Polymer Degradation and Stability 133 (2016) 36–46

Contents lists available at ScienceDirect

Polymer Degradation and Stability

journal homepage: www.elsevier.com/locate/polydegstab

The degree of acetylation affects the microbial degradability of mannans

Ran Bi [a, 1], Jennie Berglund [a, 1], Francisco Vilaplana [a, b], Lauren S. McKee [a, b], Gunnar Henriksson [a, *]

[a] Wallenberg Wood Science Centre (WWSC), Department of Fiber and Polymer Technology, School of Chemical Engineering, KTH, Royal Institute of Technology, 100 44 Stockholm, Sweden
[b] Division of Glycoscience, School of Biotechnology, KTH, Royal Institute of Technology, AlbaNova University Centre, 106 91 Stockholm, Sweden

ARTICLE INFO

Article history:
Received 19 January 2016
Received in revised form
30 June 2016
Accepted 11 July 2016
Available online 13 July 2016

Keywords:
Wood biodegradation
Acetylation
Hemicellulose
Mannan
Microorganism
Biodegradability

ABSTRACT

Hemicelluloses as major components of plant cell walls are acetylated to different extents. The biological functions of acetylation are not completely understood but suggested that one reason is to decrease the microbial degradability of cell walls. Model seed galactomannan and glucomannan, which are structurally similar to an abundant class of wood hemicelluloses, were acetylated to various degrees and used as sole carbon source on agar plates for microbial growth. When soil samples were inoculated on the plates, significantly fewer strains grew on the agar plates with highly acetylated mannans than with slightly acetylated or non-acetylated mannans. One filamentous fungus isolated and identified as a *Penicillium* species was shown to grow faster and stronger on non-acetylated than on highly acetylated mannan. The data therefore support the hypothesis that a high degree of acetylation (DSac) can decrease the microbial degradability of hemicelluloses. Possible mechanisms and the technological significance of this are discussed.

1. Introduction

Wood, the name broadly used to refer to the lignified cell walls of plants during secondary cell wall growth, has a central position in the carbon cycle. Due to its huge abundance, a large part of the carbon dioxide that is fixed during photosynthesis ends up in wood cell wall polymers. Thus, this material is attractive as a nutrition source for microbes, and a struggle between plants and microbes has been going on for hundreds of millions of years. Microorganisms have evolved sophisticated enzymatic strategies for degrading plant cell walls to obtain soluble carbohydrate nutrients, while plants have developed in response a strong and complex cell wall, which affords very good mechanical properties and strong resistance to microbial degradation [1]. For instance, the softwood California redwood (*Sequoia sempervirens*) can reach over 100 m height and stand for thousands of years, largely thanks to the properties of its wood.

Therefore, it is not surprising that wood, although it occurs in several phylogenetically distinct groups of plants, has some common chemical properties. In practice, wood consists of a composite of *cellulose*, *hemicellulose*, *lignin* and *extractives* (low molecular weight components such as fatty acids, sterols, terpenoids and waxes) [2]. Cellulose, a homopolymer of glucose (β-1 → 4-glucan), forms crystalline fibril structures and is largely responsible for the mechanical properties of wood [3]. Although its macromolecular structure makes it much more resistant to enzymatic degradation than for instance the structurally related polysaccharide amylose (α-1 → 4-glucan), tissues consisting of more or less pure cellulose are much more quickly degraded than whole wood, particularly by specialized fungi such as *Trichoderma reesei* [4,5]. The high resistance of wood to microbial degradation is often attributed to the presence of extractives and lignin in the wood [6]. The extractives are a complex and heterogeneous group of organic chemicals and many of them are poisonous to microbes (some are therefore important medicines), thereby protecting the wood from degradation [7]. Polymeric lignin is also believed to contribute to the resistance of wood in another way, by cementing and covalently cross-linking other polymers [8]. The tight interactions of lignin with wood polysaccharides make the lignified cell wall structure so compact that molecules in the size range of proteins cannot easily

* Corresponding author.
E-mail address: ghenrik@kth.se (G. Henriksson).
[1] Contributed equally to this work.

http://dx.doi.org/10.1016/j.polymdegradstab.2016.07.009
0141-3910/

penetrate into the cell wall [9]. The racemic and complex structure of lignin probably also contribute to its degradative recalcitrance. The covalent crosslinking of polysaccharides by lignin probably also plays an important role for the properties of wood, and only specialized organisms with effective systems for lignin degradation using low molecular weight reactants are able to degrade lignin [10].

The hemicellulose components of wood are, despite their abundance (between a quarter and a third of the cell wall typically consists of hemicellulose [2]), much less well understood in terms of mechanical and potential anti-microbial functions. As with wood extractives, hemicelluloses are a heterogeneous group, with a composition which varies greatly between different phylogenetic groups. Some common features do however exist; hemicelluloses are normally relatively short polysaccharides with a degree of polymerization of 150–300 monosaccharide residues. The main chain is – as in cellulose – connected mainly with β-1 → 4 glycosidic bonds in equatorial configuration, but there are also commonly short side chains or individual sugar residues attached to the main chain [11]. The most abundant hemicellulose in secondary cell walls of wood in conifers and eudicotyledons is either mannan or xylan. A property that differentiates hemicellulose from cellulose is that a portion of the component monosaccharides in many hemicelluloses are *O*-acetylated (here also referred to as simply acetylated) [12]. The reason for hemicellulose acetylation is unclear, but some studies indicate that plants transiently modify the degree of acetylation of cell wall polymers [13,14], perhaps to modulate cell wall solubility or flexibility during cell growth, development and differentiation. That acetylation may be of importance for the cell wall formation is indicated from a previous study by Kim et al. which suggests that some acetyl groups are removed from glucomannans after cell wall formation in wood [15]. Furthermore, a study on xylan shows that acetylation can partly hinder hydrolysis by glycoside hydrolase enzymes, and that the combination of de-acetylating esterases and backbone-cleaving xylanases results in synergistic boosts to the breaking down of xylan polymers [16]. Suggested explanations as to why acetylation hinders enzymatic breakdown are that acetyl groups act as steric hindrances to enzyme access [17], or that they directly inhibit certain enzymatic reactions [18]. These effects may prevent degradation of wall polymers by invading pathogens through fine-tuning of the wall polymers' degree of acetylation [17]. This could be because the non-covalent interactions between polysaccharides might be altered by acetylation, and that acetyl side groups contribute to a complex matrix structure in a more energy-efficient manner than could be achieved by depositing greater amounts of carbon in the cell wall in the form of larger side groups such as arabinose. Acetylated wood has also shown increased resistance to fungal attack [18]. One of the mechanisms of this is believed to be that increased acetylation leads to decreased moisture sorption, and that the sorption level drops below the level required for microbial attack. Pure hemicelluloses are normally much more quickly degraded by appropriate hydrolytic enzymes than cellulose is by cellulases [19]. Multiple enzymatic activities are required for the full degradation of cellulose [20], but whereas cellulose comprises only one sugar and one linkage type, hemicelluloses are more complex. Therefore, complete degradation of hemicelluloses requires a consortium of enzymes with different specialized activities for deacetylation, side group removal and degradation of the main chain, enzymes which often target different sugars and different linkages [21,22].

Galactoglucomannans (GGM) are the most common hemicellulose in softwood (conifers). The backbone consists of β-1 → 4 linked mannosyl and glucosyl units and is substituted with 1 → 6 linked α-galactosyl units. The mannose:glucose:galactose ratio is suggested to be 0.1–1:1:3–4 [2]. Additionally, the C2 and C3 positions are partially acetylated with about one acetyl group per 3–4 hexose units which is the same as a degree of acetylation (DSac) of 0.25–0.33 [2], corresponding well to additional studies on spruce GGM showing a DSac of 0.24–0.37 [23–25]. Two similar polysaccharides are galactomannan from locust bean (LBG) and glucomannan from konjac (KGM), which are in fact seed nutrition components, but have similar structures to the main hemicellulose in softwoods, and are often used as models for GGM in biochemical studies [26]. The backbone of LBG is exclusively composed of β-1 → 4 linked mannosyl units, and is substituted with 1 → 6 linked α-galactosyl units [27,28]. The ratio between galactose and mannose is about 1:4 (Locust bean gum from *Ceratonia siliqua* seeds, Sigma Aldrich). KGM consists of a backbone of β-1 → 4 linked glucosyl and mannosyl units with a ratio of about 1:1.6, with an 8% degree of branching with β-1 → 6 linked glucosyl units [29,30], and can also be slightly acetylated [28]. Structures of these three mannans are presented in Fig. 1.

In this work we investigate the effect of acetylation on the microbial degradation of the mannans LBG and KGM. The polymers were acetylated to varying degrees and tested in two microbiological experiments. First, microbial colony numbers were noted during cultivation of a soil sample on agar plates containing mannans with different degrees of acetylation as the sole carbon source. Second, the growing rate of one particular isolated organism was compared on mannans with different degrees of acetylation as the sole carbon source.

2. Materials and methods

2.1. Materials

Locust bean gum galactomannan from *Ceratonia siliqua* seeds (LBG) was purchased from Sigma Aldrich, and glucomannan from konjac roots (KGM) was obtained from Konson Konjac Gum Co., Ltd, Wuhan, China. The mannan substrates were used as purchased for the chemical acetylation procedures. Cellobiose was from Merck, Darmstadt, Germany.

2.2. Sugar analysis

The sugar compositions of LBG and KGM were analysed in duplicate by acid hydrolysis using the SCAN-CM 71:09 procedure. In short about 100 mg of dry sample was treated with 1.5 mL 72% sulphuric acid in a vacuum desiccator for 80 min, thereafter it was diluted with MilliQ water to 4% sulphuric acid and autoclaved at 125 °C for 1 h. The solution was diluted and filtered through a 0.2 μm nylon filter prior to analysis. Sugars were quantified by high-performance anion-exchange chromatography (HPAEC) equipped with a pulsed amperometric detector (PAD) (Dionex, Sunnyvale, CA, USA) using a CarboPac PA-1 column (4 × 250 mm), by comparing retention time and peak area with those of known sugar standards. The system was equilibrated with 260 mM sodium hydroxide and 170 mM sodium acetate for 7 min, and with MilliQ water for 6 min. During the analysis the eluent was MilliQ water at a flow rate of 1 mL/min. Before the PAD detection 300 mM sodium hydroxide was added to the column effluent at a flow rate of 0.5 mL/min. Quantification of glucose, mannose, galactose, arabinose and xylose were made with calibration curves of different concentrations. The concentration of a single sugar in the calibration solutions ranged from 0.15 mg/L to 180 mg/L and linear calibration curves were obtained for all sugars. Data were processed with the Chromeleon 7.1 software. The amount of pentoses and hexoses were adjusted with a factor of 0.88 and 0.9, respectively, to compensate for the accumulated water during the hydrolysis reaction.