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INVESTIGATION OF SILICA-LIGNIN INTERACTION. APPLICATION OF AFM AND FLUORESCENCE TECHNIQUES

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Abstract

In this study, we investigated, in an in vitro system, the interaction of SiO₂ (as a complex with NH₄OH) with the peroxidase-catalyzed polymerization of lignin monomer into lignin model compound DHP, imitating conditions of the last step of lignin formation in the cell walls. The structure of obtained polymer was monitored using fluorescent spectroscopy and AFM 30 minutes after the start of synthesis. We studied effect of two different Si concentrations, 0.6 mM and 6 mM. Analysis of the DHP fluorescent spectra, and the application of mathematical decomposition clearly shows that structure of lignin model compound depends on Si concentration in the reaction mixture. The amount of Si affects the distribution of electrons in the DHP polymer. When silicon is present at a concentration of 6 mM, the position of the longest-wavelength APD band shifts towards shorter wavelengths compared to the position of the APD band for 0.6 mM Si. This indicates that during polymer synthesis, higher concentration of silicon may inhibit the formation of pi-electronic structures, which are responsible for electron delocalization. AFM images also show differences in the size and regularity of DHP globules.

Keywords: lignin, Silica, fluorescence spectroscopy, deconvolution, AFM.

INTRODUCTION

Silicon (Si) holds a significant position in the mineral world as the second most abundant element in the Earth's crust [1]. Silica (SiO₂) is a naturally occurring mineral that can be taken up by plants and incorporated into their tissues, including the cell wall (CWs) [2–5]. Silica exists as silicic acid (Si(OH)₄) in the soil (ranging from 0.1–0.6 mM), which can be absorbed by the roots of plants and transported and deposited in the CWs as amorphous silica [6]. Such silica has a non-crystalline form. Plants have developed mechanisms for Si uptake, translocation, and deposition within their tissues. Silica in plant CWs can provide structural support, improve resistance to biotic and abiotic stress, and contributes to plant growth and development. Depending on the plant species, soil properties, Si source and Si amount, Si content in planta can vary from 0.1% (near the detection limit) to 10%, on a dry weight basis [7].

Lignin is a major component of the secondary CWs of many plant species and plays an important role especially in the responses to various types of biotic and abiotic stress and it provides rigidity and strength to the CWs [8–11]. It is a natural phenolic polymer with high molecular weight, complex structure and composition.

Si is predominantly accumulated in CWs, its localization and content being influenced by the chemistry and structure of lignin [12–14]. In this study, we aimed to explore the interaction between Si and the lignin formation process in CWs. Using an *in vitro* system, we examined the interaction between SiO₂ and the peroxidase-catalyzed polymerization of a lignin monomer to create a lignin model compound, thus simulating the conditions of the final step in lignin formation in CWs. DHP is a lignin model compound that allows us to investigate the behavior of lignin, changes in its structure, and its interaction with different molecules.

Fluorescence spectroscopy is a valuable technique for tracking structural changes in complex molecules like DHP through its autofluorescence. It enables monitoring and analyzing variations in molecular structure with high precision. AFM microscopy complements spectroscopic analysis by providing visual confirmation of structural features. It allows us to obtain detailed imaging and visualization of the analyzed structures, further validating the findings obtained through fluorescence spectroscopy.

Some studies have suggested that silica and lignin may interact in the cell wall, but the exact nature of this interaction is not well understood [15]. The interaction between silicon and lignin holds potential significance for the development of silicon-lignin composites, which could have intriguing applications as hydrogels for drug release or sensors in the field of biomedicine.

MATERIALS AND METHODS

DHP synthesis in presence of Si

The lignin model compound, dehydrogenative polymer (DHP), was synthesized according to the procedure of Radotić *et al.* [16]. DHP was synthesized from coniferyl alcohol, using horseradish peroxidase as an enzymatic catalyst. The reaction mixture contained $5 \cdot 10^{-3}$ M coniferyl alcohol, 5×10^{-3} M H₂O₂, and 2.5×10^{-8} M horseradish peroxidase (all from Fluka Chemical Corp., New York) in 50 mM phosphate buffer pH 7, in the absence or presence of SiO₂-NH₄OH (in further text SiO₂) in the concentrations 0.1 mM, 0.6 mM and 6 mM. All reaction components were added simultaneously to the mixture. After mixing, the solution was shaken constantly for 24 h. Polymerization occurs in the solution phase, at a temperature of 25 °C. During the reaction, aliquots of the reaction mixture were taken 1 h after the beginning of synthesis and lyophilised. After 24 h, the precipitate was washed twice in 50 mM phosphate buffer, and twice in deionized water, and finally dried in a desiccator.

Fluorescence spectroscopy

The fluorescence spectra were collected using an FL3-221P TCSPC (HORIBA Jobin Yvon, France) spectrofluorometer. For each sample, we collected 12 spectra at different excitation wavelengths with a 5-nm step, to record the emission of all fluorophores contained in the polymer. The excitation range was from 360 nm to 415 nm, and the emission range was 380 nm to 600 nm. The obtained spectral series for each sample were analyzed by deconvoluting the original spectra into an optimal number of log-normal components corresponding to different fluorophores in lignin. Non-linear fitting of all fluorescence spectra was performed using Nelder-Mead algorithm implemented in Matlab, as described by Kalauzi

et al. [17]. Thus, the histograms of component maxima were obtained, and the corresponding approximate probability distribution (APD) was calculated by weighted averaging of histogram values for a set of histograms of varying abscissa intervals [17].

Atomic Force Microscopy

The surface topography of samples was characterized by Atomic Force Microscope (NT-MDT Ntegra SPM, Russia) in Semicontact Error Mode. The scanning frequency during the measurement was maintained at 0.5 Hz, while the step size in the recorded images was $7 \times 7 \mu\text{m}$, respectively. The cantilever oscillation amplitude (Set Point) during the measurements was 10. The recorded images in the Height mode correspond to the surface topography, while the images in the Mag (Magnitude) mode are related to the error signal which gives higher contrast for sharp objects in the scanned area.

RESULTS AND DISCUSSION

Our investigation focused on the interaction between (Si) and a lignin model compound at the midpoint of synthesis, specifically 30 minutes after start of the reaction. We examined the changes in autofluorescence in samples containing two different concentrations of Si (0.6 mM and 6 mM) (Figure 1).

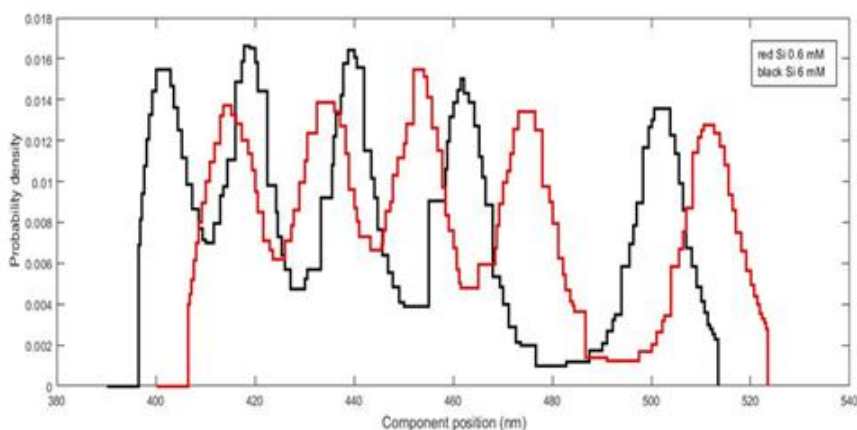


Figure 1 APD for DHP synthesizes in presence of 0.6 mM (red), and 6 mM (black) SiO_2

By employing a fluorescent spectroscopy technique and varying the excitation wavelength by 5 nanometers (ranging from 360 nm to 415 nm), we recorded 12 spectra for each sample. Utilizing the Nelder-Mead algorithm implemented in Matlab, we analyzed all spectra and determined the optimal number of components for spectral deconvolution, based on the minimum fitting error. Consequently, a five-component analysis was applied to all the samples.

The final results obtained from the analysis of the emission spectra using the APD technique revealed that higher concentration of silicon exert an inhibitory effect, hindering the formation of double bonds and conjugation in the lignin molecule during the synthesis process. This effect is manifested in the final polymer structure. Notably, the APD results demonstrated a blue-shift in the emission maximum of the green spectral component

(500–520 nm) for the sample with a higher Si concentration (6 mM). Previous research has indicated that lignin fragments containing conjugated -C=C- and -C-C- bonds emit in the green region of the spectrum, and the degree of red shift in their emission maximum is proportional to the length of these conjugated fragments.

Additionally, the AFM images (Figure 2) provided valuable insights into the influence of silicon concentration on the appearance and formation of the lignin model compounds. The microscopic images clearly depicted a more pronounced regularity in the structure and well-defined shape of DHP globules at a silicon concentration of 0.6 mM.

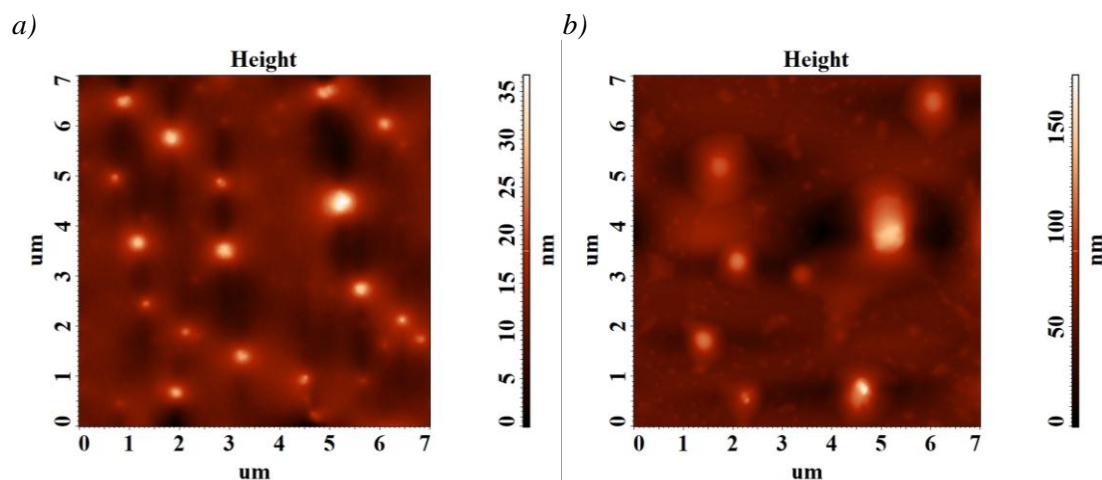


Figure 2 AFM micrography of DHP synthesized in presence of SiO_2 a) 0.6 mM; b) 6 mM

CONCLUSION

Our findings highlight the significant role of silicon in shaping the structure of the lignin model compounds during polymer formation. Fluorescence spectroscopy and AFM technique clearly show that structure and arrangement of lignin model compound depend on concentration of Si in the reaction mixture. During polymer synthesis, 6 mM silicon may inhibit the formation of pi-electronic structures, which are responsible for electron delocalization. The obtained results provide an encouraging basis for further exploration of the interactions between silicon and lignin, as well as the effects of silicon on the structure of cell walls (CWs). This opens up opportunities to deepen our current understanding of how silicon influences the overall CW structure.

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