A genetic strategy for avoiding formation of hexenuronic acid in kraft pulping?

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Abstract

By using model compound experiments we have found that the methyl group on 4-O-methylglucuronic acid is of central importance for the formation of hexenuronic acid from xylan during kraft pulping, and that unmethylated glucuronic acid virtually not form hexenuronic acid. A literature survey indicate that xylan with unmethylated glucuronic acid may fulfill the biological functions of the hemicellulose, and therefore genetic manipulation of trees in order to avoid methylation of the glucuronic acid side chain of xylan may be a strategy for avoiding formation of hexenuronic acid in kraft pulping.

Keywords: Hexenuronic acid; kraft pulping; β elimination; transgenic trees.

Introduction

Genetic manipulation of trees for better performance in pulping has gained large interest the last years. Examples of goals are increased cellulose content, and modification of the lignin structure so that the pulping will be more efficient. Less focus has been on the hemicellulose structures, although many important chemical reactions involve hemicelluloses during chemical pulping. The probably most important reaction is the formation of hexenuronic acid from 4-O-methyl glucuronic acid, a side group on xylan[1]. This structure is responsible for a large part of the kappa number in the unbleached pulp, especially in hardwoods as eucalyptus, and requires relatively strong bleaching agents for efficient removal[2]. Furthermore, remaining hexenuronic acid in the bleached pulp may cause post yellowing[3].

Several bleaching methods are efficient for removing hexenuronic acid, such as warm acid wash (A*), ozone (Z) and peracetic acid (T), but this processes removes also charges from the pulp, which is negative for many applications[2,4,5]. A better strategy should be to prevent the formation of hexenuronic acid from 4-O-methylglucuronic side group on xylan. This reaction is an alkali catalyzed β-elimination, and the methoxy anion is a good leaving group (Figure 1). In this study, we investigate if unmethylated glucuronic acid side groups have less tendency for forming hexenuronic acid under alkaline conditions.

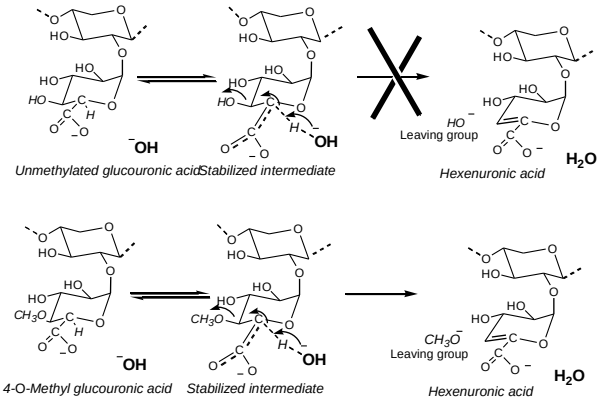

Figure 1. Formation of Hexeneuronic acid from 4-O-methyl glucuronic acid side chain on xylan below. Above is unmethylated glucuronic side chain on xylan shown. This structure form less hexenuronic acid due to that the hydroxyl ion is a less good leaving group than methoxy anion.

Experimental

Xylan from birchwood (Sigma) was reduced with $NaBH_4$ as follows: 1g of xylan was suspended in 25ml water was mixed with 25 ml 2%$NaBH_4$ in 1M NaOH. Reduction was continued for 1h at room

temperature. Reduced xylan was recovered by centrifugation, and used as model system for 4-O-methyl glucuronic acid. 1-methyl glucuronic acid was used as model system for unmethylated glucuronic acid. (The 1-methyl group represents the main chain of xylan.) Both model systems were subjected to simulated kraft cook, i.e., heated to 120℃ for 5 – 250 min. in 1 M NaOH. Formation of HexA was measured by UV spectroscopy at 260 nm as describe elsewhere [6].

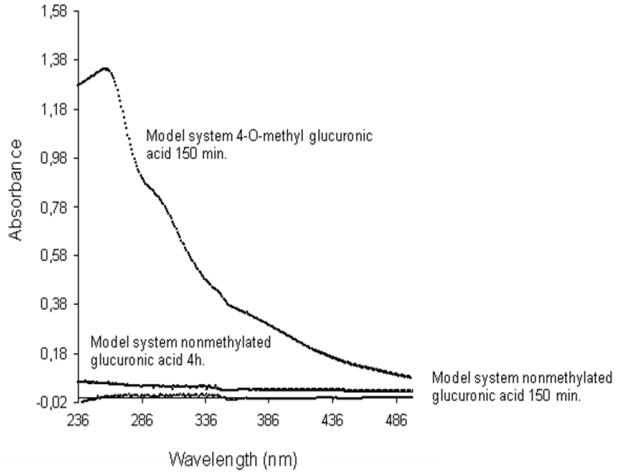

Figure 2. UV spectra detection of Hexenuronic acid formation during alkaline incubation. As expected hexeneuronic acid was formed from the methylated model system. No detectable hexenuronic acid was found from the unmethylated system even after 4h incubation.

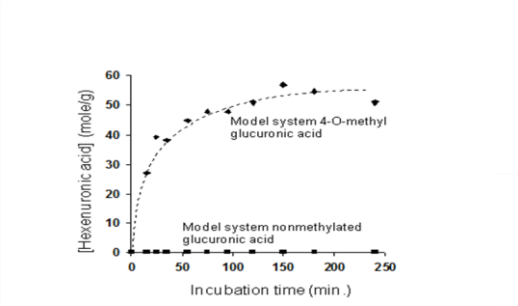

Figure 3. Kinetics of Hexenuronic acid formation during alkaline incubation. Hexeneuronic acid was readily formed from the methylated model system, whereas No detecteble hexenuronic acid was found from the unmethylated system.

Results and Discussion

Hexenuronic acid was created from the xylan, containing 4-O-methylglucuronic acid as side chain (Figure 2, 3). However, no detectable amounts of hexenuronic acid was discovered from the model system for unmethylated glucuronic acid (1-methyl glucuronic acid), and it was confirmed that most of the –methyl glucuronic acid remained in the solution after incubation (not shown). Thus, it appeared as the 4-O-methoxy group is of fundamental importance for the β-elimination forming the hexenuronic acid (Figure 1). One explanation for this may be that the methoxy anion might be a better leaving group than hydroxide anion under strongly alkaline conditions. This is also expected, since methanol has a lower pKa (15.20), than water (15.74).

Thus, it should be expected that no or little hexenuronic acid should be produced in kraft pulping from a wood which xylan contained unmethylated glucuronic acid instead of 4-O-methylated glucuronic acid. Such pulp should be expected to have a fundamentally lower kappa number after pulping – as much as 40%

of the kappa number of unbleached hardwood kraft pulp can be due to hexenuronic acid[7]. Therefore, such a pulp should be considerably easier to bleach, than normal hardwood pulp, and TCF bleaching[2] might be sufficient. Furthermore, such a bleached pulp can retain high surface charges, which is beneficial for paper properties, since charges lead to increase fiber flexibility and surface swelling of fibers[8], without large loss of brightness stability (Hexenuronic acid cause post yellowing[3]).

A specific removal of the 4-O-methyl group from xylan should therefore be technically very important, but this must be done before the kraft pulping, which practically excludes enzymatic treatments, since the wood has such a compact structure that enzymes cannot get access to the xylan[9]. A better strategy is to use genetic manipulation for altering the xylan structure. This raises two questions:

I. Which gene shall be altered for getting a xylan without 4-O-methyl groups on glucuronic acid?

II. Can a tree survive without the methyl group?

I. Hemicellulose is synthesized inside plant cells in the Golgi apparatus by specific enzymes from activated monomers, such as sugar dinucleotide phosphates. There are specific enzymes that synthesize the main chain of xylan, and other enzymes that anchors the side groups and again different enzymes that modifies the sugar residues by acetylation and methylation[10]. To our knowledge, the details in exactly which enzyme that perform the methylation of glucuronic acid are unknown, but this protein and its gene could most likely be found if a well equipped laboratory looked for it. If a knockout mutant for this gene is performed, the chances for producing a xylan without methyl groups seem to be good. An alternative strategy is to introduce a gene for an enzyme specifically removing the methyl group into the plants genome.

II. The question if a plant will survive with this altered hemicellulose structure is associated with the question about the biological role of the chemical modification of hemicelluloses. Surprisingly little literature is available on this issue, but substituent have been suggested to decrease the possibility for hemicellulose to form hydrogen bonds, and thereby make it more hydrophobic and amorphous[11], which fits well with the idea of hemicellulose as a flexible matrix. Under all circumstances, the methyl group is a rather minor part of the xylan structure. In support of that a plant can survive without the methyl group on xylan, there are report that several plants have incomplete methylation of the glucuronic acid side chains of xylan [12,13,14].

Conclusions

The methylgroup on 4-O-methyl glucuronic acid side chain of xylan has been shown to have a central role as leaving group in the formation of hexenuronic acid. Therefore formation of hexenuronic acid during kraft pulping may be avoided to large degree if this methyl group could be removed prior to pulping. One intresting strategy to do this is to identify the responsible enzyme(s) for this methylation, and suppress its treanscription in some interesting hardwood, such as *Eucalyptus*. Plantation cultivation of such genetic modified trees may provide a raw material for kraft pulping that is easy to bleach and have high surface charge combined with good brightness stability of the bleached pulp.

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