CELL WALL BIOSYNTHESIS IN DEVELOPING COTTON FIBERS: GLYCAN OLIGOMERS

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Abstract

Cellulose is a basic cell wall constituent and an abundant natural product of economic significance. Bolls picked at different times of the day and at various stages of development were quickly frozen in the field, stored frozen, and freeze dried. Later the fibers are re-hydrated, and extracted with cold water, to recover the soluble sugars including sucrose, the sucrosyl oligosaccharides and other hypothesized precursors of cell wall biosynthesis. A second extraction with hot, dilute HCl removed a series of oligomers, which consist primarily of glucose, but also may contain small amounts of mannose, galactose and arabinose. The relative abundance of the oligomers vary with time of day, suggesting a diurnal rhythm of cell wall biosynthesis. The extracted oligomers are most abundant at the onset of secondary cell wall biosynthesis, and then decline with boll maturation. In this research, we subjected the oligomers to additional fractionation by alcohol precipitation to yield populations eluting across a time gradient in high pH anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The oligomeric precipitates were subsequently resuspended and degraded by a specific endo ß-1,4-glucanase to yield glucose and the first oligomer eluted in the series. The abundance of these glycans corresponds with developmental age. In the early stages of secondary cell wall biosynthesis the relative abundance of the shorter chain length glycans is relatively high, then decreases from 21 to 25 (21>22>25) days post anthesis (DPA). During the same period the relative abundance of the longer chain length glycans increases, with a maximum at 22 DPA, then decreases (21<22>25 DPA). This pattern suggests that there are populations of oligomers differing in chain length. The populations appear to shift progressively to those of longer chain length as development proceeds. These data are consistent with a model wherein the oligomers are synthesized and incorporated into the secondary cell wall by means of a linkage or linkages that are not cleaved by the dilute acid extraction. Alcohol fractionation of the oligomers increases our ability to monitor cotton fiber development and to determine environmental effects on fiber yield and quality.

Introduction

The period of cotton lint development, from pollination to dry, mature fiber extends only about 45 days. The rates of cellulose deposition vary, with an early maximum rate at 16 days post anthesis (DPA) and a second, greater maximum rate at 28 DPA (Meinert and Delmer, 1977). However, only after the first 21 days have elapsed, can fibers be excised from developing bolls. A practical way to investigate fiber development is to freeze bolls in the field, and subsequently lyophilize them. Fractionation of the re-hydrated, developing fibers yields mono- and oligosaccharides, oligomers, an oligomer-protein complex, as well as insoluble fiber material. We have identified a series of glucose containing oligomers that are ubiquitous in all plant tissues investigated to date, including monocots, dicots, and marine algae. The quantitative distribution and presence of specific oligomers appears to be unique to each plant and tissue as well as to the developmental stage. The oligomers can be extracted using dilute acid from developing cotton fibers, mature cotton fibers, cotton dust and cotton fabric at various stages of processing as well as from old fabric, paper and wood.

Our interest is in understanding the dynamics of carbohydrate metabolism during cotton fiber development. Since a plant cell must synthesize cell wall material in order to grow and develop, knowledge of the events in cell wall biosynthesis can be used to monitor plant growth and to detect aberrations in growth due to environmental influences (Murray, 1998). During the period of fiber elongation, the fiber cell is predominantly synthesizing primary cell wall (Westafer and Brown, 1976). Following the period of cell elongation, the fiber cell thickens by synthesizing secondary cell wall, which consists almost entirely of cellulose. We believe that the glycans, constitute part of the cell wall biosynthetic process. Since they can be extracted from developing cotton fibers, mature cotton fibers, and aged cotton fibers in fabric, they may be fundamental sub-units of the cotton fiber. Since they have been found in every plant cell wall material examined, their ubiquity suggests that they are fundamental elements occurring with cellulose.

Methods

Gossypium barbadense var. Pima S-7 was grown at the University of California, West Side Research and Extension Center. Bolls, of approximately 24 DPA were collected at 4am, 6am, 8am, 12noon, 4pm, 730pm, and 12 midnight. Cotton, *Gossypium hirsutum* var. DP-50, was grown in the Mississippi Delta region. Bolls were collected at 21, 22 and 24 DPA. At both locations the bolls were placed directly in a dry ice chest, transported to the laboratory and freeze dried. The lyophilized fibers were first extracted with water at 0° to remove soluble oligosaccharides and monosaccharides (Murray, 1998). Following the cold water extraction, the fibers are subjected to 0.1N HCl in a boiling water bath for 30 minutes to extract the glucose containing oliogmers (Murray, 2000). The mono- and oligosaccharides extracted by the cold water procedure include *myo*-inositol, galactinol, arabinose, glucose, fructose, melibiose, sucrose, manninotriose, verbascotetraose, raffinose, stachyose, verbascose and, tentatively, ajugose. The relative abundance of these oligosacchardies can be used as indicators of fiber development (Murray, 1998, 2000). The oligomers extracted by the 0.1N HCl procedure can also be used as indicators of cell wall biosynthesis and fiber development (Murray, 2000). The HCl extracts were neutralized with 1N NaOH prior to HPAEC-PAD. Alcohol precipitations were performed using ethanol or n-propanol. In each case, the neutralized HCl extract was made up to 80% ethanol or n-propanol. The precipitate formed immediately and was collected by centrifugation at 8000 x g for 10 minutes. The supernatant was then frozen at –80°C overnight (16 hr.) and re-centrifuged. Chromatography was done on a Dionex CarboPac PA-1 column. The eluent was 150mM sodium hydroxide, isocratic up to 5 min, then a linear sodium acetate gradient from 5 min. to 40 min., increasing from 0 to 500mM. Subsequently, the precipitated oligomers were dissolved and treated with a highly purified cellulase (endoß-1,4-glucanase, Megazyme, Ireland) which apparently reduces the chain lengths, and yields peaks with shorter retention times. The only monosaccharide released by this cellulase treatment was glucose.

Results

Time of Day

The oligomers display retention times in HPAEC–PAD that are distinctly later than those of the oligosaccharides. The oligomers extracted from Pima S-7 are most abundant at 9:30pm and 6:00 am, with intermerdiate levels observed at midnight (Fig.1.) While the cause of the relatively low levels

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of oligomers at 4:00 am is unknown, consideration of the full circadian cycle in these data, and in previous results, suggest that oligosaccharides accumulate during the daylight period, and that synthesis of oligomers proceeds during periods of darkness (Murray and Munk. 2000). These results are consistent with those previously obtained from three collection times a day (Murray and Sassenrath-Cole, 1999).

Alcohol Precipitation

To facilitate isolation of the oligomers, the neutralized HCl extracts were subjected to alcohol precipitation to separate the larger from the smaller oligosaccharides and oligomers. The ethanol precipitates of the HCl extracts of DP-50 fibers from bolls collected at 21, 22 and 25 DPA are shown in Figure 2. The relative abundance of the glycans varies with age (Murray and Sassenrath-Cole, 1999). The relative abundance of the shorter chain length glycans decrease from 21 to 25 (21>22>25) DPA. During the same period the relative abundance of the longer chain length glycans increase, then decreases with a maximum at 22 DPA (21<22>25 DPA). This pattern is suggestive of a population of glycans increasing in chain length as development progresses, up to a point where the extraction method no longer removes the glycans. Propanol precipitated quantitatively more oligomers than did ethanol, and the discrimination between the first and second precipitate was greater (data not shown).

Cellulase Treatement of Oligomeric Precipitates

Propanol precipitates of the oligomers were subjected to cellulase for incubation times up to 90min. Such treatment results in a shift of the populations toward earlier eluting peaks, as shown in Figure 3. It was notable that the first oligomeric peak, which elutes at 14 min. was not produced by 90 mins. of treatment with cellulase. . Because of the absence of the 14-min peak, the precipitates were subjected to longer incubations. The results of the incubation of the propanol precipitates of 21 DPA fibers for various periods up to 18 hr. are shown in Figure 4. The 14-min. peak does not appear, until most of the later eluting peaks are much diminished. In all cases, glucose was the only monosaccharide released. The monosaccharide peaks are not shown in their full magnitude in order that the oligosaccharide peaks can be shown. The use of the cellulase treatment to produce earlier eluting peaks from later eluting peaks will facilitate isolation of the oligomers for further analysis by mass spectrometry. These data are consistent with an hypothesis wherein glycans of progressively greater chain length are sequentially synthesized and incorporated into the secondary wall by incorporation in a linkage or linkages, that are not cleaved by the dilute acid extraction. It is possible that such incorporation could involve spatial-physical well as chemical aspects, and could include processes as simple as physical entrapment of long chain oligomers by cellulose microfibrils.

Summary

Glucose containing oligomers have been isolated from developing cotton fibers by extraction with dilute HCl. The oligomers are most abundant in fibers collected during evening and early morning hours. To facilitate purification and isolation of the oligomers, they were precipitated by two different reagents either 80% ethanol or n-propanol. The precipitated glycans from fibers 21, 22 and 25 DPA were characterized by a decreasing abundance of the shorter chain length oligomers from 21 to 25 DPA. In a corresponding manner, the longer chain length oligomers increased in abundance from 21 to 22 DPA, and then decrease from 22 to 25 DPA. The longer chain length oligomers were then incubated with cellulase that cleaved them to the shorter chain length oligomers. These shorter chain length oligomers have been isolated and are the subject of further analysis.

Acknowledgement

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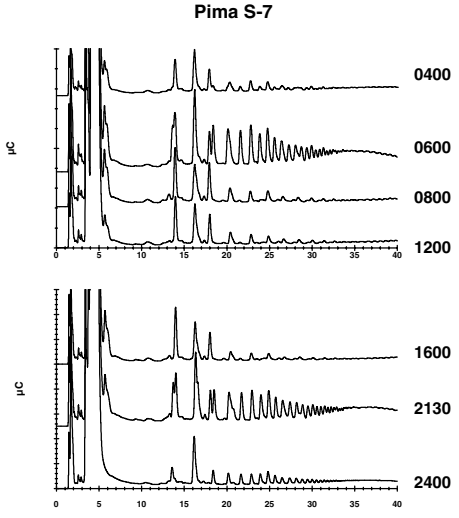

Figure 1. Oligomers extracted from developing fibers of Pima S-7 collected at different times of day.

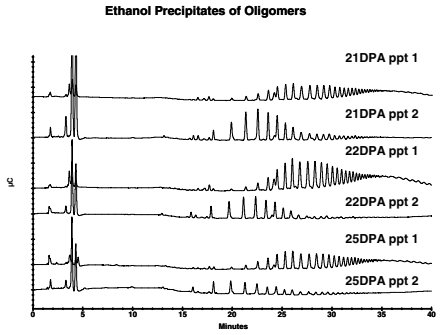

Figure 2. The oligomers from ethanol precipitates of the HCl extracts of DP-50 fibers from bolls collected at 21,22 and 25 DPA.

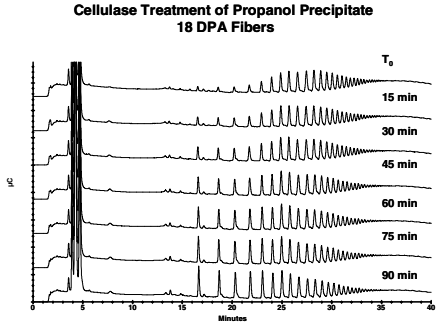

Figure 3. Oligomers from propanol precipitates of 18DPA fibers following treatment with cellulase for time periods up to 90 min.

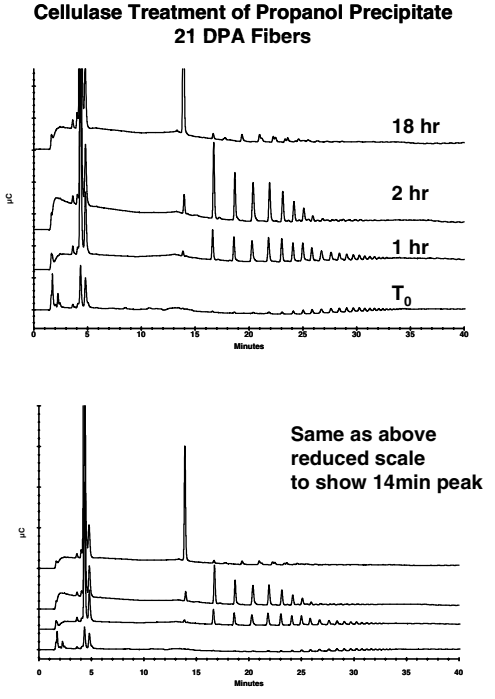

Figure 4. Oligomers from propanol precipitates of 18DPA fibers following treatment with cellulase for time periods up to 18hr.