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EFFECTS OF MILD ACID, HEAT AND SALT ON GLYCAN OLIGOMERS FROM DEVELOPING COTTON FIBERS

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Abstract

Extraction of developing cotton fibers with 0.1 N HCl at 100^0 C, or of a particulate precipitate released from the fibers by sonication in cold water using the same method, produces a series of oligomeric glycans. In order to purify the oligomers for determination of structure and more accurately quantify their concentrations, research was done to optimize their extraction. Such investigations suggested that the solubility of the oligomers in mild acid is inversely proportional to their inferred molecular weight, but much greater than expected when compared to the known soluabilities of cellooligosaccharides. Cellooligosaccharides of degree of polymerization (DP) 7 and above are reported to be insoluble. However, the glycan oligomers isolated by these techniques are estimated in the range of DP10-25 and are still soluble in water at a concentration of 2mg/ml (0.2%). These findings suggest that the oligomers may be labile in the in the cell wall biosynthetic environment, but become insoluble at a critical degree of polymerization where upon they may be joined to a developing microfibril. In related research, it was determined that the same glycan oligomers that are typically released with dilute acid, are present in the cold water extract of cotton fibers which have been heated to 165°C in the absence of oxygen. Further characterization of the glycan oligomers will clarify their relation to fiber biosynthesis, facilitate their use in monitoring fiber development, and possibly associate their *in vivo* dynamics with progression to fiber maturity.

Introduction

The utility of glycan oligomer analysis for investigation and identification of cotton fibers and textiles has been previously described (Murray and Nichols, 2004; Murray, *et. al*., 2004; Murray, 2003). Previous research indicates that such oligomeric glycans are most abundant at about 25 days post anthesis (DPA) when fiber cell wall elongation is declining and secondary cell wall deposition is increasing. The relative concentrations of the oligomeric glycans vary in response to physiological (time of day), developmental (DPA), and environmental (stress) variables, and have been hypothesized as probable indicators of relative fiber growth and maturity, and as a means of identifying differences among certain cotton cultivars as well. Disassembly of the oligomers using a highly purified endo-ß-1,4-glucanase indicates that they are composed of the monosaccharides glucose, galactose, and mannose, and a residue of crystalline cellulose. A similar set of products may be found by hydrolysis with 2N trifluoroacetic acid.

Materials and Methods

Cotton, *Gossypium hirsutum* cv. 'DP-50', was grown in the Mississippi Delta region. Bolls were placed on ice and frozen on dry ice as soon as possible following collection. Cold water extraction of developing cotton fibers and extraction of the glycan oligomers was done as previously described (Murray, et. al., 2001). Fibers were heated in a vacuum oven which was evacuated, purged with argon, and evacuated again prior to heating. In order to collect quantities of individual oligomers, a more shallow sodium acetate gradient (1.43%/min. vs. 2.86%/min.) was employed to increase the separation of the oligomers and minor constituents. Fractions were collected by hand to optimize separation of products for each respective peak that was observed. Since the fractions were in 150mM NaOH plus some sodium acetate, they were neutralized with 0.1N HCl very gradually with very complete stirring using a battery powered electric stirrer with a Teflon blade. The pH was checked using 5µl aliquots on pH strips and the samples were taken to pH 5.0-6.0. The samples were then concentrated on a Speed-Vac prior to desalting.

Results

A typical chromatogram for the oligomers extracted from 21 DPA fibers is shown in Figure 1.

The effects of conditions on the extraction of the oligomers were examined using concentrations of HCl of 0.05, 0.1, 0.25, 0.5 and 1.0 N and for varying periods of 10, 20 and 30 minutes at 100°. The results of the extractions at the varying HCl concentrations for 30 minutes are shown in Figure 2. From the perspective of current research, the concentration 0.1N HCl may be a useful means to extract a detectable quantity of product, without the appearance of major degradation of individual oligomers. However, use of lower concentrations of acidity suggests that the oligomers may be subject to disassociation at pH's at and below 1.0, and are completely disassociated in 1.0 N HCl. Basically the oligomers are increasing disassociated with decreasing pH, with the largest oligomers progressively degraded to yield the smaller ones. Clearly there is degradation of the oligomers at 0.25N HCl, and there may be some degradation at 0.1N HCl. It is unknown if the additional, minor peaks, preceding and following the first oligomer extracted at 0.1NHCl, are due to degradation of the primary product, or to more complete extraction that yields naturally occurring glycans with small differences in retention time.

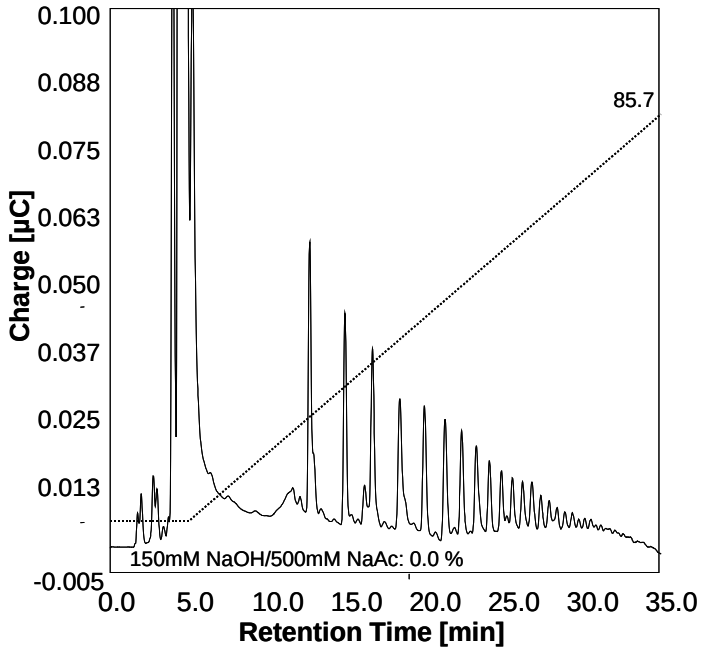

Figure 1. Chromatogram of 21DPA fibers extracted with 0.1 N HCl for 30minutes.

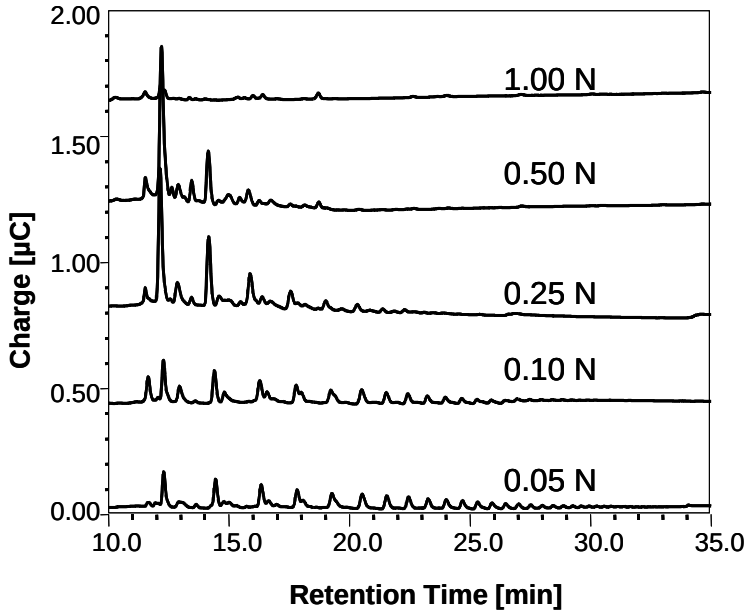

Figure 2. Chromatogram of 21DPA fibers extracted with 0.05, 0.1. 0.25, 0.5 and 1.0 N HCl for 30 minutes.
0.2.

Samples of some oligomeric fractions extracted in 0.1N HCl were concentrated from 800-900µl down to 150-200µl, but not taken to dryness. The samples were then chromatographed. The fractions which had not been taken to dryness, now contained some monosaccharides, but only the oligomer originally collected and the next smaller oligomer, although one such sample had one other smaller oligomer with a trace of a second one. These chromatograms are shown in the top four traces in Figure 3. The results suggest that the collection procedure was efficient for collection of a single peak, but that some instability of product was either occurring in solution or due to some aspect of sample handling, as evidenced by observation of the monosaccharides and the appearance of next smaller principal peak. Our interpretation is that a certain fraction of the product was conserved, but another fraction was probably losing one hexose. In contrast, samples of certain oligomeric fraction extracted in 0.1N HCl that had been taken to dryness were re-dissolved in 250µl of water and then re-chromatographed to verify that the product representing a primary peak had not been altered prior to structural studies. The samples displayed profiles indicating the presence of the full series of oligomers including those larger than the fraction isolated. This is shown in the bottom two chromatograms of Figure 3. These results suggest that taking the samples completely to dryness in the presence of NaCl and a small amount of sodium acetate results in disruption and reassembly of the whole series of oligomers. This effect of dehydration in the presence of salt is the subject of ongoing research.

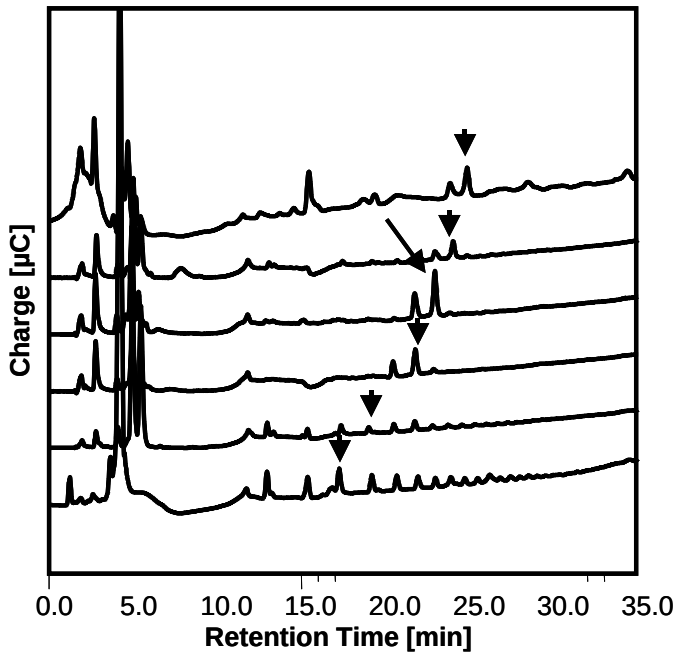

Figure 3. Chromatograms of oligomers which had been isolated and concentrated in the top four plots and those which were taken to dryness and re-dissolved in the bottom 2 plots.

In another research approach, suggested by related work on fossilized cellulose (Data not shown here.) dried fibers were heated to 166°C for 70 and 120 min under an atmosphere of argon. Following the heating, it was found that some of the oligomers were present in the cold water extract (0°C for 15 min) which precedes the 0.1N HCl extraction. The chromatograms for the 70 and 120 min heat treatments are shown in Figure 4. One can assume that a greater quantity of oligomers would be released with longer heating periods up to some point at which they may begin to be degraded. Such was observed in the related work when tissue was heated to 200°C for 200 hours. (Data not shown.)

Discussion

The apparent high-degree of lability of the glycan oligomers to weak acid solutions is striking. The extracted oligomers are much more sensitive to acid than would be expected for ß-1,4-glucans. Their solubility is particularly

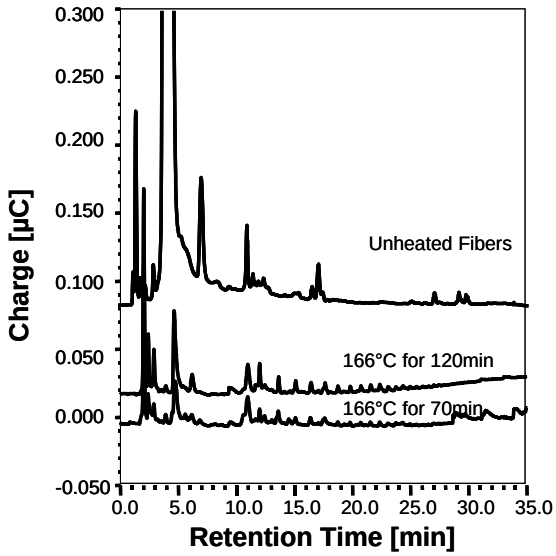

Figure 4. Chromatograms of cold water extracts of 18DPA fibers unheated or heated to 166°C for 70 or 120 minutes.

unusual since ß-1,4-glucans above DP-7 are thought to be insoluble. The glycans, extracted by these procedures, are soluble in water up to an estimated DP-25 at 2mg/ml.

The behavior of the isolated oligomers following neutralization and drying was also unexpected. Single oligomers were relatively stable in concentrated solution and when taken to dryness in a salt-free environment. Complex fractions of 10-12 oligomers have been desalted, eluted from chromatography on BioGel P-4 or Sephadex G-25, taken to dryness, and re-dissolved without any noticeable change in their distribution. (Data not shown.) However, single-peak collections that were dried before de-salting regenerated both larger and smaller glycan oligomers following rehydration and chromatography. The fact that such generation of the oligomeric series was not observed for oligomers that were only concentrated but not taken completely to dryness suggests that the molecular rearrangement is due to the effect of salt on crystallization and re-soluablization of the material. The possibility that salt crystals interfere with possible crystallization of the oligomers is worthy of further investigation. When salt-contaminated, dried extracts of a natural fiber reported to be greater than 95% cellulose, were re-hydrated, spontaneous generation of multiple macromolecules, with inferred DP's greater than those of the original extracted were observed. These chromatographs provide indirect evidence that physiochemical self-assembly of possible subunits of cellulose, the most common cell wall constituent, did occur.

The release of the glycans from fibers by dry heat was also unanticipated. These results are difficult to interpret, but further suggest that the oligomers are naturally occurring subunits of cellulose.

To this point, research with the oligomers suggests that they are consistently found in natural celluloses, that they may be readily extracted from cellulose sources, and that their properties suggest they may function as subunits with a propensity to disassemble or reassemble in response to small changes in physiochemical, and we hypothesize, biochemical conditions. The growing body of information about the glycans, their properties and transformations will contribute to our understanding of their specific chemical identities and biological functions.

Acknowledgement

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