Biodegradation of lignin-carbohydrate complexes

Thomas W. Jeffries
Institute for Microbial and Biochemical Technology, USDA Forest Service, Forest Products Laboratory, One Gifford Pinchot Drive, Madison, WI 53705-2398 U.S.A.

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Abstract

Covalent lignin-carbohydrate (LC) linkages exist in lignocellulose from wood and groups herbaceous plants. In wood, they consist of ester and ether linkages through sugar hydroxyl to the α-carbanol of phenylpropane subunits in lignin. In grasses, ferulic and p-coumaric acids are esterified to hemicelluloses and lignin, respectively. Hemicelluloses also contain substituents and side groups that restrict enzymatic attack. Water-soluble lignin-carbohydrate complexes (LCCs) often precipitate during digestion with polysaccharidases, and the residual sugars are more diverse than the bulk hemicellulose. A number of microbial esterases and hemicellulose polysaccharidases including acetyl xylan esterase, ferulic acid esterase, and p-coumaric esterase attack hemicellulose side chains. Accessory hemicellulases include α-L-arabinofuranosidase and α-methyl-glucuronosidase. Both of these side chains are involved in LC bonds. β-Glucosidase will attach sugar residues to lignin degradation products and when carbohydrate is attached to lignin, lignin peroxidase will depolymerize the lignin more readily.

Abbreviations: APPL – acid precipitable polymeric lignin; CBQase – cellobioquinone oxidoreductase; LC – lignin-carbohydrate; LCC(s) – lignin-carbohydrate complex; DHP – Dehydrogenative polymerise; DMSO – dimethylsulfoxide; DP – degree of polymerisation; MWEL – milled wood enzyme lignin; MWL – milled wood lignin (not digested with carbohydrases)

Introduction

This review explores the characteristics and biodegradation of bonds between lignin and carbohydrate. Lignin-carbohydrate complexes (LCCs) can be isolated as water-soluble entities from the walls of gymnosperms, angiosperms, and graminaceous plants (Azuma & Koshijima 1988), and they can be separated by gel filtration into three fractions. The component of lowest molecular weight consists mostly of carbohydrate, the two larger components mostly of lignin. Lignin-carbohydrate bonds are presumed to exist in higher molecular weight lignin fractions that are water insoluble. Softwood LCCs are distinct in that their carbohydrate portions consist of galactomannan, arabinino-4-O-methylglucuronoxylan, and arabinogalactan linked to lignin at benzyl positions (Azuma et al. 1981; Mukoyoshi et al. 1981). In contrast, carbohydrate portions of hardwood and grass LCCs are composed exclusively of 4-O-methylglucuronoxylan and arabinio-4-O-methylglucuronoxylan, respectively (Azuma & Koshijima 1988). Trans-p-coumaric and p-hydroxybenzoic acid are esterified to bamboo and poplar lignins, respectively (Shimada et al. 1971), and trans-ferulic acid is ether linked to lignin (Scalbert et al. 1985). Many different types of LC bonds have been proposed, but most evidence exists for ether and ester linkages.

Relatively little attention has been given to en-
zymes capable of cleaving the chemical linkages between lignin and carbohydrate, the LC bonds. Such bonds occur in low frequency. They are heterogeneous; many are easily disrupted by acid or alkali during isolation, and they are still poorly defined. Although many sorts of linkages have been proposed, two have some substantive evidence. They link the α position of the phenyl propane lignin moiety to either carboxyl or free hydroxyls of hemicellulose through ester or ether linkages, respectively. Various chemical and enzymatic procedures have been used to isolate LC complexes, and a few biological systems have been shown to solubilize lignin preparations. No enzymes specific to LC bond cleavage have been described. The objective of this review is to focus our knowledge of the heterogeneous structures that comprise LC bonds, and to sort out the enzymes that attack related structures. A recent review of LCCs has been completed by Koshijima et al. (1989).

**Chemical characteristics of LC bonds**

Ester linkages (CO-O-C) occur between the free carboxy group of uronic acids in hemicellulose and the benzyl groups in lignin. Some are present as acetyl side groups on hemicellulose, others are between uronic acids and lignin, and still others occur between hemicellulose chains. Monomeric side chains in wood xylans consist of 4-O-methylglucuronic acid units, and some 40% of the uronic acid groups in birch are esterified. In beech, one-third of the glucuronic acids present in LCCs are involved in an ester linkage between lignin and glucuronoxylan (Takahishi & Koshijima 1988b). However, many glucuronic acid groups may be esterified within the xylan polymer (Wang et al. 1967).

Direct evidence for the chemical nature of ester linkages between lignin and carbohydrate in pine has been obtained through the selective oxidation of carbonyls in lignin. Watanabe & Koshijima (1988) proposed that the 4-O-methylglucuronic acid residue in arabinoglucuronoxylan binds to lignin by an ester linkage in *Pinus densiflora* wood. The linkage position is probably the α or conjugated γ position of guaiacylalkane units (Fig. 1). Watanabe et al. (1989) found that mannose, galactose, and glucose are O-6 ether linked and xylose is O-2 or O-3 ether linked to the α benzyl hydroxyl in a neutral fraction of pine LCC.

Watanabe et al. (1989) also studied alkali-stable LC linkages. The alkali-stable linkages in LCC prepared from *Pinus densiflora* consist of acetyl glucomannan and β-(1→4) galactan bound to the