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Protection Group Effects during α,γ-Diol Lignin Stabilization Promote High-Selectivity Monomer Production

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Abstract: Protection groups were introduced during biomass pretreatment to stabilize lignin's α , γ -diol group during its extraction and prevent its condensation. Acetaldehyde and propionaldehyde stabilized the α , γ -diol without any aromatic ring alkylation, which significantly increased final product selectivity. The subsequent hydrogenolysis catalyzed by Pd/C, generated lignin monomers at near-theoretical yields based on Klason lignin (48% from birch, 20% from spruce, 70% from high-syringyl transgenic poplar) with – in the case of the poplar – high selectivity to a single 4-*n*-propanolsyringol product (80%). Unlike direct hydrogenation of native wood, hydrogenolysis of protected lignin with Ni/C also led to high selectivity to this single product (78%), paving the way to high-selectivity lignin upgrading with base metal catalysts. The use of extracted lignin facilitated valorization of biomass's polysaccharides, leading to high yields of all three major biomass polymers to a single product.

Due to limited fossil reserves and increasing environmental concerns over petroleum use, converting renewable resources such as lignocellulosic biomass into chemicals, materials, and fuels has attracted substantial attention in recent years^[11]. Lignin, a heteropolymer of phenylpropanoid units, is one of the three major components in lignocellulosic biomass and is the most abundant natural source of aromatic molecules^[21]. For this reason, lignin is often presented as a future feedstock for aromatic chemicals^[3]. However, few commercial processes or biorefinery schemes are being developed for lignin compared to other bio-polymers like cellulose or hemicellulose. These polysaccharides can be upgraded at high yields (~70-100%) to simple sugars such as glucose and xylose using relatively simple processes^[4]. In contrast, there are limited practical processes that can convert lignin at high yields and high selectivity to a narrow set of products, while remaining compatible with sugar production.

This production of sugars often involves biorefinery fractionation processes that use organic solvents^[5] or water^[6] at high temperature with mineral acids to effectively remove lignin (and hemicellulose). But the resulting lignin systematically suffers from irreversible repolymerization that limits its ability to be upgraded at high yields. Several studies have developed so-called "lignin first" extraction methods, where native wood is treated in the presence of a heterogeneous metal catalyst that allows for the rapid hydrogenation of lignin intermediates before they condense^[7]. However, these methods introduce catalyst separation issues from unreacted wood and usually result in at least partial conversion of the hemicellulose fraction of biomass, which limits their integration into current biorefinery schemes.

We recently proposed a method enabling efficient lignin extraction and depolymerization that was compatible with the traditional upgrading of biomass polysaccharides^[8]. Formaldehyde added during lignin extraction reacted with the α,γ -diol on the side chain of lignin by forming a 1,3-dioxane structure. This protection group completely suppressed undesirable repolymerization, providing high monomer yield (47-78%) in the following hydrogenolysis with a precious metal catalyst. However, alkylation of aromatic rings by formaldehyde and unselective hydrogenolysis complicated the product mixture leading to the production of 6-10 major monomers. Here, we report for the first time that by modifying the protection group on α,γ -diol protected lignin, high yields and selectivities can be achieved to only two products (4-*n*propanolguaiacol (POHG) and 4-*n*-propanolsyringol (POHS)). Furthermore, when a transgenic high-syringyl poplar is used, only a single major product (POHS) can be obtained.

We investigated the effect of adding different diol protection reagents during the lignin extraction process on the final lignin monomer yield after hydrogenolysis (Figure 1 and Table S1). As a control, we performed the same extraction without a protection reagent in triplicate and the average yield was 14 wt% with a 95% confidence interval between 13-15 wt%. Therefore, any experiment resulting in a yield value outside of this range was very likely due to the effect of protection reagents. The first reagents that we tried were aldehydes that form cyclic acetal with the α,γ -diol of the lignin side chain. All aldehydes except for hydroxymethylfurfural led to a statistically significant improvement in monomer yield. In the case of acetaldehyde and propionaldehyde, the monomer yields were 37 and 42 wt%, respectively, which were comparable to that obtained with formaldehyde (46 wt%). Interestingly, no alkylated aromatic monomers were detected, resulting in higher selectivity to ethylsyringol and ethylguaiacol. Ketal groups, especially acetonide, are some of the most common diol protection groups in carbohydrate chemistry. Therefore, acetone, butanone and other related reagents that protect 1,2 and 1,3-diol were also tested. In all of these experiments, some protective effect was detected during lignin extraction, resulting in moderate but statistically significant monomer yield improvements after hydrogenolysis. We also tested carbonate and boronate protection groups that were known to react with alcohol groups under alkaline conditions. The use of dimethyl carbonate and phenylboronic acid as protection reagents resulted in monomer yields of 16 and 27 wt%, respectively (above the yield of the control experiment, see Section 2.2 in the SI). This study demonstrated that while all diol protection groups were effective in stabilizing lignin to some degree, aldehydes were the most effective. Both acetaldehyde and propionaldehyde showed especially remarkable activity in protecting the $\alpha.\gamma$ -diol position and preventing condensation during lignin extraction and pretreatment conditions.

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Figure 1. Hydrogenolysis of birch lignin extracted with the addition of different protection reagents in dioxane. Extraction condition, 80 °C, 5 hours; Hydrogenolysis condition, Ru/C, 250 °C, 40 bar of H₂, 15 hours. Monomer yields are given based on klason lignin content (see Table S1 for details)

To better understand the reaction mechanism, we performed reactions using the common lignin dimeric model compound veratrylglycerol- β -guaiacyl ether (VG) and acet- or propionaldehyde. The yields of ethylidene acetal and propylidene acetal protected VG were 90% and 86%, respectively, after a 30-min reaction, slightly lower than that of methylene acetal protected VG (92%). The formation of the acetal protected structures were confirmed by NMR (Figure 2, A and B).

According to the HSQC spectra, the major compound of the mixture was the acetal protected VG with trace amounts of unreacted VG. A detailed inspection of the HSQC and COSY spectra revealed no peaks originating from –CH₂OH/–or CH₂CH₂OH in their corresponding region (green dash line), indicating that no hydroxyethylation or hydroxypropylation occurred during the reaction. After a subsequent hydrogenolysis reaction, no products with additional ethyl/propyl side

COMMUNICATION



Figure 2. HSQC spectra of the product mixture resulting from the reaction between veratrylglycerol- β -guaiacyl ether and (A) acetaldehyde, (B) propionaldehyde, or isolated birch lignin extracted in the presence of (C) acetaldehyde or (D) propionaldehyde. The contour areas were integrated to calculate the S:G ratio and interunit linkage distributions. S:G equals (S_{2/6})/2:(G₂); AA α , A α , plus C α equals 100%. The green dashed line indicates the region of hydroxypropyl and hydroxyethyl correlations.



Figure 3. Reaction mechanism for the hydrogenolysis of form-/acet-/propionaldehyde stabilized lignin. When using formaldehyde during lignin extraction, alkylation on the aromatic rings occurred and the corresponding products were generated. When using Ru/C as the catalyst, propanolphenolic monomers were largely converted to ethylphenolic monomers and demethoxylation of syringyl units occurred; when using Pd/C as the catalyst, these reactions were mostly suppressed and the major products were propanolphenolic monomers.

chains on the aromatic ring were detected (Figure S3), confirming the results obtained with native lignin in birch (Figure 1). Lignin extracted from birch in the presence of both acet- and propionaldehyde were also

prepared for NMR characterization (Figure 2 C and D) and confirmed the structure observed with the model compound. Contours corresponding to each structure were integrated to calculate the S/G ratio

COMMUNICATION

and the percentage of different inter-linkages. Results showed that more than 90% of the β -O-4 structure was protected by an ethylidene or propylidene acetal group, which was consistent with the model compound study.



Figure 4. Monomer yields (on the basis of klason lignin) and product distribution resulting from the hydrogenolysis of propylidene acetal protected lignin extracted from F5H poplar (see Table S2 for details). *Direct hydrogenolysis. The molecules corresponding to each color are given in Figure 1.

The absence of aromatic ring alkylation when acet- or propionaldehyde were used as protection reagents instead of formaldehyde reduced the number of products by half, allowing for high selectivity lignin upgrading (Figure 3). We (and others) had previously shown that hybrid poplar in which the ferulate 5-hydroxylase (F5H) gene was overexpressed contained a much higher proportion of syringyl units in the lignin (more than 95%)^[9], resulting in higher content of cleavable β -O-4 ethers^[8]. We therefore used F5H poplar as a feedstock to get higher monomer yields and a higher product selectivity to syringyl monomers. The results of hydrogenolysis of lignin extracted with the addition of propionaldehyde are shown in Figure 4 (and Table S2). Using the same extraction conditions, a 54% monomer yield was obtained with propionaldehyde compared to 75% for formaldehyde. With formaldehyde, long extraction time benefited lignin extraction. However, considering that propylidene acetal is less stable than methylene acetal, a longer extraction time could favor the partial cleavage of the protection groups and lead to some lignin repolymerization. Therefore, we reduced the lignin extraction time to 2.5 h, which increased the monomer yield to 65 wt%. Under these conditions, about 20% of guaiacyl monomers were obtained, which is higher than what would be expected based on the native lignin (~5%). This discrepancy was likely due to the demethoxylation of syringyl units during hydrogenolysis over Ru/C. We also assumed that ethylphenols were mainly produced from propanolphenols after cleaving of the -CH₂OH group over Ru/C (Figure 3). Model compound studies using POHS and propylsyringol (PS) as reactants for hydrogenolysis demonstrated the cleavage of -CH2OH and demethoxylation under the same reaction conditions (Figure S4). We lowered the temperature

and/or time of hydrogenolysis to suppress this demethoxylation and CH₂OH cleavage (Figure 4). When decreasing the reaction time from 15 h to 5 h, a 73% monomer yield was obtained. Additionally, the POHS and POHG products were favored at a lower temperature: 60% selectivity to POHS was reached at 200 $^{\circ}$ C for 15 h over Ru/C and the fraction of the syringyl products increased to 88%.

To further increase the selectivity to POHS/POHG, Pd/C was used as the hydrogenolysis catalyst due to its low alcohol hydrogenation activity^[10]. By running hydrogenolysis at 200 °C for 20 h, a yield of 70% monomers was obtained with 80% selectivity to a single POHS product (89% to POHS+POHG) (Figure 4), which is a potential precursor of adhesives and polymers. HSQC spectra of the hydrogenolysis product mixtures further confirmed the monomers structure, and demonstrated full conversion of β -O-4 linkages of the lignin polymer (Figure S5). This result is in agreement with previous work by Sels and colleagues, which also reported a high selectivity to POHG and POHS when treating birch sawdust mixed with Pd/C in MeOH to directly upgrade native lignin^[7a]. Here, we have achieved similar results with extracted lignin, as well as significantly higher selectivities to a single product (POHS) when using F5H-poplar. Ni has often been proposed as a non-noble metal hydrogenolysis catalyst for lignin, producing mainly propylphenolic monomers during direct hydrogenolysis of untreated wood. When F5Hpoplar was used during direct hydrogenolysis of untreated wood with Ni/C in MeOH, a relatively low product selectivity was obtained (79% monomer yield with a 59% selectivity to PS and only 29% to POHS). Interestingly, when propionaldehyde-protected extracted lignin was used, a selectivity of 78% to POHS was achieved with an only slightly diminished yield of total monomers (72%) (Figure 4). The acetal protection group is likely causing a suppression of alcohol hydrogenation over Ni, but further study of the mechanism is still underway.



Figure 5. Monomer yields (on the basis of klason lignin) and product resulting from the hydrogenolysis of lignin extracted from F5H poplar, birch, and spruce with addition of acetaldehyde (AA) and propionaldehyde (PA). The extraction conditions were 80 °C, 2.5 hours for F5H poplar and birch, and 3.5 hours for spruce. The hydrogenolysis was performed at 200 °C, with 40 bar of H₂ for 15 hours with Pd/C (see Table S2 for details). The molecules corresponding to each color are given in Figure 1.

COMMUNICATION

We also performed lignin extraction with acet- and propionaldehyde as well as hydrogenolysis with Pd/C with birch and spruce (Figure 5 and S6). In all cases, more than 91% selectivity to POHS and POHG monomers was achieved. Total monomer yields were those that are typically expected for both wild type species based on their fraction of inter-unit ether linkages: 48% and 20% for birch and spruce, respectively (extraction yields were within 10% of the yield from direct hydrogenolysis of untreated wood^[11]). The relatively low yield from spruce is consistent with the low β -aryl ether content in softwood lignin, as reported elsewhere^[11a, 11b]. Nevertheless, due to softwoods having only guaiacyl linkages, the selectivity to the single POHG product was 91%.

Besides lignin depolymerization, recovery of carbohydrates was also evaluated. Unlike direct hydrogenolysis of native wood, which causes irreversible conversion of some of the carbohydrates, this method generated clean cellulose or acetal-protected carbohydrates (mostly xylose). The latter could be easily deprotected by aqueous acidic treatment (77% of xylose was obtained after 10 min at 120 °C in 6 wt% H₂SO₄, Figure 6 and S7 A). The leftover solids after pretreatment could undergo a typical enzymatic hydrolysis process without any further acid treatment, generating an 80% yield of glucose (Figure S7 B). The high enzymatic hydrolysis yield without any further acid treatment was a significant improvement over our previous work with formaldehyde^[8], where the resulting cellulose required a harsh acid treatment to remove formyl groups that caused significantly reduced digestibilities (~10%). We suspect that any binding of propionaldehyde to cellulose was much more easily reversible than that of formaldehyde, and was likely removed even under the mild conditions (50°C, pH 5).



Figure 6. Mass balance of polysaccharides (glucan and xylan) and lignin fractions of biomass (Table S3, entry 14 and Figure S7).

In summary, we developed a high yield lignin extraction and depolymerization process that is compatible with most biorefinery schemes and can, depending on the biomass source, produce a single product at high selectivity. To our knowledge, we are reporting the highest selectivities obtained to a single product from hardwood lignin depolymerization, and the instance of a non-precious metal being used for high-selectivity lignin hydrogenolysis with molecular hydrogen. Because lignin is extracted under typical pretreatment conditions, both xylose and glucose can be produced at high yields as well, leading to mostly single products from all three major biopolymers.

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COMMUNICATION

Entry for the Table of Contents

COMMUNICATION



Lignin extraction in the presence of acetaldehyde or propionaldehyde prevents lignin condensation and facilitates the subsequent hydrogenation, generating 4-*n*-propanolphenolic monomers at >90% selectivity with high monomer yields (>70%). Because lignin is extracted under typical pretreatment conditions, both xylose and glucose can be produced at high yields after acid hydrolysis of the liquor and enzymatic hydrolysis of the remaining solids.

Wu Lan, Masoud Talebi Amiri, Christopher M. Hunston, Jeremy S. Luterbacher*

Page No. – Page No.

Protection Group Effects during α,γ-Diol Lignin Stabilization Promote High-Selectivity Monomer Production