Ferulate Polysaccharide Esters as Nucleation Sites for Lignification

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Introduction

Biomimetic lignification systems and polysaccharide-lignin isolates from grasses have revealed that ferulate polysaccharide esters are oxidatively coupled to lignin. Model studies with maize cell suspensions were conducted to determine if ferulates act as nucleation sites for lignification and to provide detailed information on the types of cross-products formed with ferulate esters and coniferyl alcohol.

Methods

Cell walls isolated from maize cell suspensions were treated with dilute hydrogen peroxide and synthetically lignified with 0 to 8 equilavents of coniferyl alcohol per unit of cell wall ferulate. Cell walls with γ–^{13}C labelled ferulate were treated with dilute hydrogen peroxide and lignified with one-equilavent of coniferyl alcohol or β-5 dehydrodiconiferyl alcohol per unit of wall ferulate. Cell walls were subjected to alkaline hydrolysis at room temperature or at 170°C to release ferulates and cross-products formed between ferulates and lignin. Hydrolysates were acidified and extracted with ethyl acetate. Extracts were analyzed by GC-FID, GC-MS, and NMR.

Results and Discussion

Nonlignified walls were incubated with an excess of H_2O_2 to stimulate oxidative coupling of ferulate monomers into dehydrodimers by wall-bound peroxidase. Dehydrodimers comprised 55% of total

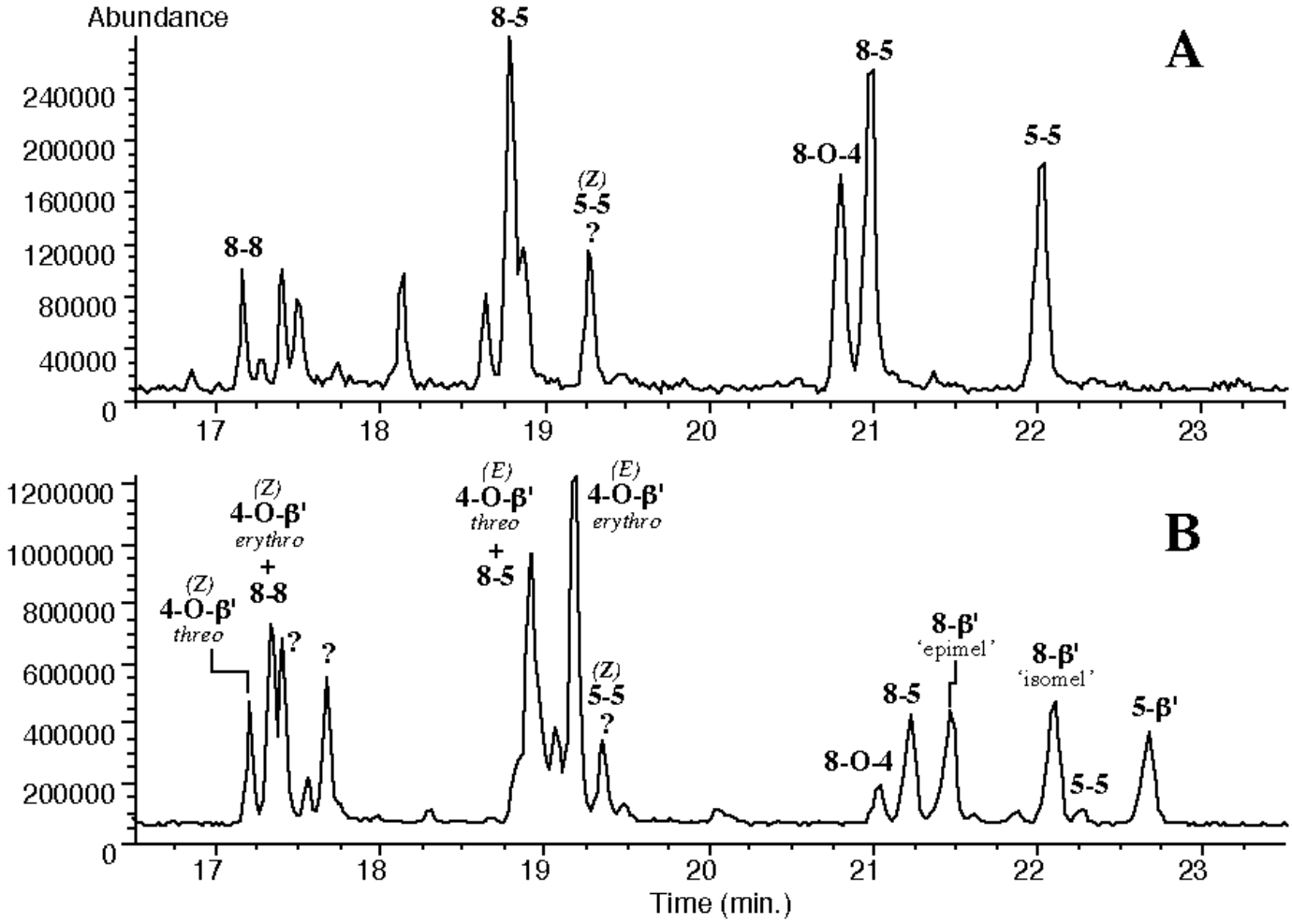

Figure 1. GC-MS total ion chromatogram of ferulate and ferulate-coniferyl alcohol cross-products recovered after saponification of (a) nonlignified maize walls and (b) maize walls lignified with coniferyl alcohol.

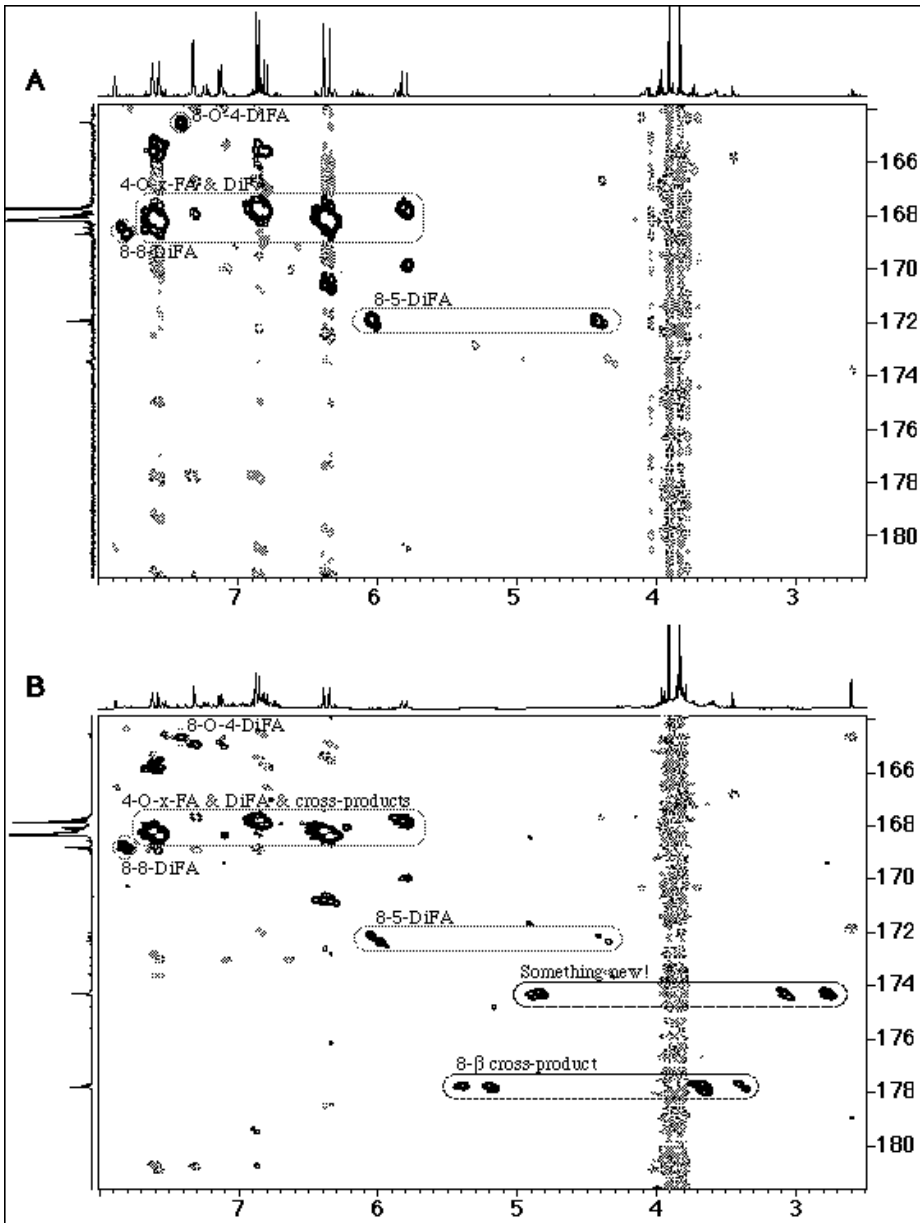

Figure 2. C-H correlation (HMBC) spectra of ferulates and ferulate-lignin cross-products recovered after saponification of (a) nonlignified maize walls and (b) maize walls lignified with coniferyl alcohol. Folded-in peaks are grayed. Correlations corresponding to a new product are currently under investigation.

ferulates in H_2O_2-treated walls. This high degree of dimerization is remarkable considering the low concentration of ferulates (17 mg/g) in cell walls. Ferulate moieties of dehydrodimers were coupled primarily by 8-5 linkages (53%) followed by 8-O-4 (20%), 5-5 (17%) and 8-8 (10%) linkages. Ferulate monomers and 5-5 coupled dehydrodimers were extensively incorporated into lignin (95%). In contrast, dehydrodimers coupled at the 8-position had a lower propensity to incorporate into lignin (78 to 90%). The incorporation of dehydrodimers into lignin is probably controlled by steric factors and by the number of phenols available for radical coupling reactions.

GC-MS and NMR analyses of wall extracts and authentic compounds revealed ferulate-coniferyl alcohol dehydrodimers were coupled exclusively by 4-O-β', 8-β', and 5-β' linkages (Fig. 1). (In labeling cross-products, the first term indicates the coupling site on the ferulate ester, and the primed term indicates the coupling site on the lignin moiety.) The absence of 8-5', 8-O-4' and 5-5' coupled cross-products indicates that ferulates couple exculsively to the β–position of coniferyl alcohol monomers; coupling at other positions occurs only with coniferyl alcohol dimers and oligomers in which the conjugated side chain is no longer present. Ferulate monomers and dehydrodimers differed substantially in their propensity to form ether-linked structures with lignin; high temperature alkaline hydrolysis released 30% of ferulate monomers compared to 28% of 5-5, 41% of 8-8, 61% of 8-O-4, and *ca* 60% of 8-5 coupled dehydrodimers.

Conclusions

At the initiation of lignification, arabinoxylans become extensively cross-linked by oxidative coupling of ferulate monomers into dehydrodimers. Ferulate monomers and dehydrodimers act as nucleation sites for lignification and their incorporation into lignin results in further cross-linking of the cell wall matrix. Ether linkages comprised 30 to 60% of the linkages between ferulates and coniferyl alcohol.