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Interaction of Lignin with Cellulase, a Key Role in Cellulosic Ethanol Production

Lan Yao[1,2], Haitao Yang[1] and Arthur J Ragauskas[2,3]*

[1]*Hubei Provincial Key Laboratory of Green Materials for Light Industry, Hubei University of Technology, China*

[2]*Department of Chemical and Biomolecular Engineering, The University of Tennessee, Knoxville, TN, USA*

[3]*Department of Forestry, Institute of Agriculture, The University of Tennessee, Knoxville, TN, USA*

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***Corresponding author:** Arthur J Ragauskas, Department of Forestry, Wildlife and Fisheries, Center of Renewable Carbon, Department of Chemical and Biomolecular Engineering, Institute of Agriculture, Knoxville, TN 37996-2200, USA, The University of Tennessee, Knoxville, TN 37996-2200, USA, Email: *arthur.ragauskas@chemistry.gatech.edu*

Opinion

Cellulosic ethanol, which is usually generated from lignocellulosic material, is considered as an alternative to petroleum-based fuels in recent years. However, the commercialization of this bio-conversion process is still significantly hindered by the cost associated with cellulases in enzymatic hydrolysis [1]. Lignin is an amorphous and cross-linked polyphenolic polymer and accounts for 20-30% of the plant cell wall. It can physically limit cellulose accessibility and adsorb enzymes irreversibly, thus it decreases the effectiveness of enzymes. In recent years, tremendous numbers of publications have been focused on lignin-cellulase interactions, especially the non-productive adsorption of cellulase to lignin. Due in part to the heterogeneity of lignin, the mechanism is not fully understood yet. Research on cellulase binding to lignin are usually explored from two different perspectives: lignin and cellulase. The former approach is often centered on the role of lignin in the interaction. With the help of modern spectroscopy and chromatography techniques, such as Fourier-transform infrared spectroscopy (FT-IR), gel permeation chromatography (GPC) and nuclear magnetic resonance (NMR), physicochemical properties of lignin and the correlations of these characterizations with cellulase adsorption have been analyzed accordingly. It was found that several functional groups of lignin are involved in the lignin-enzyme interactions.

First of all, phenolic hydroxyl groups of lignin could interact with cellulase by forming hydrogen bonding with amino acid residues in the enzymes and interfere with the enzymatic hydrolysis of cellulose [2]. On the other hand, the presence of carboxylic acids group in lignin could reduce the negative effects of lignin on enzymatic hydrolysis of biomass by increasing hydrophilicity of lignin [3]. Lignin S/G ratio was also correlated with the lignin-enzyme adsorption to some extent. However, the literature results are not all fully consistent. Most studies indicated that due to the higher affinity of the G unit over S units to cellulase, S/G ratio was negatively correlated with cellulase adsorption onto lignin [4]. While some other studies concluded that lignin with higher S/G ratio showed greater enzyme adsorption ability [5]. Other lignin features such as surface area and surface charge could also affect cellulase binding. It was reported that lignin with larger surface area led to increased cellulase binding [6], while others concluded that the surface area of lignin had limited influence on enzyme adsorption [7]. Surface charge of lignin also affected cellulase binding to lignin by electrostatic force [8]. Recently, influence of phenolic compounds on their cellobiohydrolase (CBH) binding was investigated with lignin fractions recovered by different organic solvents [9,10].

The latter approach seeks to elucidate the mechanism from the perspective of cellulase. Cellulase is composed of three main components: cellobiohydrolases (CBHs), endo-β-1, 4-glucanases (EGs) and β-glucosidase. In particular, CBHs showed notable interaction with lignin [10]. CBHs are made up of catalytic domain (CD) and a smaller carbohydrate-binding module (CBM) connected by a glycosylated linker peptide. Previous study reported that CBM-less or CBM-lacking cellulase could decrease the non-productive binding and may lead to novel means of reducing cellulose charges [11]. Prediction of binding sites on CBM of CBH by molecular docking showed that Lys 57 played a key role in interaction between lignin and CBM [12]. Other studies indicated that pH and temperature could also affect adsorption of cellulase onto lignin by changing hydrophobicity and electric potential of cellulase surface [13]. Selecting and/or developing engineered enzymes with less lignin affinity could be a feasible way to reduce non-productive adsorption of cellulase. Due to some contradictory results existing in the literatures, there might be a long way to go to fully understand the mechanisms involved in the lignin-enzyme interactions. There are two main ways to overcome this negative lignin effects on cellulosic ethanol production: one is the pretreatment method which could reduce and/or modify lignin to have less binding ability to protein; the other way is by using protein engineering to obtain cellulase which exhibits higher affinity to cellulose than lignin.

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