Order and Randomness in Lignin and Lignification: Is a New Paradigm for Lignification Required?
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Lignification and the Definition of Lignin

Lignification is a process essential to the nature and evolution of vascular plants that is still poorly understood, even though it has been studied for more than a century. Lignin is unusual compared to other abundant natural polymers due to the apparent low degree of order and the high degree of heterogeneity in its structure. It is also unusual as a plant polymer in that there are no plant enzymes for its degradation.

Definitions of lignin that are both specific enough and general enough are difficult to find. For example, a recent book on “Lignin and Lignans” does not provide such a definition even in the first chapter, entitled “Lignin and Lignan Biosynthesis: Distinctions and Reconciliations” (Lewis et al. 1998). Attempts to define lignin in terms of its function within the plant are also not clear-cut. Lignin is often defined for specific applications, e.g., it is often regarded as little more than the (non-extractable) phenolic component that must be fragmented or degraded to produce pulp and paper. We prefer to consider lignin as an operational term for a diverse class of naturally occurring phenolic polymers that need to be more precisely defined in each case, based upon the source and the method of purification or analysis. It is often difficult to distinguish cell wall lignin from other polymerized infusions, e.g., normal lignification vs. wound-response lignin. Until there is better agreement on how lignin should be defined, it will not be surprising if there is continued controversy about its origin, composition, properties and biosynthesis.

Order in Lignin

The issue of randomness or the degree of order in lignin formation remains controversial. Researchers occasionally speculate about the possibility of crystallinity. Studies on simple lignin trimers led one of us to describe lignin as a stereochemical nightmare with crystallinity in the traditional sense being astronomically improbable (Ralph 1993). That is not to say that other aspects of order in the polymer, such as the alignment of aromatic rings indicated by Raman spectroscopy (Atalla and Agarwal 1985), are impossible. Randomness has been used to imply that the process appeared to have no direct enzymatic control. Obviously, the polymerization of lignin is not completely random, and only a subset of the possible linkages are found. In fact, prior descriptions as ‘random’ presumably never implied a totally random distribution of coupling products; clearly, the coupling of two monomers must be weighted by the propensity for each type of coupling. However, lignin formation has little to do with monomer coupling — lignification, unlike lignan formation, almost entirely involves coupling of a single monomer to a growing oligomer. The idea that the coupling would depend on the types of units involved, their concentrations, the matrix and so on are all issues that can determine what type of coupling arises at any step.

Order in the lignin polymer could result from processes of self-assembly, or alternatively, specific finely orchestrated steps might be involved in the assembly. Lewis has argued that, “It is inconceivable that lignin formation would be left to the vagaries of such a wide range of enzymes or be realized in a haphazard manner” (Davin et al. 1997). A more general opposing view is succinctly stated by Denton (Denton 1998), who argued that “Biological defense is well served by loosely ordered chemistry. Where many randomly linked products are needed, sloppy pathways are economical.”

A New Paradigm for Lignification?

Lewis et al. claimed that we have argued (Gang et al. 1998, Lewis et al. 1998) for a new and unsupported paradigm for lignin polymerization from our findings relating to lignin-biosynthetic-pathway mutants (Ralph et al. 1997) and transgenics (Ralph et al. 1998). This is clearly not the case. Rather, we believe that the properties of abnormal lignins are simply due to changes in the relative abundance of precursors likely
to be found in normal lignins. For example, the lignin in *Arabidopsis* can be converted from a guaiacyl-syringyl lignin to a predominantly guaiacyl lignin or to an almost exclusively syringyl lignin by modifying the level of expression of the gene for ferulate-5-hydroxylase (Meyer et al. 1998). Much of the variation in lignin, whether due to genetic or environmental causes, can be readily explained by a change in the relative abundance of the precursors delivered to the lignifying zone. All that is required beyond delivery of precursor is that the mechanism for polymerization be a general one.

Our results do challenge the need for the new paradigm proposed by Lewis and Davin (Lewis and Davin 1998). Recent isolation of a “dirigent” protein from *Forsythia* which facilitates coniferyl alcohol radial coupling to produce the lignan pinoresinol in a regio- and stereoselective manner has led to a proposed new mechanism for lignin biosynthesis because of the similarity of the phenolic precursors. According to their model, lignins form from template arrays of dirigent proteins and are synthesized with absolute structural control. With the possibility that the synthesized lignin chain then structurally dictates the next chain by a template-polymerization process (Sarkanen 1998), the model resembles the mechanism of biosynthesis of more highly ordered biological polymers. This idea is intriguing, but highly speculative and currently devoid of evidence.

Exquisitely synthesized polymers should produce an array of discrete products following degradation by such procedures as ether cleaving reactions (acidolysis, thioacidolysis, the DFRC method, or high temperature base). Instead, such degradative methods produce a continuous array of oligomers with no members of the series obviously missing. We have not been able to detect any hint of optical activity in various isolated lignins nor in degradation products which retain the optical centers produced in the coupling step [see p. 35]. The new paradigm proposal cites two possible explanations for the “perceived lack of optical activity of lignins” (Lewis and Davin 1998). One explanation is that “two distinct types of proteins each encoding formation of complementary chains that effectively cancel out any measurable optical activity.” This idea requires that the plant would go to the energetically extreme measure of creating an optically active lignin polymer only to carefully negate that structural feature via a complementary set of proteins for which additional (complimentary) biochemical pathways must also be supported. This effort is to produce two complementary lignin polymers when each has *identical physical properties*, identical to those of the racemic mixture — some reason would have to be envisaged, although producing a variety of structures/stereochemistries is an asset in defense. The template argument (that “complementary mirror images form via template replication”) has less serious detractions, although no evidence of any structural replication ability has yet appeared. Since lignins are found intimately associated with hemicelluloses, it is not clear how discontinuities in the alignment of one lignin chain might contribute to excess optical activity (of the incompletely replicated section). We look forward to cogent arguments, rationales and diagnostic experimental evidence for the complex issues involved in the proposed new lignification paradigm. Until experimental evidence is provided, the extension of the lignan dirigent protein observations to lignin, as a paradigm for lignification, is without substance.

**Control of Monomer Supply**

We have not seen any experimental data that require a precisely controlled synthesis of structurally-defined lignin, *i.e.*, data that cannot be supported by simply recognizing that the plant does exquisitely, temporally and spatially, control the supply of monolignols, oxidizing enzymes and oxidative species. The matrix environment of the polymerization may alter the interunit linkage composition, as has been well demonstrated in synthetic lignification experiments. The regulated differences in lignification in various cells and various regions of the cell, in wounding or in stress, demand little more than changes in monomer supply.

**Conclusions**

Lignification is a complex process that currently defies exact definition. Evidence to date points to lignin as being a product of chemically (rather than
biochemically) controlled radical coupling reactions. A recently proposed new paradigm which aims to bring lignin into the realm of other exquisitely synthesized biological polymers is currently devoid of evidence. In fact, all of the variation in lignin composition and structure, including the dramatic changes that occur in lignin-biosynthetic-pathway mutants and transgenics, appears to be the result merely of controlled precursor supply (including oxidases, and co-factors, e.g., peroxide) to the lignifying zone. How the plant controls the temporal and spatial aspects of precursor supply will presumably be subjected to more extensive study in the future. Although interesting, there are overwhelming problems with the proposed new paradigm for lignification resulting from extrapolation of lignan observations. No evidence currently available challenges the established views on lignification.

References


