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Lignin-carbohydrate complexes in forages: structure and consequences in the ruminal degradation of cell-wall carbohydrates

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Summary — Lignin-carbohydrate complexes (LCCs) are recognised as key structures in forage degradability. Apart from ester bonds involving phenolic acids, which seem to play a major role in grasses, little is known about the other types of linkages that must exist but have proved difficult to demonstrate. The chemical nature of possible LCC linkages is presented and the various mechanisms through which LCCs in the cell-wall architecture may interfere with carbohydrate utilisation by rumen microorganisms are discussed.

lignin-carbohydrate complexes / cell wall / forage / digestibility

Résumé — Les complexes lignines-polyosides des fourrages : structures et conséquences sur la dégradation des parois végétales dans le rumen. Les complexes lignines-polyosides (LCC) sont considérés comme des structures clés pour la dégradabilité des fourrages. En dehors des liaisons ester par l'intermédiaire d'acides phénoliques, qui jouent probablement un rôle majeur chez les graminées, on sait peu de choses sur les autres types de liaisons qui devraient exister mais sont très difficiles à mettre en évidence. Dans ce document, nous décrivons les différentes liaisons chimiques proposées, et discutons les mécanismes par lesquels les LCC peuvent interférer avec l'utilisation des polyosides par les microorganismes du rumen.

complexes lignines-polyosides / parois végétales / fourrages / digestibilité

INTRODUCTION

It is well known that lignins restrict cell-wall carbohydrate degradation in maturing forages. Several studies have shown a negative correlation between lignin content and cell-wall digestibility (Jarrige, 1980; Minson,

1982). However the mechanisms involved in the disproportionate effect on cell-wall digestibility exerted by relatively small amounts of lignins remain unexplained. The effect of lignins (see review by Besle *et al*, 1995) depends on plant variety and tissue. This is more marked with primary than with secondary walls (Engels and Schurmans,

1992). The chemical nature of lignins and how they are linked with the other cell-wall polymers seem as important as the total amount of lignin present. This organisation takes place when lignins become anchored to the primary wall (Yamamoto *et al*, 1989; Terashima, 1993), develops as lignification proceeds (Jung and Deetz, 1993), and continues during cell-wall ageing. It is hypothesized that the lignin-carbohydrate complexes (LCCs) included in the wall structure are key elements in explaining the impact of lignins on cell-wall degradation. Soluble LCCs with predominant lignin moieties have been isolated from the rumen liquor (Gaillard and Richards, 1975; Lomax *et al*, 1984). In a mechanistic model, Chesson (1993) explained the release of soluble LCCs as a consequence of the degradation of the surrounding carbohydrates. Cell walls were represented as discrete blocks with different compositions. As suggested by Wallace *et al* (1991), primary layer LCC structures differed from those of the secondary layer. While other aspects of direct and indirect roles of LCCs in cell-wall degradation have been reviewed by Chesson (1988) and Jung and Ralph (1990), this paper presents the state of knowledge about LCC structures in forages and recent concepts concerning their role in cell-wall degradation.

NATURE OF THE LINKAGES BETWEEN LIGNINS AND CARBOHYDRATES

There are numerous possibilities for the formation of lignin-carbohydrate linkages in cell walls. Firstly, the polymers themselves (polysaccharide and lignin) contain numerous functional groups: primary and secondary alcohols, carboxyls and carbonyls. In grasses, the phenolic hydroxyls of esterified phenolic acids constitute an additional type of functional group. Secondly, enzymes participating in building the cell-wall architecture, such as glycosyl-transferases and

peroxidases, are present in multiple forms. Finally, the free radicals and quinone methides produced at the time of lignin polymerisation are very reactive species (see review by Leary, 1980), and have long been suspected of reacting with carbohydrates (Freudenberg and Neish, 1968). In this paper, frequent references will be made to radical and quinone methide reactions. Those reactions have been summarised in figure 1 to illustrate the mechanisms described in the text. Additional information can be found in the review by Ralph and Helm (1993). Other hypothetical reactions, which could lead to the formation of LCC bonds, and which are unrelated to lignin synthesis or occur in the cytoplasmic compartment, are also described.

Linkages involving phenolic acids

Phenolic acids are precursors of the phenylpropane units of lignins although free phenolic acids are rarely found in cell walls (Newby *et al*, 1980). However, such acids esterified to lignins or polysaccharides (compound 2 in fig 1) are relatively abundant in grasses and are often referred to as 'non-core lignins' (an inappropriate terminology as discussed by Ralph and Helm, 1993).

It is now well known that grass arabinoxylans are esterified with ferulic acid. Smith and Hartley (1983) purified the first 'FAX' fragment (*O*-[5-*O*-(*trans*-feruloyl)- β -L-arabinofuranosyl]-(1 \rightarrow 2)-D-xylopyranose) from wheat bran enzyme hydrolysate. Similar structures, but with α -(1 \rightarrow 3)-linked arabinose have since been isolated (table I). A feruloylated xyloglucan fragment 'FXG' (*O*-[4-*O*-(*trans*-feruloyl)- α -D-xylopyranosyl]-(1 \rightarrow 6)-D-glucopyranose), has also been isolated from bamboo by Ishii *et al* (1990). In addition to ferulic acid, grass arabinoxylans have been shown to contain *p*-coumaric esters, and corresponding typical fragments (eg, 'PAXX': *O*-[5-*O*-(*trans*-coumaroyl)- α -L-

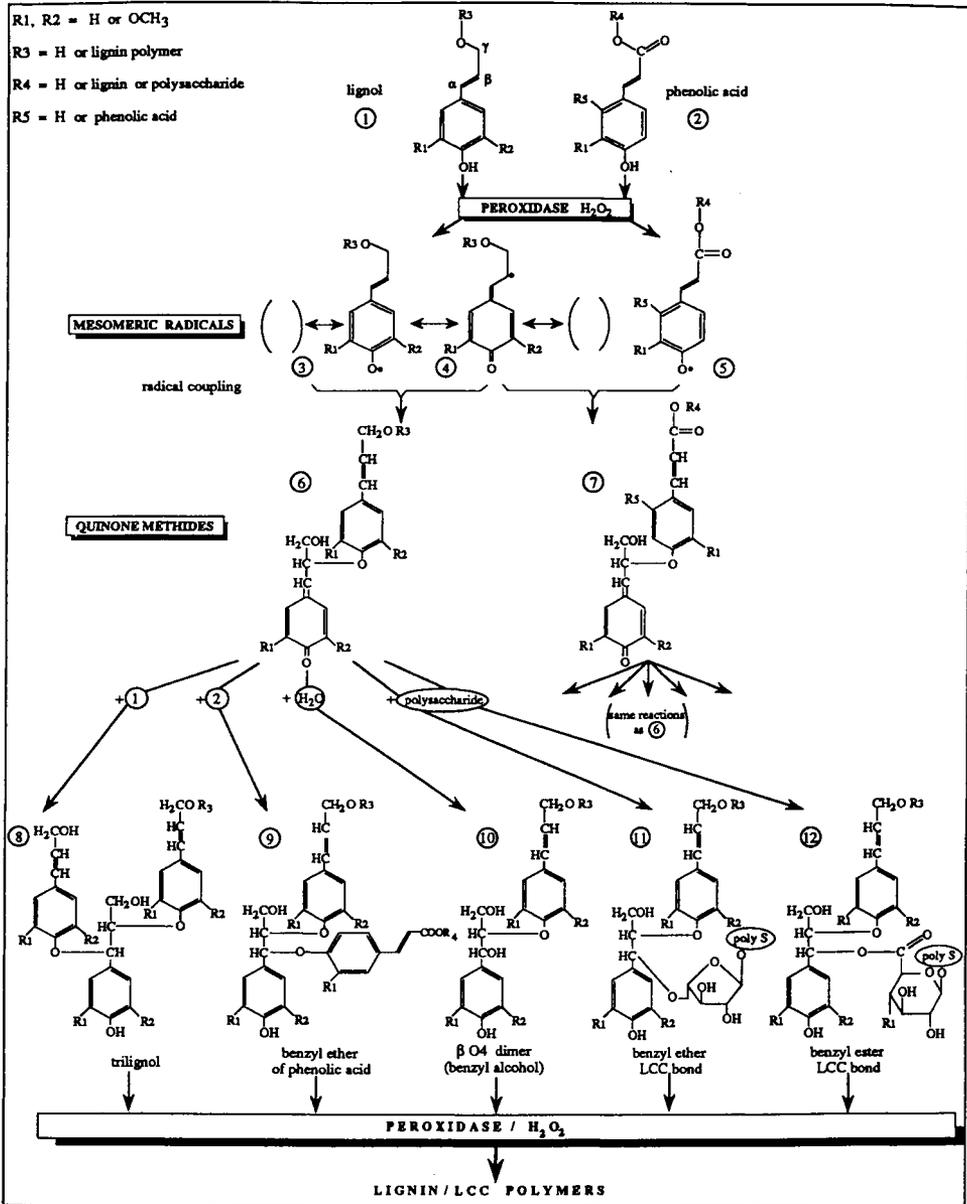


Fig 1. Radical coupling and quinone methide condensation are recognised reactions for binding polysaccharides and phenolic acids to lignins.

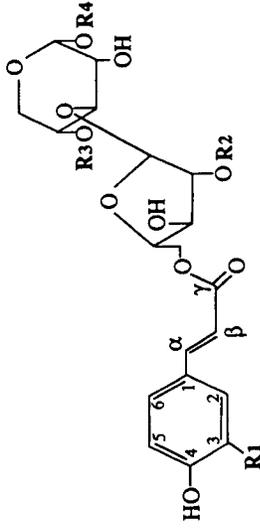


Table 1. p-Coumaroylated and feruloylated xylan fragments purified from monocots.

Reference	R_1	R_2	R_3	R_4	Origin
Kato and Nevins (1985)	OCH ₃	H	H	4-O-Xyl	Oxalic acid- and driselase-treated zea shoot cell walls
Mueller-Harvey <i>et al</i> (1986)	OCH ₃ PAXX	H	H	4-O-Xyl 4-O-Xyl	Cellulase-treated barley straw cell walls Cellulase-treated barley straw cell walls
Ishii <i>et al</i> (1990); Ishii and Hiroi (1990a)	H PAXX	H	H	4-O-Xyl	Driselase-treated bamboo-shoot cell walls
Ishii and Hiroi (1990b)	OCH ₃ FAXXX	H	H	4-O-Xyl 4-O-Xyl	Driselase-treated bamboo-shoot cell walls Driselase-treated bamboo-shoot cell walls
Azuma <i>et al</i> (1990)	OCH ₃ Ac FAXXX	OAc	β Xylp	4-O-Xyl	Cellulase- and hemicellulase-treated sugar cane bagasse
Borneman <i>et al</i> (1992); Borneman <i>et al</i> (1990b)	OCH ₃ PAXX	β Xylp	H	4-O-Xyl	Enzyme-treated coastal bermuda grass
Kato <i>et al</i> (1983)	OCH ₃ FAXX	H	H	4-O-Xyl	Enzyme-treated LCC fraction from bagasse
Kato <i>et al</i> (1987b)	OCH ₃ FAXXX	H	β Xylp	4-O-Xyl	Enzyme-treated LCC fraction from bagasse
Gubler <i>et al</i> (1985)	OCH ₃ FAXX	H	H	4-O-Xyl	GA ₃ -treated cultured aleurone from barley
Hartley <i>et al</i> (1990b)	OCH ₃ PAXX	H	H	4-O-Xyl 4-O-Xyl	Enzyme-treated coastal bermuda grass Enzyme-treated coastal bermuda grass

arabinofuranosyl]-(1→3)-O-β-D-xylopyranosyl-(1→4)-D-xylopyranose) have been isolated (table I). The frequency of esterification of barley arabinoxylans has been estimated to be about 1 arabinosyl residue every 15 for ferulic acid, and 1 every 31 for *p*-coumaric acid (Mueller-Harvey *et al*, 1986).

In dicots, ferulic and *p*-coumaric acids are found mainly associated with the pectic fraction and are about 10 times less abundant than in grasses (Jung *et al*, 1983). Feruloylated pectins have been identified in sugar beet (Rombouts and Thibault, 1986). Fry (1982) isolated 2 types of feruloylated disaccharides: 3-*O*-(3-*O*-feruloyl-α-L-arabinopyranosyl)-L-arabinose and 4-*O*-(6-*O*-feruloyl-β-D-galactopyranosyl)-D-galactose, which accounted for more than 60% of the total ferulic acid content of cultured spinach primary cell walls.

Feruloylation invariably occurs at the same position on polysaccharides, which strongly suggests an enzyme-mediated, site-specific phenomenon. Fry (1983) proposed an intracellular esterification at the non-reducing end of newly synthesised spinach pectins. In parsley, *in vitro* esterification of wall polysaccharides by radio-labelled feruloyl-CoA has been shown to occur in a microsomal subfraction derived from Golgi apparatus (Meyer *et al*, 1991). However, Yamamoto *et al* (1989) observed that the kinetics of cell-wall deposition in grasses were different for arabinose and ferulic acid, suggesting an extracytoplasmic esterification. Feruloyl-CoA however, has never been detected *in vivo* in the cell wall. Although further investigations are needed in order to check if transesterification can occur in the cell wall, different mechanisms could be involved for individual classes of polysaccharides; pectins, which are abundant in primary walls of dicot-cultured cells, may be feruloylated intracellularly, whereas arabinoxylans and xyloglu-

cans, predominating in the grass samples studied, could be esterified in the cell wall.

Phenolic esters on cell-wall polysaccharides may undergo 2 distinct types of dimerisation. 5,5'-dehydrodiferulic acid was obtained *in vitro* from an artificially esterified polysaccharide in the presence of peroxidase and H₂O₂ (Geissman and Neukom, 1971). Such biphenyl structures have been observed bridging polysaccharides in spinach (Fry, 1986), wheat flour (Markwalder and Neukom, 1976) and bamboo (Ishii and Hiroi, 1990a), and a diferuloyl hexasaccharide [XXAF-FAXX] has been isolated from bamboo by Ishii (1991). In the cell wall, diferulic bridges probably participate in controlling cell-wall elongation (Fry and Miller, 1989). Diphenyl structures have not been described for *p*-coumaric acid, whereas both ferulic and *p*-coumaric acids have been found in the cyclobutane dimers truxinic and truxillic acid. These dimers are the result of the photochemical coupling of esterified hydroxycinnamic acids (Hartley and Ford, 1989; Hanley *et al*, 1993; Turner *et al*, 1993). They have mainly been observed in grasses (Hartley *et al*, 1990a, b), but are also present in minute amounts in lucerne and red clover stems (Eraso and Hartley, 1990).

p-Coumaric acid may also be esterified to lignins in wheat (Smith, 1955) and bamboo (Shimada *et al*, 1971; Nakamura and Higuchi, 1978). In these and in maize lignins, evidence for ether-linked *p*-coumaric acid has also been obtained (Nimz *et al*, 1981). Ferulic acid has also been shown to be etherified to lignins in an LCC fraction from bagasse (Kato *et al*, 1987a). Scalbert *et al* (1985) observed in wheat straw that more ferulic acid (25–65%) than *p*-coumaric acid (5%) was etherified to lignin.

Most of the ferulic acid linkages with lignins are of the ether type while this acid is abundantly esterified to polysaccharides. In contrast, *p*-coumaric acid, mostly esterified to lignins, does not seem to be etherified to polysaccharides.

The location on lignin units of ester-linked *p*-coumaric acids has been studied in bamboo and grass by Shimada *et al* (1971) and Nakamura and Higuchi (1978). The resistance to methanolysis of *p*-coumaric acid esters in lignin fractions compared with that of model compounds indicates that bondings through the γ -position of the phenylpropane unit predominate over bondings through the α -position.

Yamamoto *et al* (1989) proposed 2 mechanisms for ether bond formation, involving the phenolic group of ferulic acid, which is already esterified on polysaccharide chains. The first was a radical coupling that produces an ether linkage between the phenolic group of the acid and the β -carbon of the lignin unit (compound 7 in fig 1). The second was described by Scalbert *et al* (1986), and consists of a nucleophilic reaction resulting in an α -ether (compound 9 in fig 1). Both reactions result in bridging lignins to polysaccharides. The existence of [polysaccharide-ester-ferulic-ether-lignin] structures now seems to be established for wheat (Iiyama *et al*, 1990), ryegrass (Kondo *et al*, 1990a) and *Phalaris* (Lam *et al*, 1992a).

Likewise, dehydrodiferulic bridges between polysaccharides become etherified to cell-wall polymers during maturation (Lam *et al*, 1992b), a reaction involving the free phenolic hydroxyls of the dimer and quinone methides.

Ether and ester linkages of phenolic acids can be differentiated by sequential treatments with sodium hydroxide (Iiyama *et al*, 1990). Ester-only-, ether-only- and ester-ether-linked phenolic acids can be distinguished using the method of Lam *et al* (1992a). The authors confirmed with this method that *p*-coumaric acid, unlike ferulic acid, does not form ester-ether bridges. They also measured the content of esterified and total phenolic acids in internode segments of *Phalaris* varying in maturity. Ferulic acid appeared at a very early stage of cell-

wall building and its total amount quickly stabilised. The proportion of etherified ferulic acid increased more gradually. *p*-Coumaric acid remained mainly in the saponifiable form, and its total content increased continuously during cell-wall building (Lam *et al*, 1992b). Similar observations were made in ryegrass harvested at different stages of maturity (Kondo *et al*, 1990a), and in growing culms of sugar cane and rice (He and Terashima, 1991). Ferulic acid can therefore be considered as a component of certain wall polysaccharides, acting as a group for anchoring hydrophobic lignins to hydrophilic carbohydrates, whereas *p*-coumaric acid behaves as a fourth lignin unit. The origin and significance of this individual specialisation of phenolic acids is not clear.

In dicots, the rarity of phenolic acids suggests that lignin anchoring proceeds in a different way and other types of linkages predominate. Joseleau and Gancet (1981) observed in aspen wood alkali-stable lignin-araban complexes held together by alkali-labile lignin-glucuronoxylan linkages, indicating the coexistence of esters with ethers or glycosides.

Linkages involving uronic acids

Glucuronic acid and its 4-*O*-methyl derivative occur as side groups in most xylans. In jute fibre (Das *et al*, 1984a) and in other dicots (Das *et al*, 1984b; Fry, 1986), they have been shown to be largely, if not totally, involved in ester linkages. The partner molecule was presumed to be a lignin, because lignins were solubilised by treatments that cleave ester bonds.

Esterification of uronic acid side chains of xylans may occur in the cell wall at the time of lignin polymerisation. Tanaka *et al* (1979) showed *in vitro* that carboxyl groups (in comparison to secondary and primary alcohols) are the groups most reactive with quinone

methides leading to the formation of benzylester linkages (compound 12 in fig 1).

Experiments made by Stewart (1973) on eucalyptus wood indicate that uronic acids are also involved in an indirect lignin-xylan bridging: 4-*O*-methyl-glucuronoxylans bear alkali-labile uronic acid residues (ester-linked to the xylan backbone). Some of these residues were linked to lignins by acid-resistant ether bonds. As many as 7 esterified uronic acids were estimated in each 100 xylose units, 3 of which were etherified to lignins.

Chesson *et al* (1983) measured the hemicellulosic hydroxyls liberated by an alkaline treatment and estimated that about 30% of the alkali-labile substituents in grasses as well as in dicots, were not accounted for by acetic and phenolic acids recovered in the extracts. These alkali-labile substituents could have been linked to lignins by alkali-resistant bonds.

Thus, ester linkages seem to play an important part in LCCs. Esters are relatively easy to detect, due to their characteristic infrared absorption at 1730 cm^{-1} , and their sensitivity to mild alkali treatments. It is more difficult to distinguish uronic acid from phenolic acid esters. Borohydride is known to reduce hemicellulosic uronic acid esters to give the corresponding neutral sugar residue. When applied to fibrous material, however, the low yield obtained (Das *et al*, 1984a, b), suggests a poor accessibility to some ester linkages *in situ*. Takahashi and Koshijima (1988) showed with model compounds that the use of a high pH buffer greatly enhances the reduction yield. When applied to LCC fractions from grasses, borohydride does not cleave phenolic acid esters (Morrison, 1974; Tanner and Morrison, 1983; Ford, 1989, 1990). This property, confirmed on model compounds submitted to several hydride reducers, is due to the presence of the conjugated double bond (Lam *et al*, 1992a). Finally the ester's partner molecule and its linkage position on this

molecule need to be identified. Methanolysis or mercaptolysis could prove an interesting method, since the liberated hydroxyl is consequently methylated or thioacylated. Moreover, esters on the α -position of a lignol are cleaved, whereas in the γ -position they are resistant (Nakamura and Higuchi, 1978).

Ether and glycosidic linkages

It is extremely probable that direct ether and glycosidic bonds occur in LCCs, but conclusive evidence is difficult to obtain for 2 major reasons: they are likely to be very infrequent; and their properties do not allow them to be readily distinguished from intrapolymeric linkages.

Hayashi (1961) reported that β -glucosidases released new reducing ends as well as new phenolic groups in an LCC from wheat, suggesting the occurrence of phenylglycosidic linkages. Enoki *et al* (1983) showed that a glycosidic linkage at any position of the lignol could indeed be cleaved by glycanase treatment. Ford (1990) suggested that arabinoxylans from pangola grass could be glycosidically linked to lignins, since borohydride treatment of an LCC fraction resulted in no detectable alditol. In an LCC from aspen wood, Joseleau and Kesraoui (1986) observed monomeric arabinofuranose glycosidically linked to lignin.

Soluble LCCs have been found in the rumen liquor of steers fed on tropical grass (Gaillard and Richards, 1975; Neilson and Richards, 1982). Structural investigations indicated that in these complexes, glucose, xylose and rhamnose were glycosidically linked to lignins (Lomax *et al*, 1984). In a study on soluble LCCs from the rumen of sheep fed ryegrass, Conchie *et al* (1988) found reducing xylose and glucose residues, which were thought to be ether-bound to lignins. These fractions also contained rhamnose and appreciable amounts of nitrogen.

Nordkvist *et al* (1989) obtained such soluble complexes after *in vitro* incubation. These LCCs had very low carbohydrate contents and could thus be enriched in sugars directly linked to lignins. However, it is inadvisable to draw conclusions about plant LCC from rumen-soluble LCC studies, due to the lack of knowledge on the microbial transformations that could take place. For example, in aerobic systems, glycosylations occur concomitantly with lignin degradation (Jeffries, 1990). In the rumen, however, lignins are known to be poorly degraded, and such reactions have not been reported.

Benzylethers have been demonstrated in wood lignin–glucomannan and lignin–arabinoxylan complexes by Watanabe *et al* (1986). Primary alcohol groups of glucose and mannose, and hydroxyls in positions 2 and 3 of xylose were involved, as shown by methylation. These results were obtained by using a selective degradation method with DDQ (2,3-dichloro-5,6-dicyanobenzoquinone) developed for LCC studies by Koshijima *et al* (1984). DDQ oxidatively cleaves benzylic bonds in the *para*-position with an electron-donating group. Benzylesters (Watanabe and Koshijima, 1988) and benzylglycosides (Cornu, 1989) are also cleaved by DDQ oxidation.

Model compound experiments by Enoki *et al* (1983) showed that glycosidic linkages in γ -, benzylic or phenolic positions are resistant to mild alkali except in the case of syringyl units where they are partially cleaved. Ether bonds in the γ -position are stable, while benzylethers are more labile (varying with the molecular environment); the presence of a methyl substituent on the phenolic hydroxyl considerably enhances the resistance of benzylether linkages (Enoki *et al*, 1983; Taneda *et al*, 1987). Takahashi and Koshijima (1988) observed that sodium hydroxide released significant amounts of xylose from a beechwood LCC, but only traces after methylation of the LCC. DDQ treatment released sugars in proportions

similar to those released by alkali, xylose being linked to lignins at O-3 or O-2. Thus the term 'alkali-labile linkages' includes not only esters, but also some phenolic benzylethers and glycosides involving syringyl units. Morrison (1973) found arabinoxylan-lignin complexes in ryegrass alkali-extracts, whereas Al Katrib *et al* (1988) extracted LCC from NaOH-treated straw, showing the occurrence in these plants of alkali-resistant bonds. From the model experiments of Enoki *et al* (1983), γ -ethers would be the most easily distinguishable linkages, since they resist most of the cleavage conditions tested, including strong mineral acid hydrolysis (H_2SO_4 1 N, 100°C, 6 H).

Benzylethers, like benzylesters, can arise from a reaction between polysaccharide hydroxyl or carboxyl groups and quinone methides (compound 11 in fig 1). Benzylethers also arise spontaneously when phenolic compounds are mixed with sugars (Hemmingson, 1979; Leary *et al*, 1983). The reactivity of the sugar functional groups decreases from carboxyls to secondary alcohols to primary alcohols, and benzylglycosidic linkages are not favoured (Tanaka *et al*, 1979). Glycosidic linkages, however, have been obtained *in vitro* during dehydropolymerisation of coniferyl alcohol by a crude enzyme extract from aspen in the presence of free sugars (Joseleau and Kesraoui, 1986). These authors observed a greater reactivity of arabinofuranose compared to glucopyranose.

A possible mechanism for the formation of glycosidic linkages has recently been described by Kondo *et al* (1990b), who showed *in vitro* that β -glucosidases, which occur in cell walls, catalyse the transfer of a glycosyl residue on acceptor lignols. This reaction is much more efficient if the donor molecule already contains a glycosidic linkage, but has also been observed with free glucose. Primary alcohols (γ) are more reactive acceptors than secondary ones (α), and

the presence of a phenolic hydroxyl results in greater efficiency.

ROLE OF LCC IN RUMEN DEGRADATION OF CELL WALLS

Mechanistic model of cell-wall degradation

Chesson (1993) has proposed a precise model for the degradation of lignified cell walls. Cell walls are schematised as being built of bricks representing potentially degradable polysaccharides, with other scattered bricks representing LCCs in the secondary and primary layers. Since cell-wall degradation is considered as a superficial process, the external blocks are removed first by microbial action. Some bricks representing LCCs are released in the rumen medium when the surrounding degradable carbohydrate has been removed, while others remain bound to the cell wall. As degradation proceeds, LCC bricks accumulate at the surface of plant particles, preventing further degradation. The primary wall remains almost intact, either due to it being shielded by the external layer, or because the LCC present have a different structure from those in the secondary wall and offer greater resistance (Wallace, 1989). Differences in the rate of formation of the inert layer explains differences in digestibility observed between cell walls. This model also suggests that LCCs may have both negative and positive effects on degradation.

Negative effects of LCCs

Since lignin preparations added to an *in vitro* fermentation system do not impede cell-wall degradation (Han *et al.*, 1975; Op den Camp, 1988), the inhibition caused by phenolics is evidently due, directly or indirectly, to link-

ages between phenolics and carbohydrates. Hypotheses concerning the role of the diverse structural features of cell walls in preventing polysaccharide degradation have been reviewed by Besle *et al.* (1995). In addition to the physical barrier effect of lignins, lignin-carbohydrate linkages constitute a biochemical barrier sterically hindering glycanases (Jung and Deetz, 1993). *In vitro* experiments by Gressel *et al.* (1983) have shown that polyeugenol, a lignin model polymer, inhibits cellulolysis only if it is linked to cellulose. Esterification of cinnamic acids to either isolated hemicelluloses (Jung, 1988a) or cellulose (Jung and Sahlu, 1986) will also inhibit glycanolysis. Phenyl-esterases are produced by rumen fungi (Borneman *et al.*, 1990a) and bacteria (Akin *et al.*, 1993; McDermid *et al.*, 1990). Feruloyl esterases liberate ferulic acid from xylan oligomers, synergistically with xylanases, which must first liberate oligomers in the medium (Faulds and Williamson, 1991).

Phenolic acids released in the rumen medium may have a limited antimicrobial effect, which has been shown *in vitro* (Chesson *et al.*, 1982; Jung and Fahey, 1983; review of Martin, 1990). Likewise, isolated LCCs decrease microbial activity (Cherney *et al.*, 1992). However, except in microenvironments, phenolic acids are produced in subtoxic amounts (Jung and Ralph, 1990) and transformed to phenylpropanoic acid which is considered as a growth factor (Hungate and Stack, 1982). As shown by Besle *et al.* (1988) in a semi-continuous fermentor, it is doubtful that any consequent inhibitory effect appears *in vivo*. A reduction in microbial adhesion is also possible (Varel and Jung, 1986), but this effect is not significant (Roger and Fonty, personal communication).

Positive effects of LCCs

Release of soluble LCCs could have positive effects, limiting the shielding of structural

polysaccharides by lignins. Soluble LCCs accounting for 43% of the total lignin intake have been found in the rumen liquor of steers fed tropical grass (Gaillard and Richards, 1975). LCCs were also found in the rumen of sheep fed ryegrass (Conchie *et al*, 1988) and after *in vitro* incubation of wheat straw (Nordkvist *et al*, 1989). It is not known if the carbohydrate moiety is further degraded in the rumen, but these compounds probably precipitate in the acidic conditions found in the abomasum (Neilson and Richards, 1978) and the lignin portion is indistinguishable from other lignins in the faeces. Chesson (1981) has shown that an alkali treatment releasing 40% of barley straw lignin in association with carbohydrates, was sufficient to result in nearly complete *in situ* degradation of the remaining carbohydrates. Mosoni *et al* (1993) observed a similar degradation of wheat straw apical internode after a sodium hydroxide extraction that gave 76% delignification. The positive effect of LCC release in the rumen on carbohydrate hydrolysis may therefore be high. This effect could, however, be partly counterbalanced by some inhibitory effect of soluble LCCs on rumen enzyme activities (Jung, 1988b). An analytical study of the net effect of transformations undergone by LCCs in the digestive tract should be of relevance.

Heterogeneity of the effects of LCCs

Chesson's model (Chesson, 1993) shows a possible variation of the nature and effects of LCCs within the different layers of the cell wall. Likewise, structural heterogeneity corresponding to diverse cell-wall architecture may also produce different effects on degradation between plant species. This is the case, for example, for differences in cell-wall degradation kinetics observed between grasses and legumes. After glycanalysis of Björkman LCCs, the insoluble residue from ryegrass was enriched in phenolic acids and

contained more carbohydrates (arabinose and xylose) than that from alfalfa (Kondo *et al*, 1990c). The authors suggested that ferulic acid could be responsible for the enzyme-resistant bonds in ryegrass LCC. The low phenolic acid content observed in legumes means that direct linkages between lignins and polysaccharides are enhanced. Titgemeyer *et al* (1992) identified glucuronoxylan fractions from alfalfa stems with a high resistance to degradation, probably due to ester linkages of the uronic side chains with lignins.

No soluble LCCs were found in the rumen of steers eating high quality alfalfa and coastal bermudagrass hay (Windham *et al*, 1989). Thus, the undigestible residue of these highly digestible forages may contain all the original lignin (8.7 and 3.7% respectively). This lignin could be of a different type from that found in soluble LCCs of less digestible forages. It could be localised in walls that lignify first, less in weight but more inhibitory.

It should be emphasized that the effects of LCCs on cell-wall degradation, for specific conditions of microbial attack, reflect not only the frequency of recalcitrant linkages, but also result (if a holistic approach is taken) from the combined effects of the various factors determining the cell-wall environment, namely architecture, tissue arrangement, and presence of minerals (silica).

CONCLUSION

Lignins and hemicelluloses are linked through several types of covalent bonds. Heterogeneity of linkages is observed across plant families and species and within tissues and cell walls. The types of LCCs in the wall are determined by the monomers present and by the process of lignification. Several structures have been suggested or indirectly proposed. Some of these structures are cleaved by the enzymic activities

present in the rumen and it is not known if totally resistant LCC bonds occur.

Mechanisms explaining the role of LCCs have been suggested. The superficial release of LCCs would have several consequences on cell-wall degradation. The frequency of linkages probably explains a part of the wall resistance but the importance of this resistance is related to all the factors of the cell-wall environment.

Further work is needed to define the structures and to elucidate cell-wall resistance mechanisms. Moreover, it should be worthwhile enhancing the positive effect of LCC release and studying the fate of LCCs in the digestive tract in relation to carbohydrate digestion. A more complete understanding of the nature and effects of LCCs may have diverse agronomic consequences: in plant breeding, prediction of nutritional value, and utilisation of low quality forage. It must be noted, however, that the resistance of cell walls to microbial degradation, a drawback in ruminant nutrition, may be an advantage for the plant.

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