Polysaccharide Integrity as Related to the Degradation of Lignin in Wood by White-Rot Fungi

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ABSTRACT

Previous research has shown that much of the lignin in wood can be degraded by certain white-rot fungi without proportional depletion of carbohydrates. Data are presented here which suggest that lignin in wood may be degraded by these white-rot fungi without proportional degradation of the polysaccharides as well. It follows that the polysaccharides in wood probably do not present a significant protective barrier to the degradation of lignin by white-rot fungi.

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TABLE 1. Analytical data for sound and white-rotted birch wood

Fungus	Wt. loss of wood (%)	Lignin in wood (%)	Change in lignin content[1] (%)	Holocellulose (%)	Holocellulose (% of non-lignin material)[2]	Change in holocellulose (%)[3]	$[\eta]$[4] of holocellulose ml · 100 / g	Change in $[\eta]$[5] (%)
None (control)	0	21.5	0	82.5	105	0	6.0	0
Fungi that deplete lignin and carbohydrates proportionately								
Ganoderma applanatum (Pers. ex Wallr.) Pat.	17	21.1	-2	75.3	96	-9	5.7	-5
G. applanatum	32	20.0	-7	60.2	75	-29	4.2	-30
Polyporus versicolor L. ex Fr.	21	18.7	-13	76.4	94	-10	4.5	-25
P. versicolor	36	20.6	-4	72.4	91	-13	5.0	-17
Fungi that deplete lignin preferentially								
Fomes ulmarius (Sow. ex Fr.) Gill.	15	14.7	-32	76.9	90	-14	5.6	-7
Polyporus berkeleyi Fr.	8	16.2	-25	73.6	88	-16	5.4	-10
P. berkeleyi	22	16.0	-26	68.5	82	-22	4.4	-27
P. berkeleyi	38	12.8	-40	68.8	79	-25	4.5	-25
Polyporus resinosus Fr.	11	15.7	-27	70.9	84	-20	5.3	-12
P. resinosus	22	15.4	-28	65.9	78	-26	5.3	-12

[1] $\frac{21.5\% - \%\text{ lignin in wood}}{21.5\%} \cdot 100 = \%$ change in lignin content.

[2] $\frac{\%\text{ holocellulose}}{100\% - \%\text{ lignin in wood}} \cdot 100 =$ holocellulose as % of non-lignin material.

[3] $\frac{105\% - \text{holocellulose as \% of non-lignin material}}{105\%} \cdot 100 = \%$ change in holocellulose.

[4] $[\eta]$ = intrinsic viscosity.

[5] $\frac{6.0 - [\eta]}{6.0} \cdot 100 = \%$ change in $[\eta]$.

From studies of the solubilization of wood and modified woods by polysaccharidases and by microorganisms, it appears that the lignin, physically, and perhaps to some extent chemically, serves as a rather effective barrier to the enzymatic degradation of the polysaccharides (3, 7). It is not known whether the converse is also true; i.e., whether the intact polysaccharides also serve as a barrier to the enzymatic degradation in lignin. One indication that the polysaccharides may not be such a barrier is that a substantial part of the lignin in wood can be depleted disproportionately by some white-rot fungi (4, 5). Nevertheless, it is possible that the polysaccharides must still be degraded, even though not necessarily removed, as lignin is attacked. Cowling (2) showed that the polysaccharides remaining in sweetgum wood after various extents of decay by *Polyporus versicolor* L. ex Fr. were only slightly degraded, and that in this case the lignin and polysaccharides were removed simultaneously.

In the study reported here, holocellulose preparations were isolated from birch wood decayed by several white-rot fungi that deplete lignin faster than carbohydrates (5). These were compared with holocellulose preparations from sound wood, and from wood decayed by two fungi that do not deplete lignin preferentially, in order to determine whether the lignin depletion was accompanied by a proportional loss of the integrity of the polysaccharides.

The criteria for assessing the polysaccharide integrity were (i) yield of holocellulose and (ii) intrinsic viscosity, $[\eta]$, of the holocellulose. Hollocellulose is the total polysaccharide component of wood left after chemical delignification (1, 6). Holocellulose does not necessarily include all the carbohydrates since those of low molecular weight are lost during the holocellulose preparation. Thus, the yield of holocellulose provides one indication of the polysaccharide integrity. Intrinsic viscosity, $[\eta]$,

gives an indication of the average chain length, or average degree of polymerization, and thus gives an indication of holocellulose integrity. Therefore, if lignin depletion depended on proportional degradation of the polysaccharides, a correlation should be found between the change in lignin contents of the wood on decay and (a) the change in yield of holocellulose and/or (b) the change in $[\eta]$ of the holocellulose.

MATERIALS AND METHODS.—Extracted wafers of kiln-dried birch (*Betula alleghaniensis* Britton) sapwood were decayed in modified soil-block chambers (5). Single isolates of the fungi were used. Two of the fungi, *P. versicolor* and *Ganoderma applanatum* (Pers. ex Wallr.) Pat., decomposed the lignin and carbohydrates at rates approximately proportional to the amount of each substance present, whereas *Fomes ulmarius* (Sow. ex Fr.) Gill., *P. berkeleyi* Fr. and *P. resinosus* Fr. depleted the lignin more rapidly than the carbohydrates (5). Wafers comprising narrow weight loss ranges (±1%) with each fungus were combined and ground to pass a 40-mesh screen.

Holocellulose was prepared from 2-g samples by chlorination followed by extraction with monoethanolamine and ethanol (1, 6). Six to eight chlorination-extractions were required for complete removal of lignin, determined visually by color formation on addition of the amine (1, 6). Yields of holocellulose were recorded, and intrinsic viscosities were determined with cupriethylenediamine solutions by the method of Cowling (1). Analysis of the glucose:xylose:mannose ratios in acid hydrolysates of the holocellulose was made paper chromatographically (6). Lignin was determined by the 72% sulfuric acid method (1, 6).

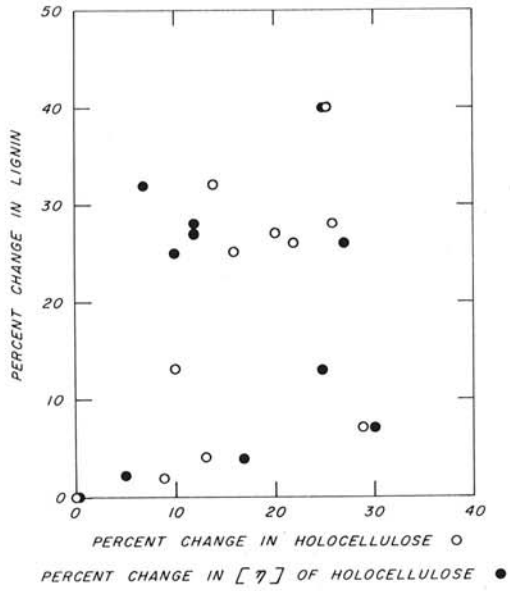

Fig. 1. Plots showing change in lignin vs. change in holocellulose (○), and change in lignin vs. change in intrinsic viscosity $[\eta]$ of holocellulose (●) for birch wood decayed by various white-rot fungi.

TABLE 2. Relative amounts of glucose, xylose, and mannose in acid hydrolysates of holocellulose samples from sound and white-rotted birch wood

Fungus	Wt loss of wood on decay (% of original wood)	Polysaccharide ratios (%)		
		Glucose	Xylose	Mannose
None (control)	0	64	33	3
Fomes ulmarius	15	71	26	3
Ganoderma applanatum	17	63	34	3
Polyporus berkeleyi	22	65	32	3
P. resinosus	22	68	29	3
P. versicolor	21	66	31	3

[a] Values expressed so that the sum glucose + xylose + mannose = 100%.

RESULTS AND DISCUSSION.—The yield of holocellulose from each of the decayed samples was lower than from the sound sample (Table 1), despite the lower lignin contents. Yields of holocellulose are expressed in two ways in Table 1: as a percentage of the sample, and as a percentage of the nonlignin material in the sample. The latter more than compensates for the higher total carbohydrates that are indicated by the low lignin contents (see ref. 5). [The 105% value for the sound wood (Table 1) probably indicates inclusion of a small amount of lignin in the holocellulose]. There was no relationship between the change in yield of holocellulose, expressed on this latter basis, and the change in lignin (Fig. 1).

The intrinsic viscosities $[\eta]$ of the holocellulose samples from the decayed wood were from 70-95% of that of sound wood (Table 1); $[\eta]$ was generally lower with increasing decay (total weight loss) of the wood samples (correlation coefficient, $r = 0.73$), a result that accords with the observation of Cowling on *P. versicolor* and sweetgum wood (2). There was no relationship, however, between the changes in lignin content of the wood samples and changes in $[\eta]$ of the holocellulose preparations (Fig. 1).

The observed general lowering of the degree of polymerization of the holocellulose on decay could reflect either degradation of the polysaccharides or a substantial decrease in the ratio of cellulose to hemicelluloses. An increase in the proportion of hemicelluloses would cause a lowering of $[\eta]$ because hemicelluloses have much lower degrees of polymerization than does cellulose. Since birch hemicellulose is mostly xylan (8), estimation of the proportion of xylose residues provides an index of hemicellulose content. From the glucose:xylose:mannose ratios in acid hydrolysates of the holocellulose samples (Table 2), it can be deduced that most of the holocellulose samples were similar in the ratio cellulose:hemicellulose; most of the decayed

samples perhaps being slightly higher in cellulose. Therefore, a simple change in the ratio cellulose:hemicellulose does not account for the changes in the degree of polymerization of the holocellulose during decay. It follows that the lowering of the average degree of polymerization, as well as the lowering of the yield of the holocellulose on decay, points mainly to some degradation of either the hemicelluloses or the cellulose, or both. In any case, the results presented here indicate that the fungi most active in the disproportionately high degradation of lignin are not necessarily most active in degrading the polysaccharides.

Previous work has shown that much of the lignin in wood can be removed more rapidly than polysaccharides by white-rot fungi (4, 5). This indicates that carbohydrate removal need not accompany lignin degradation. Results of the present study suggest that carbohydrate degradation also is not closely tied to lignin degradation. Thus, polysaccharides probably do not serve as a significant barrier to the degradation of much of the lignin in wood by white-rot fungi.

The data here indicate that *G. applanatum* and *P. versicolor* degraded the polysaccharides without a proportional depletion of lignin (Table 1). Thus, the behavior of these fungi apparently contradicts the generality made at the beginning of this paper that lignin provides a barrier to polysaccharide degradation in wood. Another apparent contradiction exists in the brown-rot type of wood decay, in which polysaccharides are severely degraded without a proportional degradation of lignin (2). With the brown-rot fungi this severe degradation of polysaccharides may be nonenzymatic (3; also J. Koenigs, *unpublished*), in which case the lignin would not be likely to protect the polysaccharides.

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