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GLYCAN OLIGOMER PROFILES OF COTTON

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Abstract

Mature cotton fibers have been described as containing as much as 98% cellulose, a β-1,4-glucan. The generally-accepted hypothesis of cellulose microfibril biosynthesis is that a membrane-bound enzyme complex adds one glucose molecule at a time to an accreting linear polymer. No intermediate products of the biosynthesis have been hypothesized. However, several carbohydrates, of varying degrees of polymerization (DP), can be extracted from mature fiber, depending on the extractant employed. Glucose, fructose, sucrose, ribose, galactitol, mannitol, arabitol, raffinose, stachyose and verbascose, among other sugars and sugar alcohols, can be extracted with cold, deionized water. A series of glycan oligomers, comprised chiefly of glucose, putatively of DP 4 to more than 25, may be extracted by mild acid, 0.1 N HCl. A similar series of oligomers also may be extracted by strong acid treatment of 80% acetic,1.8N nitric acid. The presence of the soluble sugars and oligomers can be demonstrated and their relative concentrations determined by high pH anion exchange chromatography. The oligomers liberated by mild and strong acid treatment also have been recovered from wood, paper, and textiles; and have been used to differentiate among specific types of materials within such cellulose sources. In developing cotton, the same oligomers may be found and vary in response to developmental and environmental factors. Analysis of over 15 cultivars further suggests that the profiles of oligomers from mature cotton fibers vary among cultivars, and may provide a means of cultivar identification.

Introduction

Cotton fibers are single cells that grow out from the seed coat. At maturity, each fiber is the collapsed, desiccated wall of the formerly tubular cell. Cotton Fibers have an exterior or primary cell wall an an internal secondary cell wall. While the secondary cell wall consists primarily of cellulose both the primary and secondary cell walls contain several polysaccharides, many of which have not been characterized. The biosynthesis of polysaccharides requires several sugar nucleotide donors each being the product of a series of enzymatic reactions. Glycosyl acceptors are required of which most have not been characterized. The expression of the enzyme is under genetic control but it is also subject to environmental influences. The concentrations of the substrates and products of the enzymatic pathways are governed by Michaelis-Mentin kinetics. Given the complexity of the system, it is not surprising that a number of non-cellulosic components can be identified in cotton fibers and that variability can be demonstrated in the polysaccharide composition of cotton fibers.

Materials and Methods

Cotton, (*Gossypium hirsutum*) cultivar 'Ultima' , was grown in the San Joaquin Valley of California. A bale of Ultima was shipped to Cotton Incorporated, Cary, North Carolina, and the fiber processed using manufacturing-scaleequipment, and a knit fabric made. A fabric sample was diced as finely as possible with a razor blade. A 5-mg sample of fibers was placed in a 2.0 ml screw cap plastic tub and 0.5 ml of water was added. In the refined method, the sample was homogenized with a PRO 200 Homogenizer with a Multi-Gen tip (PRO Scientific, Inc.), 7-mm in diameter, until a uniform homogenate was obtained. Using either sample preparation method, the tube was shaken, then placed in a Branson 85 W sonicator filled with ice water for 15 minutes. The tubes were centrifuged at 15,000xg for 5 minutes. The supernatant was removed with a Pasteur pipette, 1.0 ml of 0.1 N HCl was added and the tube was mixed on a vortex mixer and placed in a boiling water bath for 30 min to extract the glucose containing oligomers. The supernatant was removed with a Pasteur pipette, 1.0 ml of 80% acetic 1.8N nitric acid (Updegraff, 1969) was added and the tube was mixed on a vortex mixer and placed in a boiling water bath for 30 min to extract

the second group of glucose containing oligomers. HPAEC-PAD was performed using a CarboPac PA-1 column. The eluent was 150 mM sodium hydroxide, isocratic from 0 to 5 min then a linear sodium acetate gradient from 5 to 40 min going from 0 to 500 mM in 150 mM NaOH at a flow rate of 1 ml/min. The waveform had a potential of +0.1V from 0 to 0.40 sec, -2.0V from 0.41 to 0.42 sec, +0.6V from 0.43 to 0.44 sec and –0.1V from 0.44 to 0.50 sec with integration from 0.20 to 0.40 sec.

Results

Examples of typical chromatograms for the three extractions are shown in Figure 1. Although the oligomer profiles for the HCl extraction and the acetic nitric extraction appear to be different, some of the oligomers appear to be the same in both extracts. This similarity is based on similar retention times both between extracts and in experiments in which the extracts are mixed. Similarities of the monosaccharide composition are the subject of ongoing experiments. In the case of mature cotton or fabric, the amount of oligomer material removed by the acetic nitric extraction is much greater than in the case of the HCl extraction. In immature fiber such as 21 days postanthesis much more oligomer material is contained in the HCl extract than in the acetic nitric extract.

Discussion

Three sequential extractions, cold water, dilute HCl and acetic nitric acid followed by high pH anion exchange chromatography permit a multifaceted characterization of cotton fibers. The relative concentrations of constituents in the three extracts appear to be characteristic for a source of cotton or other cellulosic material. Although these extracts contain only non-cellulosic material, they appear to contribute to the unique characteristics of each cultivar of cotton (Murray, 2006). Although one can observe differences in the glycan oligomer profiles of cotton from different sources future work involves the development of a mathematical model for a more sophisticated analysis of the data. One goal will then be to correlate the glycan oligomer profiles with fiber characteristics.

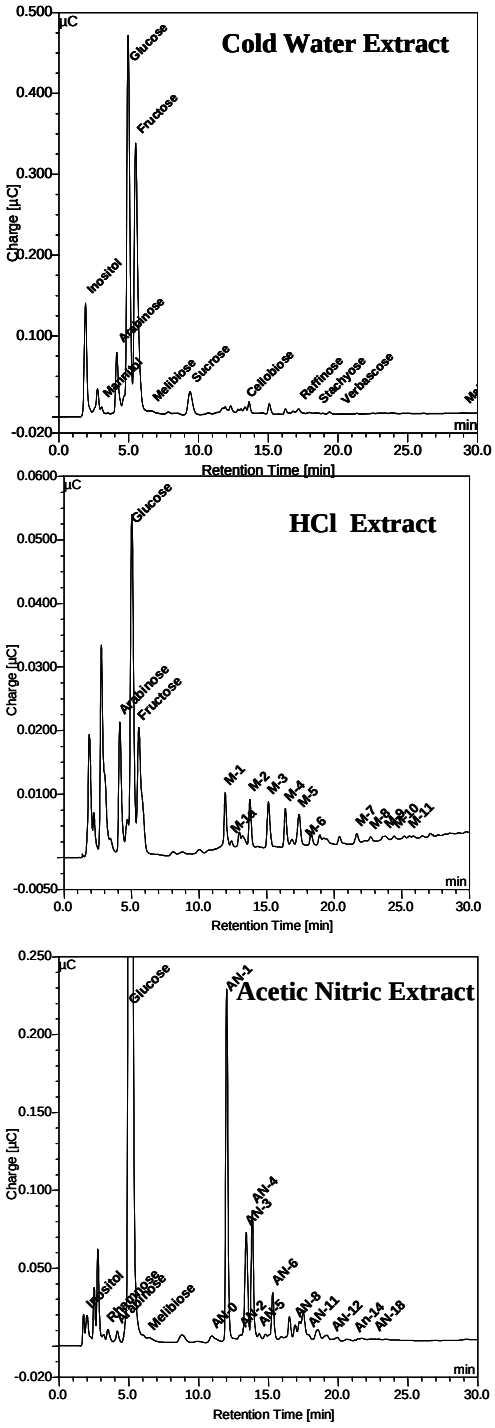

Figure 1. Chromatograms of the water, 0.1N HCl and 80% acetic 1.8N nitric extracts.

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