CELL WALL CONSTITUENTS AND ENZYME ACTIVITIES IN COTTON DUST

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Abstract

Synthesis of developing cotton fiber cell walls involve a glycosylated protein "glue" matrix, This "glue" matrix then appears to play a role in maintaining fiber integrity since its removal by specific enzymes results in a loss of fiber integrity. Oligosaccharides released from the "glue" matrix have been found in samples of cotton dust. In addition, soluble oligosaccharides, which play a role in fiber wall development, and endogenous enzymes which convert them have also been found in cotton dust. These results will be presented in the context of cotton fiber development and the potential problems presented by these constituents in cotton dust.

Introduction

The differences in glycoconjugate profiles of developing cotton fibers have been documented from either tagged flowers or from bolls of various ages (Murray, 1996, Murray & Brown, 1996, 1997, Murray *et. al*. 1997). Recent work in this laboratory has focused on oligomers of glucose released with dilute acid and a “glue” matrix protein in cotton fiber development (Murray and Sassenrath-Cole, 1999). The “glue” matrix protein is heavily glycosylated which is cellulase sensitive, indicative of a ß-D glucan, and linked to a core structure which has not been completely characterized but contains galactose and mannose. In vitro incubations of developing cotton fibers demonstrate the interconversion of the sucrosyl oligosaccharide and the fact that exogenous substrates in the form of oligosaccharides do influence the glycopeptides, which can be released by hydrolysis with dilute acid. The experiments described here were carried out to determine to what extent elements of the fiber biosynthetic apparatus are still present in cotton dust.

Methods

Samples of cotton dust (Stoneville and DB 5/89) were obtained from the USDA, ARS, SRRC, New Orleans, LA. Cotton dust was 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 was achieved under conditions of dilute acid and elevated temperature prior to HPAEC-PAD. Incubation experiments were carried out at 37°C under toluene, to prevent microbial growth, with the additions and incubation times as described in the text.

Results

Cotton dust was found to contain the same soluble carbohydrates as are found in mature cotton fibers. The question of the presence of the enzymes required to interconvert the soluble carbohydrates is of interest since the presence of the enzymes would indicate that the cotton component of the dust is biologically active and not a simple organic mixture of fiber components. Cotton dust was incubated with 3.75mg/ml bovine serum albumin (BSA) with or without 5mM glucose under the conditions described in Methods. The results are shown in Figure 1. The predominant sucrosyl oligosaccharide in the BSA boiled control (-C) is verbascose with a retention time of about 14.1min. The incubation (BSA) also has carbohydrates with retention times of about 9.5, 28.5 and 30 minutes respectively. The 28.5 and 30 minute peaks also have coincident A_{280} peaks not shown on the figure. The peak at 9.5 minutes is also found in incubations of developing cotton fibers however its identification is not complete at this time. The addition of 5mM glucose to the incubation mixture results in a several fold increase in the 9.5 minute peak as well as the peaks at 28.5 and 30 minutes. Between 13.3 and 15.5 minutes three peaks, raffinose, verbascose and ajugose (tentative identification) are also present.

The oligomers extracted with dilute acid from cotton dust and old denim fabric are shown in Figure 2. The majority of the oligomers found in fabric are also present in dust. The effect of incubation without added substrates for 24 hours prior to extraction of the oligomers, as shown in Figure 3, shows the increase in the peak at 15.5 minutes. This is indicative of the presence of enzymatic machinery to either interconvert or synthesize the amount of this glycopeptide, which is then released by dilute acid.

Discussion

The fact that the enzymatic mechanisms to interconvert the sucrosyl oligosaccharides are active in cotton dust on hydration is of interest since a similar scenario could occur in tissues of the respiratory tract if cotton dust is inhaled. The presence of the acid labile oligomers in cotton dust and the capability of the enzymes present in the dust to alter the distribution of these glycopeptides, which can be isolated from the dust, have been observed. From the standpoint of cotton fiber development, the presence of the soluble oligosaccharides as well as the acid labile oligomers and the presence of enzymes to alter both of these classes of constituents are of interest. The presence of such activities in dust leads to the conclusion that these activities are also present in mature cotton fibers and they may alter the fiber properties if the cotton is stored under conditions of temperature and humidity which would favor such

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activities. From the standpoint of the adverse health aspects of cotton dust these results indicate that constituents of the cotton fibers may have the potential to contribute to adverse health effects in addition to the microbes associated with the dust.

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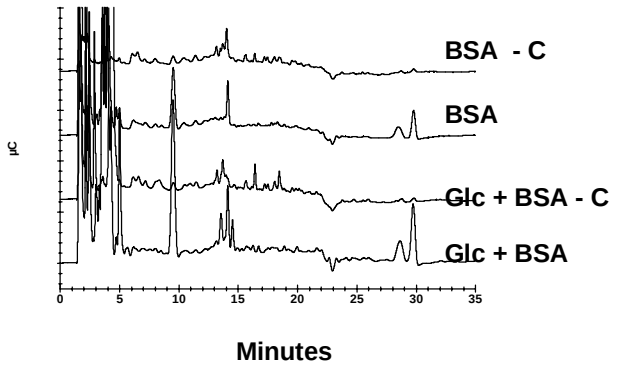

Figure 1. Effect of Incubation on Soluble Oligosaccharides Extracted from Cotton Dust (DB 5/89)

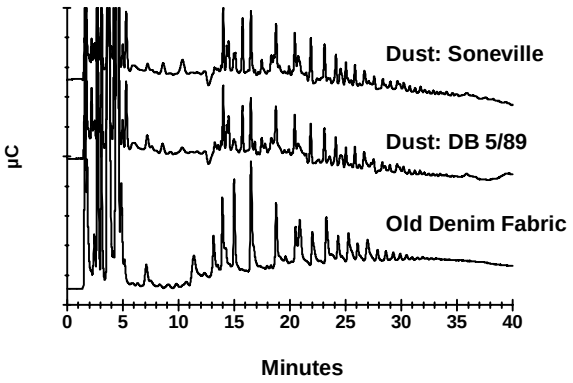

Figure 2. Oligomers Extracted from Cotton Dust and Fabric.

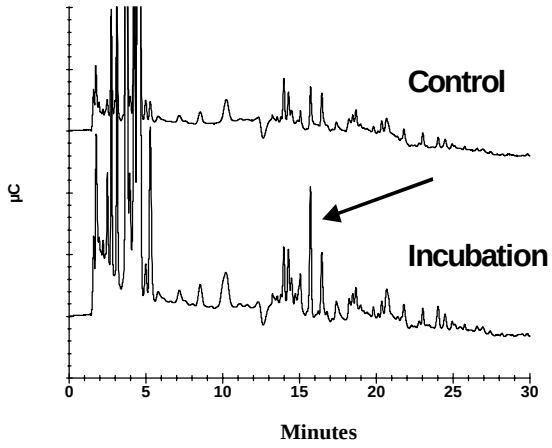

Figure 3. Effect of Incubation on Oligomers Extracted from Cotton Dust (Stoneville)