Severe Inhibition of Fiber Degradation by Cinnamyl Aldehyde-Containing Lignins

J.H. Grabber, J. Ralph and R.D. Hatfield

Introduction

Lignins are formed by peroxidase/H_2O_2-mediated polymerization of *p*-coumaryl, coniferyl and sinapyl alcohols. In plants where cinnamyl-alcohol dehydrogenase (CAD) activity is low (e.g. brown midrib mutants and CAD antisense plants), *p*-hydroxycinnamyl aldehydes probably also become a major component of lignin. It is not known whether lignin composition affects the degradability of cell wall polysaccharides. Such information would provide a rational basis for directing plant selection or molecular engineering efforts aimed at improving the bioconversion of structural polysaccharides into metabolizable energy for livestock or into ethanol fuels. We used a biomimetic model system to determine if alterations in lignin composition affect the degradability of cell walls.

Methods

Cell walls from cell suspensions (*Zea mays* L. cv. Black Mexican) were synthetically lignified with several types of monolignols. Cell walls were analyzed for neutral sugars, uronic acids and Klason lignin. Cell walls (100 mg in 10 mL of 20 mM acetate buffer, pH 4.8, 39°C) were degraded with hydrolases from *Trichoderma reesei* (4 µL of Celluclast, NOVO) and *Aspergillus niger* (4 µL of Viscozyme L, NOVO). Supernatant samples were analyzed for total sugars and uronic acids and for neutral sugars after TFA hydrolysis.

Results and Discussion

Nonlignified cell walls were rapidly and extensively degraded by fungal hydrolases (Table 1). Lignins formed with coniferyl aldehyde were much more inhibitory to cell wall degradation than lignins formed with coniferyl, sinapyl or *p*-coumaryl alcohols (Table 2 and 3). Cell-wall digestion by mixed rumen microorganisms was also severely restricted by aldehyde-containing lignins. Lignification, particularly with coniferyl aldehyde, reduced the release of all neutral and acidic sugars by fungal hydrolases, especially that of xylose. When aldehyde groups were selectively reduced to alcohols (by ethanolic-sodium borohydride) prior to enzyme hydrolysis, degradability differences were largely eliminated. This suggests that high hydrophobicity of aldehyde-containing lignin was responsible for depressed cell wall degradation.

Conclusions

Our results indicate that incorporation of *p*-hydroxycinnamyl aldehydes into lignin, via down regulation of CAD, will severely restrict the bioconversion of structural polysaccharides into metabolizable energy for livestock and into ethanol fuels. In contrast, altering the type of *p*-hydroxycinnamyl alcohol incorporated into lignin is not expected to change the enzymatic degradation of plant cell walls.

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Table 1. Lignin concentration and fungal-hydrolase degradability of cell walls synthetically lignified with coniferyl alcohol and coniferyl aldehyde.

Monolignol	Klason lignin	Total sugars released 6 h	72 h
	- - - - - - - - mg/g of cell wall - - - - - - -		
None (nonlignified)	6[a]*	421[a]	750[a]
Coniferyl alcohol	133[b]	201[b]	487[b]
Coniferyl aldehyde	141[b]	104[c]	306[c]
Coniferyl alcohol + coniferyl aldehyde (1:1 ratio)	139[b]	136[c]	400[bc]
CV%	8.7	9.0	8.4

*Means followed by the same letter are not significantly different ($P < 0.05$).

Table 2. Lignin concentration and fungal-hydrolase degradability of cell walls synthetically lignified with *p*-coumaryl, coniferyl and sinapyl alcohols.

Monolignol	Klason lignin	Total sugars released 6 h	72 h
	- - - - - - - mg/g of cell wall - - - - - - - -		
Coniferyl alcohol	111[a]	221[a]	530[a]
Coniferyl + *p*-coumaryl alcohol (1:1 ratio)	111[a]	212[a]	507[a]
Coniferyl + sinapyl alcohol (1:1 ratio)	102[a]	220[a]	527[a]
CV%	5.2	5.6	7.1

*Means followed by the same letter are not significantly different ($P < 0.05$).