from accepted views concerning the biogenesis of lignin, it provides a plausible rationale for patterns of structure distribution in native lignins. It also suggests an avenue for intracellular regulation of the assembly processes for lignin that is more in keeping with the hierarchic and sequential control of biochemical steps that is characteristic of biological systems *(26)*. That such a level of regulation is probable is evidenced by the uniformity of the structure of lignin in adjacent cells within a particular tissue. In woody plants, this uniformity manifests itself in the uniformity of structure within a particular annular ring. If the structure of lignin were determined by unregulated kinetic and microenvironmental factors, rather than being under the central control of the cells, one would expect most trees grown at latitudes outside the tropics to have lignins that differ in structure between northern and southern exposure within the same annual ring: due to the difference in exposure to solar radiation, the two regions would be expected to be at different temperatures during daylight hours. While it is not clear that this possibility has been explored experimentally, it would seem very likely that such differences would have resulted in anatomical differences that would have been Recognized long ago on the basis of visual macroscopicc examination, and certainly upon subjection to the different staining reactions that have long been used to characterize lignification in plants. That such differences have never been observed or reported can be taken as *prima facie* evidence for central intracellular regulation of the assembly of lignin.

The only plausible avenue for intracellular regulation of the structure of lignins is through the provision of templates for their spatial organization prior to the development of covalent linkages through radical coupling reactions. The most plausible templates are the domains of the polysaccharide matrix and their interior surfaces formed through the interations between cellulose and the hemicelluloses. Since mechanisms for intracellular conrol of synthesis of the polysaccharides have been articulated, and are clearly a reflection of the expression of genetic information, the pathway to regulation of the structure of the lignins is complete.

This pathway to regulation of lignin structure suggests new approaches for genetic modification of the assemb]y of lignin. This would be seen as a process requiring two complementary components wherein modification of pathways for the synthesis of phenylpropanoid structures is coordinated with modification of the assembly of the

polyssccchaides that provide the templates for organization of the lignin. Thus, any program directed at development of transgenic plants with different lignin structures must include a component addressing modifications of the hemicellulosics as well. While this adds another dimension to the complexity of such a program, it does offer the possibility of another avenue for regulation of the structure.

The significant departure from the accepted views about the biogenesis of lignin presented here can provide both an organization principle for empirical observations concerning the structure and diversity of native lignins, and a guide for future exploration of the biosynthetic pathways. One of its attractive features is that it envisions assembly processes for lignin that are consistent with the hierarchic and sequential control of elementary biochemical steps that is characteristic of biological systems *(26)*.

Wehn taken together with our observations of photoconductivity *(24)* and the coherence of order that they imply, the model suggests that cell wall systems in higher plants are hierarchically organized at the nanoscale level, and that the constituents are coupled at secondary and tertiary levels of molecular structure that are not adequately captured by the traditional models describing cell walls as two phase composite systems. In additon, they point to the possibility that the cell wall matrix pocesses functions in which electronic charge transport may play an important role The intimate relationship between lignin, which can be an electron conductor, and cellulose, which is piezoelectric, suggests the possibility that the integrated molecular composite represents an electromechanical system with properties that can translate mechanical deformation of the plant cell wall into electrical signals wthat my elicit cellular responses to mechanical deformation. Conversely, the piezoelectric character of the composite may play a key role in the response of plant tissue to photochemically generated electrical potentials that are likely to trigger many of the complex responses to light.

In summary, our understanding of the functions of lignin and the cell wall matrix needs to be re-examined. It may be well lead to the conclusioin that the wall of the developing cell is one of the most dynamic mediators between intracellular and extracellular processes.

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