

01. LIGNIFICATION: ARE LIGNINS BIOSYNTHEZED VIA SIMPLE COMBINATORIAL CHEMISTRY OR VIA PROTEINACEOUS CONTROL AND TEMPLATE REPLICATION?

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Abstract

Current debate on the mechanism of lignification needs to be scrutinized. In examining arguments raised *against* the existing Freudenberg theory of chemically-controlled combinatorial synthesis, or *for* the new contender advocating absolute proteinaceous control, we conclude that the challenger fails to displace the current theory.

Introduction

Lignification, the plant cell wall polymerization process generating lignins from monolignols, is becoming increasingly well understood (1). The current “Freudenberg” theory holds that the polymerization is a chemical process involving the radical coupling of phenols, incorporating available phenolic substrates under simple chemical and physical controls. As radicals from the monolignols and from the evolving polymers are resonance-delocalized and are capable of coupling with different regiochemistries, the process is “combinatorial” (1).

An alternative but ill-defined concept attempting to displace the current theory was recently championed in a particularly polemic article (2). Davin and Lewis propose that lignin primary structure is controlled by “proteins harboring arrays of dirigent (monolignol radical binding) sites” and template replication mechanisms. They contended that the current combinatorial free-radical coupling theory is an “unproven working hypothesis,” and unilaterally raised their own hypothesis to “theory” status. The article contained errors and misrepresentations that presented a distorted image of the current state of knowledge concerning lignin biosynthesis and chemistry.

In many articles now, the “evidence” touted to denigrate the current theory has been irrelevant or invalid. Indeed the existing theory is growing stronger as it successfully withstands such attacks. Its supposed replacement paradigm has, despite the rhetoric, no valid supporting evidence to date and has enormous difficulty in explaining many of the basic facts. Despite this, the new paradigm has come to some prominence, even appearing in a text book (in a section coauthored by one of the proponents) to the exclusion of the currently held theory (3).

The true test of any hypothesis is rigorous experimental testing designed to refute its premise. This scientific process allows rapid evaluation and refinement of a hypothesis. Once it has withstood all experimental challenges it may then, and only then, be considered for elevation to a theory. As scientists we have an obligation to objectively scrutinize our own as well as colleagues’ work to ensure science moves forward. Our presentation will be an attempt to bring balance to the current debate.

Discussion

The following are a small sample of the arguments advanced that should be more openly discussed. Many have already been addressed in reviews and commentary articles on the subject, as reviewed (1).

1. *Lignin would be an abomination if not produced in a controlled fashion.* This oft-espoused notion (see references and quotes in the expanded version of this abstract online) is merely a belief. Note that no one claims that lignification is uncontrolled; clearly the cell exerts exquisite control over the supply of monolignols (and other phenolics) to the lignifying zone, over the supply of H₂O₂ and, consequently, the radical-generation capacity. The issue is in the control over the polymerization (1). Even eminent polysaccharide chemists are willing to debate that “*all other known biochemical processes*” (4) are deftly controlled.
2. *Monomer substitution is unprecedented in a biological process.* The statement that “There is, however, no known precedent for the free interchange of monomeric units in any biopolymer assembly, then or now...” (4) has now been compellingly debunked (1). And monomer substitution was already evident in the literature previously; the substitution of L-fucose with L-galactose in fucose-deficient *mur1* mutants of *arabidopsis* occurs in polysaccharide biosynthesis which is more highly structurally controlled than in lignification (5). As Lewis has noted that their new paradigm is incompatible with the concept of monomer substitution, perhaps the debate should already be over.
3. *“Sequenced” lignin fragments require programmed assembly.* A recent contention was that putatively sequenced fragments, such as the hexamer S-(8-O-4)-S-(8-O-4)-S-(8-O-4)-S-(8-8)-S-(8-O-4)-G (c.f. Fig. 4

- in (2)), require programmed assembly by protein templates. In fact the constitution of such a hexamer in syringyl-rich lignin is entirely in agreement with the combinatorial coupling theory. Since the only coupling mode of a monomer to a syringyl unit is 8–O–4, syringyl polymers must be linear and can only contain one 8–8-unit. Additionally, the putative hexamer masses were chosen from a virtual continuum of low-abundance peaks (6). Stereochemistry is a major issue that was not addressed — there are $2^9 = 512$ stereoisomers possible for this hexamer, $2^8 = 256$ of which are physically different, and all of which have the same m/z and are indistinguishable by mass spectrometry. This challenge has *not* been met by Lewis, and to dismiss this chemical fact with ridicule, e.g. (7), instead of experimental evidence is unacceptable.
4. *Lignin deposition vs thioacidolysis monomer yield.* Evidence for the designation of well-defined primary structures was claimed from monitoring lignin deposition by thioacidolysis in *Arabidopsis* stems. This conclusion does not have any logical connection with the experimental results. On the contrary, existing data clearly and predictably show that (syringyl-rich) hardwood lignins give significantly higher yields of thioacidolysis monomers than (guaiacyl-rich) softwood lignins (8).
 5. *Monomer-independent sequences.* Among recent claims is “the existence of invariant and predetermined distributions of identical linkages with the primary lignin chains that are independent of the degree of monomer methoxyl group substitution patterns and/or lignin contents. Accordingly, the main primary chains of the biopolymers are considered to be of a predetermined regularity” (7). Such a claim contradicts known structural features of lignin. Guaiacyl lignins, for example, have some 5-10% β -5-coupled (phenylcoumaran) units. How can syringyl-rich lignins have the same sequence when syringyl units have no available 5-position for coupling? Furthermore, *p*-hydroxyphenyl-rich alfalfa lignins appear to be totally depleted in β -1-coupled products that are present in the control (9). These and other data refute any notion that there is mysterious monomer-independent control over the sequence.
 6. *Precedent for template replication.* The template hypothesis has no precedent. Of the “several thousand papers” describing template effects (2), Davin and Lewis did not cite one that pertains to lignin biosynthesis or provide a documented example from nature of a protein template producing racemic polymers. Problems facing the concept of template replication as a mechanism of lignin formation were evaded. Protein-controlled *formation of chiral centers* would not lead to racemic mixtures. In lignification, chiral centers are *generated* from achiral starting materials each time a monolignol couples with another monolignol or with the growing polymer, yet neither the polymer nor any units released from it are optically active (1).

Conclusions

To date, presentations such as (2) have failed to specify (in terms of experimental data) the shortcomings of the Freudenberg mechanism of lignification, and failed to show how the template replication hypothesis resolves these shortcomings. Presenting ambiguous or irrelevant observations as evidence cannot supplant diagnostic experiments that validate or invalidate hypotheses. And declaring that “recent progress has provided crucial evidence to support the theory that lignin primary structure is controlled at the proteaceous level” and that “evidence for control over lignin assembly has been demonstrated...” (2) simply does not make it so!

Perhaps it is time to reevaluate the elegance in chaotic systems, particularly for plant defense. We continue to marvel that plants may have chosen a route to this crucial cell wall component that allows them tremendous flexibility to respond to environmental factors, and even to have sometimes successfully thwarted the tinkering of genetic engineers bent on impeding monolignol production.

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