

Formation of a phenyl-choline ether structure in lignin reduces inhibition of cellulase activity by lignin

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Project Goals:

Ionic liquids have been extensively studied as solvents for biomass pretreatment and have been shown to be excellent solvents for selective isolation of cell wall components. Work in the deconstruction division at the Joint BioEnergy institute (JBEI) has focused on developing lower cost, biocompatible and adaptable ionic liquid technologies for application in biorefineries. Our work has demonstrated that choline-based ionic liquids are highly efficient at solubilizing lignin during biomass pretreatment. However, the mechanism by which lignin from different biomass sources is solubilized by choline-based ionic liquids is still unclear. In this study, we show that lignin is chemically modified by choline during pretreatment with choline glutamate ([Ch][Glu]) and choline α -ketoglutarate ([Ch][aKg]). Interestingly, these choline-based ionic liquids modify lignin by forming a new phenyl-choline ether (4-O-C) bond, which dramatically increases the solubility of modified lignin in aqueous solution. We also analyzed the thermal stability and inhibition kinetics of a selected endo-1,4- β -D-glucanase (Cel5A from *Thermotoga maritima*) in the presence of choline-modified and unmodified lignin. Our results show that both choline-modified lignin and unmodified lignin enhance the melting temperature of Cel5A and bovine serum albumin (used as a control), indicating a stabilizing interaction. We show that native lignin inhibits Cel5A via an uncompetitive inhibition mechanism, whereas choline modified lignin is less inhibitory, having a 10X higher IC50. These results support the development of choline-based bio-compatible one-pot pretreatment and saccharification technologies.

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