Cell Wall Subunits, "Glue" Matrix and Cotton Fiber Development

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ABSTRACT

The object of this study was to isolate and characterize the cell wall subunits and "glue" matrix involved in cell wall synthesis in developing cotton fibers. This study employed fibers taken from 25DPA bolls collected at 7am, noon and 7pm. Cold aqueous extracts of developing cotton were analyzed by HPAEC-PAD (Murray, 1998). Subsequent sequential extracts were taken on a daily basis at three temperatures, 37°, room temperature and 4°. Extracts were fractionated by centrifugation and filtration. The fractions of the extracts were subjected to alkaline borohydride, mild acid, protease digestion or glycosidase treatment followed by PAGE and HPAEC-PAD (Murray, 1998). 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 that the fibers were collected and the temperature of the extraction. The extracts demonstrated the ability to synthesize the "glue" matrix by a temperature dependent mechanism that "consumed" hexoses in the process and released apparent carriers. The "glue" matrix appears to add carbohydrate residues to the subunits in the matrix after the initial carbohydrate residues are conjugated. The enzymatic activities associated with the "glue" matrix will be discussed. (Murray, A. K., 1998, U.S. Patent No. 5,710,047).

Introduction

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

Methods

Cotton variety DP-50 was grown in the Mississippi Delta for the time of day samples that were collected at 7am, noon and 7pm at 25 DPA. DP-50 plants for sequential bolls were also grown in the Sacramento Valley, California. Sequential boll sampling was at the same times 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 ~mers was achieved with dilute acid and elevated temperature prior to HPAEC-PAD.

Results

A series of oligomers (~mers) from developing cotton fibers were extracted 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 unit monomer in size. They 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 (Figure 1). These differences are suggestive of a role in cell wall synthesis that occurs at a maximal rate at night and is at its lowest rate in the afternoon (Balls, 1928).

The ~mers were extracted from first position bolls taken from sequential fruiting branches on the same plant. Comparison were made of the ~mers extracted from normal and stunted plants from the same field (Figure 2). The stunted plants were from an area of the field with shallower topsoil above gravel so it did not retain moisture as well as other parts of the field.

Summary

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. This has been observed in the ~mers extracted from other stressed plants. The prominent initial ~mer in the series from stressed plants suggests that there is abnormal polymer elongation under these environmental conditions.

The ~mers from a large molecular complex secreted by fibers, *in vitro*, by a temperature dependent mechanism was not obtained. The relative distribution of the ~mers can vary, depending on the exogenous substrates incubated with the fibers and the time of day that bolls were collected. Under optimal conditions the presence of the ~mers in an initial soluble fraction, a secreted fraction that will not pass through a 0.2µ filter, the precipitate of the aqueous extract and the fibers themselves have been demonstrated. The ~mers appear to play a structural role in the integrity of the cotton fiber since recent experiments to extract the ~mers using specific enzymes have resulted in a striking loss of the physical integrity of cotton fibers.

References

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Abbreviations: F, Fruiting Branch; DPA, Days Postanthesis; In, Inositol; Gl, Galactinol; S, Sucrose; Mb, Melibiose; Vt, Verbascotetraose; R, Raffinose; S, Stachyose; V, verbascose; A, Ajugose

Figure 1. Sequential formation of oligosaccharides: Extracted mers from glue matrix.

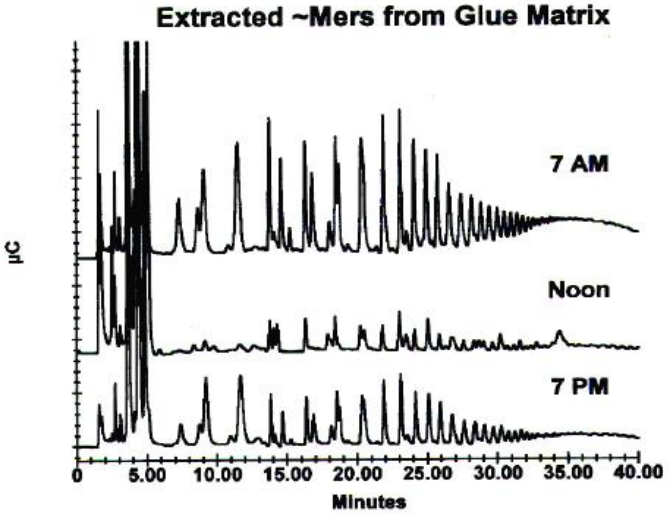

Figure 2. Chromatograms of developing fibers.