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Note

Extractability of the (1→3)-β-D-glucan from developing cotton fiber

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The developing cotton fiber (*Gossypium hirsutum* L.) contains substantial (1→3)-β-D-glucan just before the onset of secondary-wall synthesis[1]. Several investigators[2–5] have characterized amounts of the (1→3)-β-D-glucan throughout fiber development, but the location and role of this glucan during various stages of development are not clear.

The ease of extraction of the glucan from the cotton fiber could provide information pertinent to its location and role in fiber development and performance[2,5,6–8]. However, only tantalizing fragments of isolated data on extraction have been reported. Removal of a portion of the (1→3)-glucan by enzyme digestion is strong argument for the extracellular location of this fraction. A more-definitive report concerning location of the (1→3)-β-D-glucan in the cotton fiber is that of Waterkeyn[9]. By a special fluorescence method, he showed that insoluble glucan was always located in the innermost wall layer bordering the cell lumen. In addition to this insoluble fraction, he observed a soluble fraction that emanated from fibers sectioned in ethanol.

The purpose of this report is to characterize the relative ease of extraction of the (1→3)-β-D-glucan from developing cotton fibers, to employ a method specific to this glucan for the extraction, and to describe the methodology so that it may be utilized by others. A preliminary step in the method involves a low degree of derivatization of the polysaccharidic substrate to introduce *O*-(2-diethylaminoethyl) substituents. Following extraction with 2M trifluoroacetic acid, the portion of (1→3)-β-D-glucan removed from the substrate is identified by the presence of 4-*O*-substituted glucose residues in the hydrolyzate. Only a low and negligible amount of 4-*O*-substitution results from terminal glucosyl groups of the cotton cellulose molecule[5].

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EXPERIMENTAL

Plants. — Field growth and sampling of cultivar DES-56 (*Gossypium hirsutum* L.) were described earlier[5]. Samples examined in this study included fibers from unopened bolls harvested at 20, 27, 34, and 48 days post-anthesis (DPA) and from open bolls harvested at 62 and 104 DPA.

Chemicals and derivatization of the cotton fibers. — Common chemicals were reagent grade. Technical grade 2-chloroethyldiethylamine hydrochloride (Hexagon Laboratories, Bronx, NY, U.S.A.)* was purified and converted into *N,N*-diethylaziridinium chloride. The latter reacted with raw cotton fibers to introduce 2-diethylaminoethyl (DEAE) substituents at low degree of substitution (~0.05) at hydroxylic positions of the polysaccharides of the raw cotton fibers[5].

Analyses for site of derivatization. — Samples of derivatized cotton fibers were hydrolyzed to mixtures of glucose and DEAE-glucoses: total hydrolysis was with H_2SO_4 and partial extractive hydrolysis with trifluoroacetic acid (following paragraph). Details, including those of the g.l.c. analyses, have been described earlier[5].

Extraction of derivatized (1→3)-β-D-glucan. — A weighed sample (0.2500 g) of derivatized cotton was covered with 25.0 mL of 2M CF_3CO_2H in a tightly sealed, thick-walled, 50-mL serum bottle. The bottle was shaken in an autoclave for 30 min at 121°. (The shaker consisted of a pivotal stand jolted at 5 sec intervals by a solenoid-driven rod. The shaker and solenoid inside the autoclave were connected to an electrical pulse-controller outside the autoclave by thin Formex insulated wire around which the autoclave gasket formed a tight seal.) Shaking was most useful in the second and third extractions to assure maximal and even extraction of the (extensively fragmented) fiber residues. The mixture was centrifuged and the supernatant solution withdrawn by pipette to a round-bottomed flask containing 2 mg of *myo*-inositol as the internal standard. The undissolved solid was rinsed with 5 mL of 2M CF_3CO_2H. The supernatant solution, combined with rinsings, was evaporated to dryness under diminished pressure with the water bath maintained below 40°. The residue was thrice dissolved in 15 mL of water and evaporated to dryness under diminished pressure to dissociate CF_3CO_2H–glucose complexes[10]. The final residue was freeze-dried for direct silylation and g.l.c. analysis on a capillary column[5].

Reproducibility of hydrolytic extractions with CF_3CO_2H. — Reproducibility was delineated with samples that were similar to the primary samples, which were available in only limited amount. Triplicate analyses of products from hydrolysis by CF_3CO_2H gave relative standard deviations of 7% for each of the DEAE-glucoses; variations were similar for 4-*O*-DEAE-glucose from hydrolysis by sulfuric acid, but standard deviations were lower (1–3.5%) for the other DEAE-glucoses. The major

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*Names of companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned.

source of variability lay in the hydrolysis step, most probably associated with small variations in duration of reaction in the autoclave.

RESULTS

Derivatization of raw cotton fibers with *N,N*-diethylaziridinium chloride was conducted to assess a representative fraction of the total accessible hydroxyl groups. The HO-4 is unique to each glucose residue of a (1→3)-β-D-glucan and to the terminal glucosyl group of each cellulose molecule. The latter amounts to one HO-4 per 5,000 to 13,000 glucose residues and is insignificant in comparison to the former. The glucose residues of the (1→3)-β-D-glucan in the raw cotton fiber are assumed to be completely accessible, as was observed for curdlan, an available pure (1→3)-β-D-glucan[8]. The derivatization was conducted under mild conditions to facilitate reproducibility and to generate substituted polysaccharides bearing only monosubstituted glucose residues. The degree of substitution of raw cotton

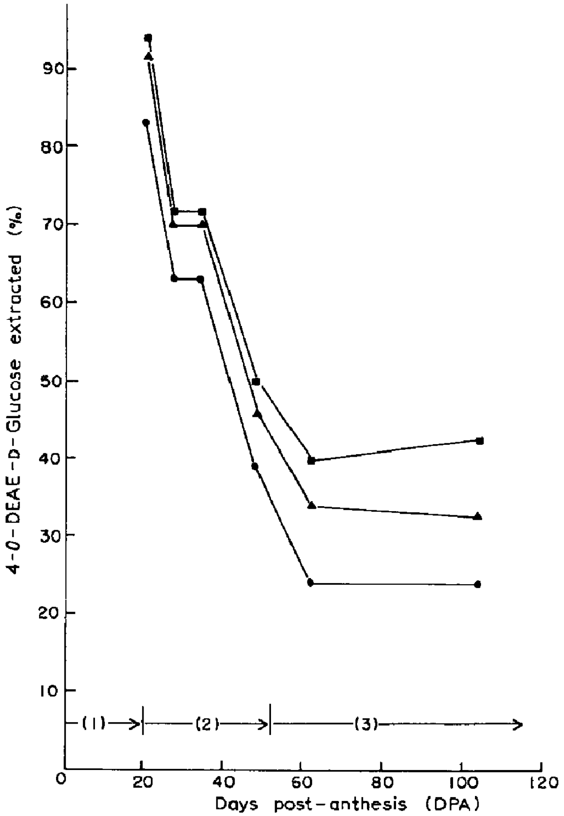

Fig. 1. Percentage of 4-*O*-DEAE-glucose extracted from derivatized raw-cotton fibers in sequential 30-min treatment with 2M CF_3CO_2H: ●, initial extraction; ▲, plus second extraction; ■, plus third extraction; (1) primary-wall deposition; (2) secondary-wall deposition; (3) boll opening and fiber desiccation.

cellulose (or of the average glucose residue) was near 0.05, but the extent of monosubstitution was a function of the accessibility of the hydroxyl groups of each substrate. To ensure a realistic assessment of accessible HO-4 present in each sample of cotton, the reagent was allowed to react with the cotton fibers in their natural states, that is, the cotton was brought directly into the reagent system without drying (for closed-boll cotton) and without rewetting (for open-boll cotton)[5].

Dried, derivatized cotton fibers subjected to 30-min treatment in 2M CF_3CO_2H at 121° were only partially solubilized in the reagent. This behavior was consistent with results of Meinert and Delmer[1] in that non-cellulosic polysaccharides were attacked and extracted, and >97% of the cellulose was resistant to the treatment. G.l.c. analysis of the DEAE-glucoses in the solubilized fraction showed that the 4-*O*-DEAE-glucose, representing the (1→3)-β-D-glucan, decreased with increasing DPA of the cotton fibers, namely, 0.458, 0.335, 0.142, 0.101, 0.027, and 0.032 mg/g of derivatized cotton at 20, 27, 35, 48, 62, and 104 DPA, respectively.

The initial content of 4-*O*-DEAE-glucose units in each sample of the derivatized cotton was measured by total hydrolysis with sulfuric acid followed by g.l.c. analysis. Relative to these total contents of 4-*O*-DEAE-glucose, the first extractions with 2M CF_3CO_2H accounted for 83% of 4-*O*-derivative at 20 DPA, 63% at 27 DPA, 63% at 34 DPA, 39% at 48 DPA, 24% at 62 DPA, and 24% at 104 DPA (Fig. 1). These numbers express decreasing extractability from the period of primary-wall formation through secondary-wall deposition to boll opening and fiber desiccation.

A second extraction of the non-solubilized fraction increased recoveries of 4-*O*-DEAE glucose by 7–9% for the immature cottons (27–48 DPA) and 9–10% for the mature cottons (62–104 DPA). A third, sequential extraction further increased recoveries by 2–4% for the immature cottons, and 6–10% for the mature cottons.

DISCUSSION

Hydrolytic extractability of (1→3)-glucan from dried cotton fibers with 2M CF_3CO_2H decreases progressively from the high level characteristic of essentially complete formation of primary wall through the deposition of secondary wall to boll opening and fiber desiccation. The stages of decrease in extractability are consistent with high exposure and accessibility of the (1→3)-glucan in the (relatively disordered) primary wall (20 DPA), with decreasing exposure and accessibility within the highly ordered, secondary wall during progressive deposition of this cellulose (20–48 DPA), and with further decreased accessibility (increased trapping) within all of the cellulose (which consists primarily of secondary wall) following desiccation and deswelling of the fiber (between 48 and 62 DPA). In each of the periods of change of extractability, the decrease is believed to result from increased association of microstructural units of the cellulose fiber to increase the degree of trapping of the (1→3)-glucan.

We propose that the first extraction readily removes the (1→3)-β-D-glucan from the primary wall and lumen (that is, the more disordered and exposed regions of the cotton fiber), and that subsequent extractions remove glucan trapped in increasingly more crystalline regions of the fiber, which are much more resistant to hydrolysis by 2M CF_3CO_2H. The slightly larger percentage increases in recoveries in the second and third extractions from the mature cottons (beyond 48 DPA) versus immature cottons (20–48 DPA) may be associated with the increasing disorder known to occur on boll opening[8].

Hydrolytic extraction, such as with 2M CF_3CO_2H, must be considered unique by comparison with an extraction that dissolves the (1→3)-β-D-glucan as an intact component away from the raw cotton fiber. The latter dissolution depends upon the release of one long-chain molecule from another. In the case of 2M CF_3CO_2H, some extraction may occur in this manner (primarily at low DPA), but the major release of (1→3)-glucan from cellulose chains is believed to occur as the molecular chain of the (1→3)-glucan is cleaved into glucose units. Thus, extractability by hydrolytic extraction with 2M CF_3CO_2H is likely to be higher than extractability realized with such agents as ethanol, Me_2SO, strong alkali, acetic–nitric acids, and ammonium oxalate–oxalic acid, which have already been explored for this purpose.

The foregoing data show that glucose units having hydroxyl groups that are readily accessible and available for derivatization are not readily susceptible to hydrolytic extraction with 2M CF_3CO_2H. This behavior is consistent with the presentation of hydroxyl groups and glucosidic linkages in the microstructure of cotton cellulose[11], in which case high accessibility of hydroxyl groups and glucosidic linkages is indicated for those glucose residues on one of the two types of accessible surfaces of fibrils. The other type of accessible surface is characterized by decreased accessibility of hydroxyl groups and very low accessibility of glucosidic linkages. A generally similar behavior may characterize the (1→3)-glucan that is trapped within the cellulosic microstructure.

Extractability of the (1→3)-β-D-glucan from cotton fibers with 2M CF_3CO_2H is consistent with location of a fraction of the glucan in the lumen[9] and a fraction within the primary wall[7], and it is indicative of the location of a substantial fraction (presumably between layers) in the secondary wall of the mature of near-mature fiber. The fraction more-resistant to extraction with 2M CF_3CO_2H is believed to be that within the crystalline, secondary wall.

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