CELL WALL SUBUNITS, "GLUE" MATRIX AND COTTON FIBER DEVELOPMENT

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Abstract

This study employed fibers taken from 25DPA bolls collected at 7am, noon and 7pm. A series of oligomers was extracted from fibers with dilute acid. In addition, a high molecular weight "glue" matrix was extracted from fibers, isolated and cleaved with either proteases or dilute acid to yield a similar series of oligomers. The cell wall subunits and "glue" matrix were found to vary both in the quantity extracted and in the character of the "glue" matrix extracted. This variability is dependent both on the time of day which the fibers were collected. The "glue" matrix appears to add carbohydrate residues to the subunits in the matrix after the initial carbohydrate residues are conjugated.

Introduction

The probability that cellulose microfibrils of the cell wall are embedded in a glue matrix has been proposed by a number of investigators over the years. The nature of such a glue matrix has been the subject of considerable discussion but there has been no characterization of such a matrix material. The presence of cell wall subunits, in cotton fibers, was proposed by W. Lawrence Balls (Balls, 1928). This work is an extension of work in this laboratory to characterize soluble oligosaccharides and the sucrosyl oligosaccharides in particular which appear to be involved in developmental changes of the cotton fiber (Murray, 1996, Murray & Brown, 1996, 1997, Murray *et. al.* 1997).

Methods

Cotton plants were DP-50 grown in the Mississippi Delta region for the time of day samples which were collected at 7am, noon and 7pm at 25 DPA. Plants for the sequential bolls were also DP-50 grown in the Sacramento Valley of California and sequential bolls were taken at the same time on the same plant. Cotton fibers were subjected to aqueous extraction and analysis of the soluble carbohydrates by high pH anion chromatography with pulsed amperometric detection (HPAEC-PAD) (Murray, 1998). Additional extraction of the oligomers (~mers) was achieved under conditions of dilute acid (0.1N HCl) and elevated temperature (100°C for 30 min) prior to HPAEC-PAD. The glue matrix was isolated by prolonged aqueous extraction and filtration (0.22μ). The ~mers were released from the glue matrix by either chymotrypsin or by the dilute acid extraction.

Results

We have been able to extract a series of oligomers (~mers) from developing cotton fibers by both chemical and enzymatic methods. These ~mers have retention times of 14 minutes and greater under the conditions analyzed. The regular spacing of the peaks is indicative of a series of oligosaccharides varying by a repeating unit in size. These ~mers are heteropolymers with a repeating glucan unit extending from a core glycan structure. The nature of the extracted ~mers varies both quantitatively and qualitatively depending on the time of day when the bolls were collected. The ~mers extracted from fibers of bolls collected in the early morning and the evening are quantitatively greater, on a per mg fiber basis, and their distribution is somewhat different than the ~mers extracted from fibers of bolls collected in mid day. This difference is shown in Figure 1. These differences are suggestive of a role in cell wall synthesis which occurs at a maximal rate at night and is at its lowest rate in the afternoon (Balls, 1928).

The ~mers were extracted from fibers from first position bolls taken from sequential fruiting branches on the same plant at the same time. These were extracted from normal plants and stunted plants from the same field The stunted plants were from an area of the field with more shallow topsoil above gravel so it did not retain moisture as well as other parts of the field. The comparison of the ~mers from normal and stunted plants is shown in Figures 2 and 3.

The isolated ~mers have been subjected to degradation with ß-glucosidase and with a cellulase (*T. reesei*). The ß-glucosidase appeared to attack all of the ~mers resulting in a decrease in the quantity of each while the cellulase degraded the ~mers down to the core structure with a retention time of approximately 14 min. Based on this susceptibility to degradation by cellulase, cotton fibers were subjected to enzymatic degradation. Immature fibers from 25 to 39 DPA were treated with either cellulase followed by several proteases or the order of treatment was reversed. Fibers treated with cellulase followed by protease for 24hr per treatment lost their structural integrity. However, fibers treated with protease first followed by cellulase did not lose integrity. In the case of mature fibers from open bolls, the enzyme treatment was repeated for a total of four treatments (cellulase, protease, cellulase, and protease). The oligosaccharides released from mature fibers by the different enzyme treatments are shown in Figure 4. Following the enzyme treatments the fibers lost structural integrity and yielded white rod-like particles. These particles appear to be highly crystalline cellulose based on their resistance to hydrolysis in 2N TFA at 100°C for 2 hrs and their complete hydrolysis in 6N HCl at 100°C for 2 hrs, which released only glucose.

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Summary

We have been able to obtain the ~mers from a large molecular complex which is secreted by fibers, *in vitro*. The relative distribution of the ~mers can vary depending on the time of day which the bolls were collected. Under optimal conditions we have been able to demonstrate the presence of the ~mers in an initial soluble fraction, a secreted fraction which will not pass through a 0.2μ filter. The ~mers appear to play a structural role in the integrity of the cotton fiber since extraction of the ~mers using specific enzymes results in a striking loss of the physical integrity of cotton fibers.

Discussion

The striking difference in the distribution of ~mers from normal and stunted plants suggests that there is an abnormality in the fiber wall synthesis under stress conditions. The prominent initial ~mer in the series from the stressed plants supports a theory that there is an abnormality in polymer elongation under these environmental conditions. The fact that

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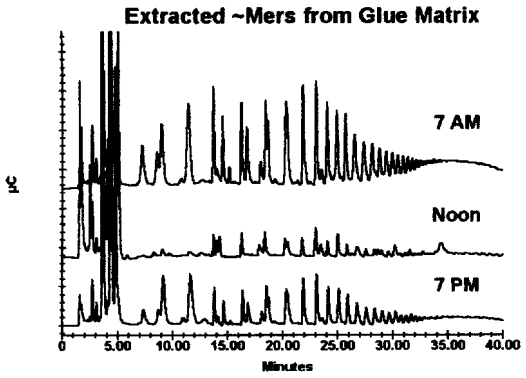

Figure 1. Oligomers (~mers) extracted from glue matrix.

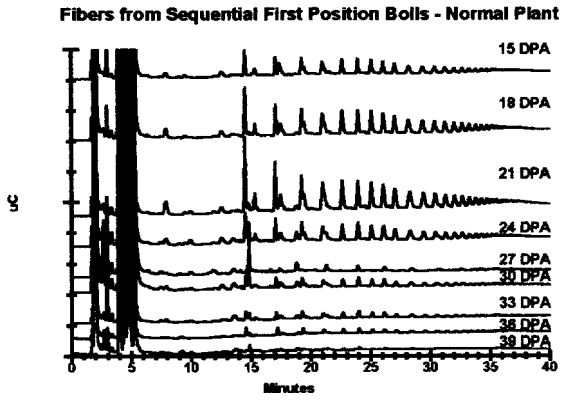

Figure 2. Oligomers (~mers) extracted from fibers from normal plant.

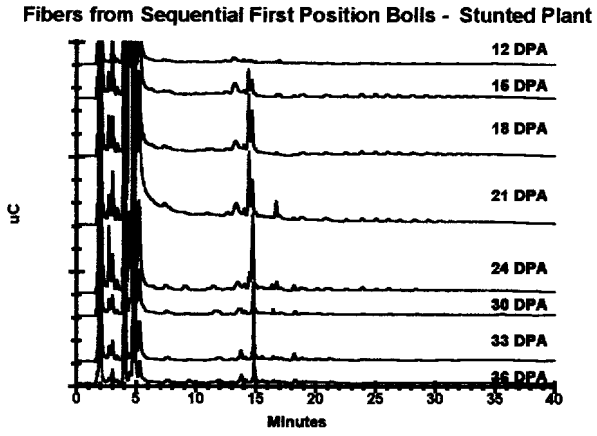

Figure 3. Oligomers (~mers) extracted from fibers from stunted plant.

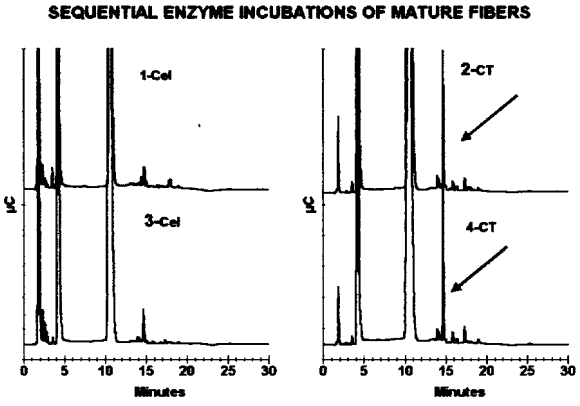

Figure 4. Oligosaccharides released from mature fibers by enzyme treatments. (Cel:cellulase, CT: chymotrypin).