Direct Observation of the Main Cell Wall Components of Straw by Atomic Force Microscopy

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ABSTRACT: Cellulose, hemicellulose, and lignin are the main cell wall components of straw. After removal of the wax and the major portion of lignin, the remaining components of the cell wall surface of straw were determined by atomic force microscopy, which revealed a network structure of cellulose and hemicellulose, and some lignin localized on the surface of the network, consistent with the cell wall model suggested by other researchers. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 88: 2055–2059, 2003

Key words: atomic force microscopy (AFM); morphology; biopolymers; microstructure; straw

INTRODUCTION

The cell wall of plants is often described as a more-or-less random network of cellulose microfibrils associated with other polysaccharides and protein complexes.[1]

Although the main chemical structures of wood and straw constituents are well characterized (i.e., lignin, hemicellulose, and cellulose can be successively separated by chemical treatment), it is still difficult to relate the chemical and morphological structure of wood and straw. Unfortunately, this lack of knowledge means that today's pulp and paper industry is still the source of considerable pollution, especially black liquor. Because the exact location of each component in the ultrastructure of fibers has not been possible to delineate, it has been difficult to measure the interactions among the chemical components of an individual fiber wall.[2]

It has been suggested that in a plant's cell wall, many parallel cellulose molecules are held together by hydrogen bonds between hydroxyl groups of the glucose monomers to form microfibrils, and several intertwined microfibrils form a cellulose fibril.[3] Recent research has shown that the microfibrils are crosslinked and stabilized by shorter molecules, such as hemicellulose, which order the cellulose microfibrils into a network, as shown in Figure 1. The lignin component orients randomly and fills in some of the gaps in the network structure.[4]

In our study we used atomic force microscopy (AFM) to measure the structure of the cell wall of straw, to observe its structure more directly.

EXPERIMENTAL

Dewaxing of straw

Wheat straw was collected from a farm field in eastern China. After the straw was cleaned and dried it was milled into powder (<1.0 mm). The dried powder was extracted with toluene/ethanol (2 : 1, v/v) to dewax in a Soxhlet apparatus for 6 h,[5] after which it was dried in an oven at 50°C for 16 h before use.

Isolation of lignin and hemicellulose from straw

Dewaxed straw (5.0 g) was treated with 150 mL of ultrapure water (>18 MΩ, Milli-Q system; Millipore, Bedford, MA) containing 2% hydrogen peroxide (H_2O_2, wt/v) in a jacketed reaction vessel with a water bath and a heater; 4*M* sodium hydroxide was gradually added into the reaction suspension to maintain the pH 11.5 of the system. It was stirred gently and the extraction temperature was maintained at 50°C for 16 h.

After extraction the suspension was filtrated. In the insoluble residue, there were mainly cellulose with some hemicellulose and slight amounts of lignin. It was washed with ultrapure water several times to

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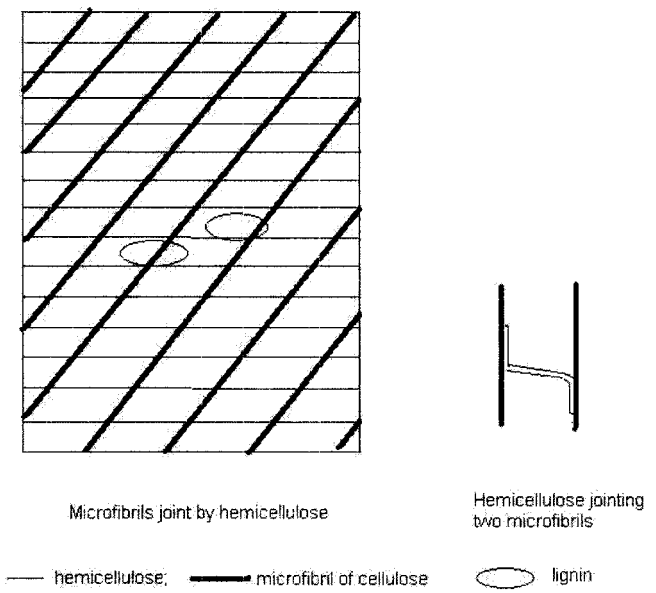

Figure 1 Structural model of plant cell wall suggested by Vincent.[4]

remove dissoluble chemicals and then dried. The filtrate was composed mainly of lignin and hemicellulose.

Separation of lignin to hemicellulose

The filtrate was collected and neutralized to pH 5.5 with 10% hydrogen chloride and then concentrated. After the concentrated filtrate was poured into three volumes of ethanol, a hemicellulose precipitate was obtained. The hemicellulose was then filtrated from the solution, and the residual solution was composed mainly of lignin.

Samples prepared for AFM studies

The insolvable cellulose residue was resuspended in Milli-Q water and the final concentration of the suspension was about 1% (wt/wt). For imaging by AFM, the suspension was droplet-applied onto the surface of freshly cleaved mica. The aperture of a Gilson pipette was enlarged by making an oblique cut across the end of a pipette tip because the residue could not be pipetted by conventional methods.[6] The resulting wet deposits on mica were carefully blotted to remove excess liquid by high purity nitrogen. AFM imaging was carried out immediately after the deposit was dried.

Hemicellulose was resolved in Milli-Q water and stored at 4°C for at least 24 h before use. For single-molecular imaging the concentration was controlled at about 1–10 μg/mL. The solution was also droplet-applied onto the surface of freshly cleaved mica and dried in air.

Lignin samples were prepared by a method similar to that for hemicellulose, although the concentration was slightly greater than that of hemicellulose.

AFM measurements

A NanoScope IIIa multimode AFM (Digital Instruments, Santa Barbara, CA) was used in all the measurements. All samples were imaged in tapping-mode AFM. Both height and phase images were captured. Silicon cantilevers with a resonance frequency of 250–300 kHz were used, the scan rate ranged from 0.5 to 3.0 Hz, and the scan size varied from 500 nm to 5.0 μm. All images were measured in air at 512 $\times$ 512 pixels and analyzed by both microscopy-suitable software and PhotoShop 5.5 software (Adobe Systems, San Jose, CA).

RESULTS AND DISCUSSION

Figure 2 shows the morphology of straw after dewaxing. The surface is covered by many irregular aggregates, most of which should be lignin; in addition, the fibrillar structure of cellulose, marked by an arrow, is also shown. Figure 3 shows the AFM image of insolvable cellulose residue; height and phase data were recorded synchronously. The image clearly shows not only that many fibers are oriented in the same direction, constituting the main bundles of microfibrils, but also their rough surface structure. Their diameters range from 20 to 100 nm. It was previously suggested that the cellulose microfibril is composed of 60–80 cellulose molecular chains and that individual microfibrils are supposed to be about 20 nm in diameter.[2,3] From Figure 3, one can note the aggregates of grains

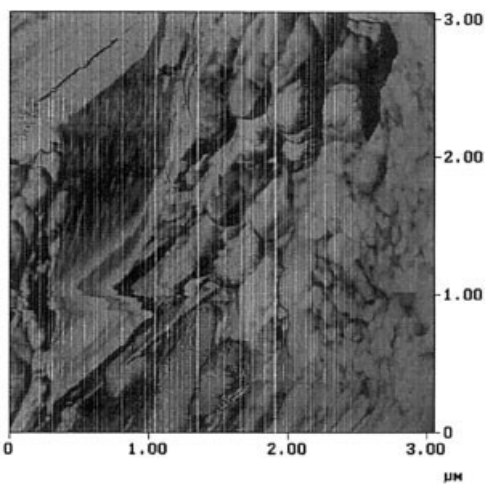

Figure 2 AFM image of straw dewaxed by toluene/ethanol.

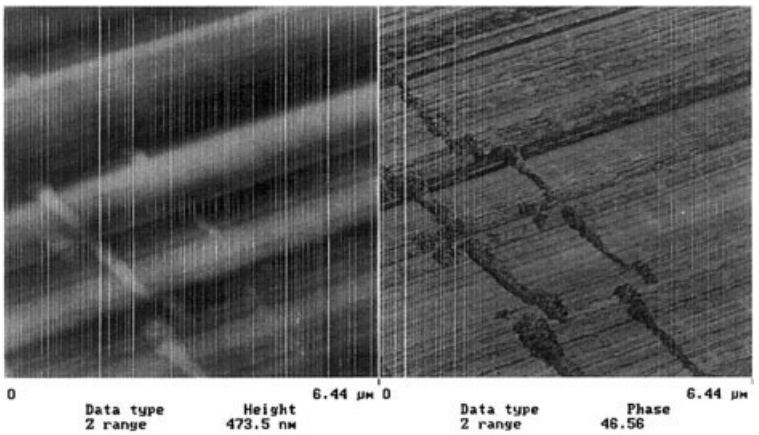

Figure 3 AFM images of insolvable cellulose residue of straw extracted by toluene/ethanol and hydrogen peroxide.

on the surface of the cellulose microfibril bundles, whose grain size was in the range of 50–200 nm. By comparing Figure 3 with Figure 2, it can be said that these may be lignin residues on the surface of cellulose after H_2O_2 treatment. The FT-IR spectrum in Figure 4

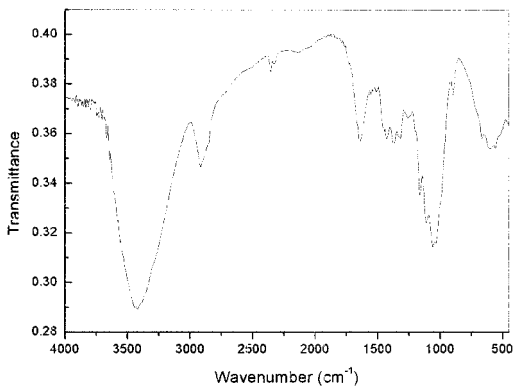

Figure 4 FT-IR spectrum of residue of straw after H_2O_2 extraction.

shows there is still some lignin in the residue. The typical peak of lignin is near 1514 cm^{-1}. The three-dimensional model for lignin proposed by Jurasek suggests that a lignin molecule of MW ~ 55,000 (DP = 300) would have a diameter of about 20 nm.[7] For lignin in straw, whose molecular weights have a wide distribution, it is believed that the observed grains correlate to aggregates of lignin.

Partial, enlarged images of Figure 3, shown in Figure 5(a) and (b), reveal more detailed structures of the cell wall of straw. Some lines that are nearly perpendicular to the direction of cellulose microfibrils are shown to form a network structure. These newly appeared chains are thinner than cellulose microfibrils with diameters of about 5–10 nm. By comparing the network structure to the theoretical model shown in Figure 1, it can be suggested that these chains nearly perpendicular to cellulose microfibrils may be hemicellulose. Figure 5(a) clearly shows that experimental results are consistent with the theoretical model. Although the samples were prepared by separating lig-

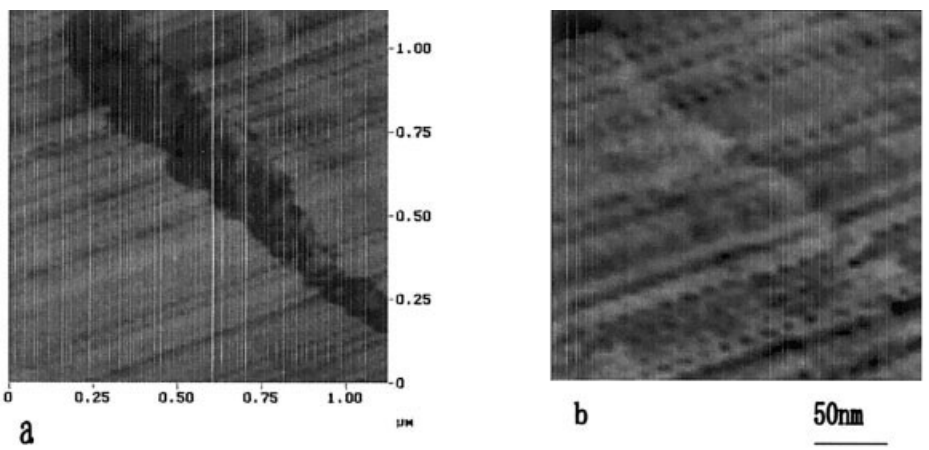

Figure 5 Partial, enlarged images of Figure 3: (a) structural relationships of cellulose microfibrils, hemicellulose, and lignin; (b) network structure of cellulose fibrils and hemicellulose.

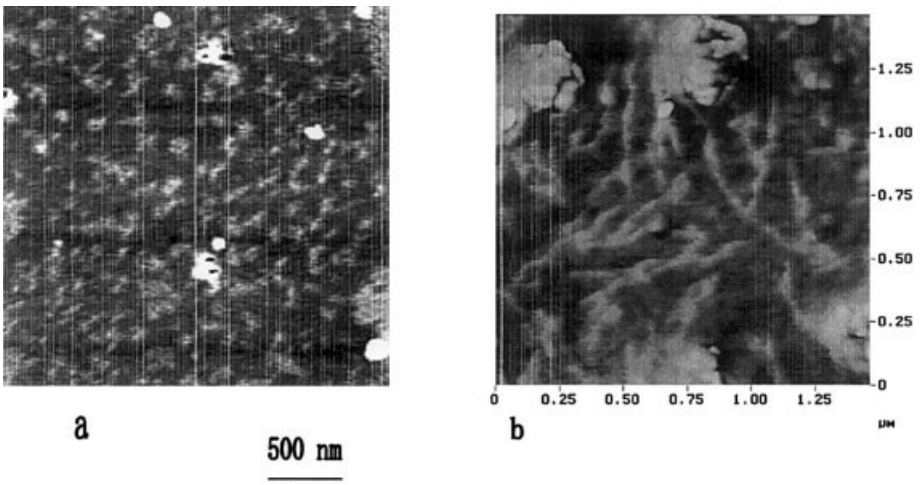

Figure 6 AFM image of hemicellulose separated from straw by H_2O_2 extraction: (a) 1.0 μm/mL; (b) 10.0 μm/mL.

nin and hemicellulose, some portions of these components still exist that retain the structural relation of these main components of the cell wall of straw. Thus the AFM images reveal the true structure of straw.

It is known that hemicellulose is composed of different monosaccharide units such as D-glucose, D-mannose, D-galactose, D-xylose, and L-arabinose with branched structure.[8] To observe its structure, a single-molecular solution of hemicellulose must be prepared. The solution of hemicellulose was prepared by solving the hemicellulose separated from straw in Milli-Q water at a concentration of about either 10 or 1 μg/mL. After being deposited on the surface of freshly cleaved mica and dried in air, the samples were determined by tapping-mode AFM; AFM images of hemicellulose at 1.0 and 10.0 μm/mL are shown in Figure 6(a) and (b), respectively. At lower concentrations, single hemicellulose molecules show a coiled structure. The widths of these chains are larger than the real width of single hemicellulose, which is attributed to the tip-broadening effect.[9] At high concentrations several chains with branched structure are clearly discerned. The length of the main chain is about 500 to 750 nm, and the branch-chain lengths range from 200 to 500 nm. Figure 6 also shows that on one main chain there are perhaps one or two branches, or new branches forming on existing branches, which may be attributed to the tangled network of polysaccharide molecules.

Figure 7 (1) shows the AFM image of lignin separated from straw, with grains ranging in size from 50 to 100 nm, consistent with the size of grains shown in Figure 3; and (2) verifies that the aggregates on the surface in Figure 3 are composed of lignin.

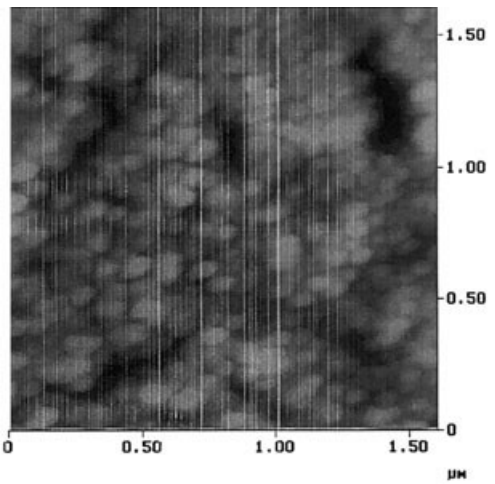

Figure 7 AFM image of lignin separated from straw by H_2O_2 extraction.

CONCLUSIONS

Wheat straw was successively extracted by toluene/ethanol, hydrogen peroxide, hydrogen chloride, and ethanol. Samples of dewaxed straw, insolvable cellulose residue, hemicellulose, and lignin were collected. AFM studies show that the main structure of the cell wall of straw is composed of bundles of cellulose microfibrils, and hemicellulose links them together to form a network structure on the surface of the lignin aggregate network to form a protective layer. The AFM results are consistent with the theoretical model of the cell wall proposed by Vincent.

Examination of the structure of separated lignins shows that their size ranges from 50 to 100 nm, which is also consistent with computer-simulated results.

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