Enhanced Enzymolysis of Never-Dried Cotton Fibers Belonging to Different Species

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SYNOPSIS

Enzymolysis of cotton cellulose in their never-dried state, belonging to all the four cultivated species of cotton, was carried out with the enzyme derived from *penicillium funiculosum* F_4. Hydrolysis to reducing sugars was almost complete for all the cottons in 6 h, though glucose percentage varied. X-ray characterizations of the residues obtained, which were both after enzyme and acid hydrolysis, showed significant differences between both hydrolyses, as well as differences in the behavior of different cotton fibers towards enzyme action. These differences have been attributed to the different structural organization of cellulose in the secondary cell wall of cotton fibers.

INTRODUCTION

Our earlier studies have shown that the enzymolysis of cotton cellulose of DCH-32, an interspecific hybrid (*Gossypium hirsutum* × *Gossypium* barbadense), in their never-dried state was significantly faster and different when compared with a similar action performed on their dried counterparts.[1] It was shown that these fibers could be completely converted to sugars within 6 h of treatment with enzyme. It was also noted that the never-dried fibers became further disordered after enzyme treatment, contrary to an ordering usually found in normally dried cotton fibers during a similar treatment.[2] The disorder occurring during enzyme hydrolysis was traced to the simultaneous access by the enzyme to the variously ordered regions of cellulose due to the highly open nature of the substrate.

In this article we have extended the above study to cottons belonging to all the four cultivated species in order to understand the enzyme action on never-dried fibers and thereby to gain knowledge about the structural organization of different species in cotton fibers. Some of the pertinent results are presented here.

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EXPERIMENTAL

Materials

Never-Dried Bolls

Green bolls (40 days postanthesis) from potted plants belonging to all the four species viz. G-27 (*G. arboreum*), DB 3-12 and V.797 (*G. herbaceum*), Laxmi (*G. hirsutum*), and Suvin (*G. barbadense*) were collected from several plants and were preserved in 10% formalin until use. The bolls were opened under water and the fibers were separated gently and were washed several times to remove the preservative. After centrifuging at 5000 rpm for 10 min, the fibers were weighed and transferred immediately to the enzyme system.

Enzyme Hydrolysis

Penicillium funiculosum Thom F_4 was used for the production of cellulose. Enzymolysis was carried out at 50°C in an acetate buffer system of pH 4.8. In all the sets, the enzyme activity was adjusted to 0.277 FPU by manipulating the amount of buffer solution. The enzymolysis was arrested after different periods by suitable denaturing treatments to the enzyme solution. The residues collected by filtering were dried to constant weight using standard procedures.

The total reducing sugars and glucose were esti-

mated according to the methods of Somogyi[3] and Bergmeyer et al.[4]

Acid Hydrolysis

The acid hydrolysis of never-dried fibers was carried out in IN HCL at 60°C for 24 h. After the desired interval, the filtered and washed fibers were dried to constant weight.

Methods

X-Ray Diffraction Measurements

Residues obtained after enzymatic and acid hydrolysis, as well as untreated control fibers, were subjected to X-ray examination using a Philips stabilized X-ray generator that was fitted with a diffractometer and with recording accessories. Substrates or residues were cut, if required, to pass through a 40 mesh screen and the fine powder filling a rectangular holder was pressed under nominal pressure and was used for the X-ray examination.

The crystallinity calculations were made using the intensity profile obtained between 2θ values of 10°–40° (2θ), based on the method standardized at CTRL earlier by Chidambareswaran et al.[5] The half breadth, $\beta_{1/2}$ of the (200) peak was also measured to characterize the lattice order and perfection of crystallites in the fiber.

Degree of Polymerization. The degree of polymerization was calculated for all the samples before and after enzyme hydrolysis by measuring their viscosities using the procedure outlined in the earlier publication.[1] For the fibers G–27, the viscosity measurements were made using a Cuprammonium solvent and the DP values so computed were later converted to the Cuene-scale using a regression equation formulated from the results of Pandey,[6] where a comparative evaluation of both the solvents for different varieties of cotton fibers was presented.

RESULTS

The results on sugar yield, amorphous content, and degree of polymerization obtained after the enzymolysis of all the varieties are given in Table I. It can be noted that the total reducing sugar obtained after 6 h incubation in the case of old-world cottons (G–27, DB 3–12, and V.797) was around 90%, as opposed to 100% with those of *G. hirsutum* and *G. barbadense*. The glucose yield from the former group was around 15% as compared with 25% with the latter species.

Amorphous contents measured using X-ray techniques indicated that there are significant differences in the decrystallization during enzymolysis of different fibers. While DCH–32 recorded a decrys-

Table I Data on Sugar Yield and Structural Characterization of Enzymatically Hydrolyzed Cotton Fibers Belonging to Different Species

Variety	G–27 Mature Fibers = 92%				DB 3–12 Mature Fibers = 72%				V–797 Mature Fibers = 22%			
Treatment	RS (%)	Glucose (%)	AM (%)	DP	RS (%)	Glucose (%)	AM (%)	DP	RS (%)	Glucose (%)	AM (%)	DP
None	—	—	38	4039	—	—	30	2557	—	—	44	2959
Enzyme treated (6 h)	92.9	17.0	42	1047[a]	90.5	16.5	37	605	94.4	14.4	51	300
Variety	Laxmi Mature Fibers 51%				Suvin Mature Fibers = 78%				DCH–32 Mature Fibers = 66%			
	RS (%)	Glucose (%)	AM (%)	DP	RS (%)	Glucose (%)	AM (%)	DP	RS (%)	Glucose (%)	AM (%)	DP
None	—	—	30	1809	—	—	35	2076	—	—	40	2978
Enzyme treated (6 h)	100	24.7	36	469	100	26.2	40	408	100	25.5	59	803

[a] This DP value is for sample treated with enzyme for 3 h.

tallization of 50% as compared to control, G–27 and Suvin cotton fibers showed minimum changes in amorphous contents (10% and 8%, respectively, over their untreated controls); the other cottons showed intermediate levels of disordering. In general, cotton belonging to *G. arboreum* showed less decrystallization than those of *G. hirsutum* and hybrid.

The amount of mature fibers expressed as a percentage, shown in Table I, did not seem to influence greatly either the course of the enzyme hydrolysis or the final structural order obtainable after the enzymatic attack.

The degree of polymerization was also found to decrease considerably during enzyme action on cotton fibers: the exact change in DP seemed to be dependent on the initial value, that is, variety. The initial DP values among cottons were also found to have a wide range. Since DP values for G–27 were measured for fibers subjected to enzymolysis only up to 3 h, a comparison of 3 h treated samples indicated that, while enzyme treated DB 3–12 fibers showed only 59% decrease in DP, those of Laxmi showed the very high decrease in DP of 83%. The differences between cottons, however, were found to decrease to some extent after 6 h enzymolysis, as shown in Table I.

In order to understand the course of enzyme hydrolysis on different cottons, a rate curve of sugar production has been schematically presented in Figure 1. Whereas Figure 1 (A) shows the production of total reducing sugars as a function of time, Figure 1 (B) depicts a similar variation in the glucose produced. These curves are somewhat different for the various cottons, illustrating the fact that the enzyme attack is different in these varieties; the difference in curves also may point to the fact that the substrates have different structural organizations.

Figure 2 (A) and (B) depicts the changes in the amorphous content and DP values during the course of enzymatic hydrolysis. Whereas the initial amorphous content among cottons varied only by 10% (30 to 40), the disordering could be seen to have taken place to different extents, and after 3 h enzymolysis, the range has widened to 29%–50%; it continues to remain at 35%–59% even after 6 h of treatment. The widening of the gap is particularly due to the creation of large amounts of amorphous material in DCH–32 during enzymolysis. These facts clearly show that the lateral order distribution present in cotton fibers is responsible for providing such a disordering pattern after enzymolysis and, further, that the lateral order distribution could be different among cottons.

The above observations have been corroborated by the DP rate curve shown in Figure 2 (B). The widely different initial DP values for these fibers

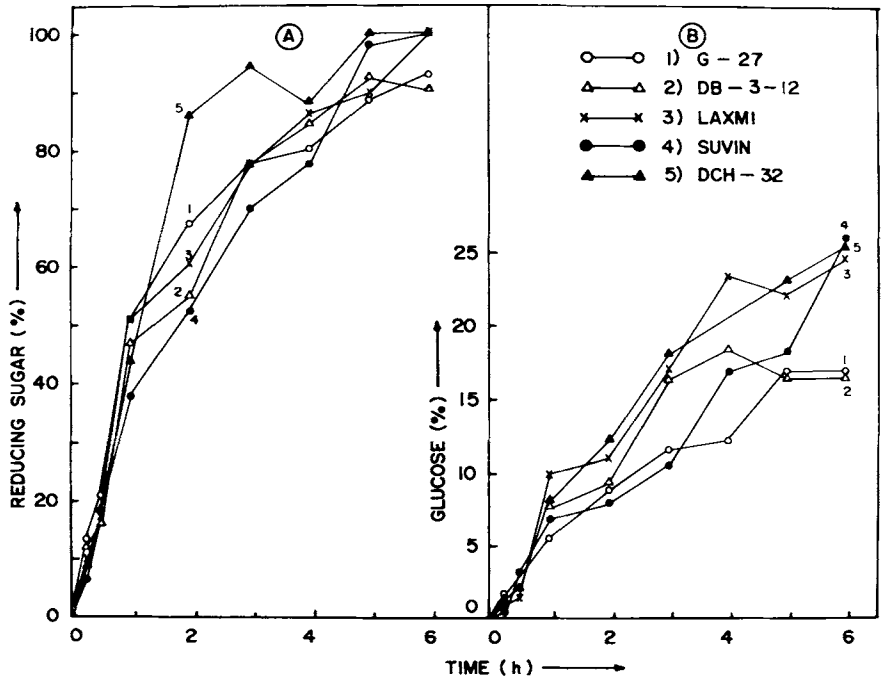

Figure 1 Rate curve of enzymolysis of different Cottons: (A) Reducing sugar (%) and (B) Glucose (%).

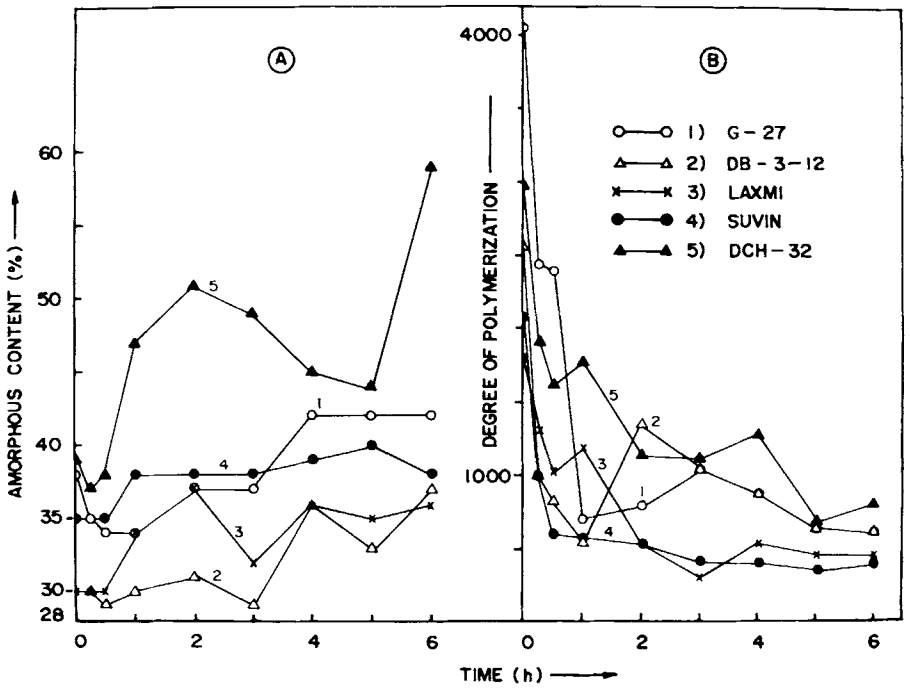

Figure 2 Change in the (A) Amorphous content (%) and (B) Degree of polymerization values with time of incubation during enzyme hydrolysis.

probably point out that the chain length distribution could be widely different in these cottons. Further, since chain length is one of the essential factors governing hydrolytic action, the DP curves are markedly different for the different cottons. Because the substrates are in the never-dried form, the accessibility for the enzyme, even to the most organized parts of the fiber, is ensured. As a result, the chain scissions and conversion to lower molecular substances would take place simultaneously in all regions, leading to a continuous change in the DP distribution in the substrate. Since the rate curves are found to be different for various substrates at all times of hydrolysis, this denotes that the chain length distribution could be different, not only in their untreated, never-dried state, but also in their hydrolyzed residues.

The results obtained after acid hydrolysis, carried out on the various cottons in their never-dried state, showed that regardless of their initial crystallinity and DP, all fibers recrystallized after hydrolysis. This is made clear in Figure 3, in which the final crystallinity of the hydrolyzed residues are plotted against their initial values. The mild hydrolysis conditions used here caused very negligible weight loss; earlier studies[7,8] have shown that recrystallization does occur during such a process. This crystallization is a result of the mobility conferred during acid attack to chain segments constituting fringes of crystallites before hydrolysis, enabling them to come closer in order to form stronger hydrogen bonding. This process leads to a lengthening and broadening of crystallites. Although irrespective of cotton, all samples showed recrystallization after acid hydrolysis; the extents of crystallization are different as shown in Figure 3. The different extents of recrystallization obtainable for different varieties could be due to different lateral order distribution in these cottons.

The decrystallization of cotton fibers during enzymatic hydrolysis, and a recrystallization during hydrolysis using acid, could be due entirely to different mechanisms of action of these reagents on never-dried cotton fibers.

DISCUSSION

One of the significant results in the present study is that the reducing sugar and glucose yields obtained were lower in cottons belonging to *G. arboreum* and *G. herbaceum* than those of *G. hirsutum* and *G. barbadense*. This result could be due to the differences in the organization of cellulose in the secondary cell wall between cottons. Further, the optimum chain length required, especially for glucose production after enzymolysis, could also be different in these cottons. Earlier work in this laboratory by Betrabet

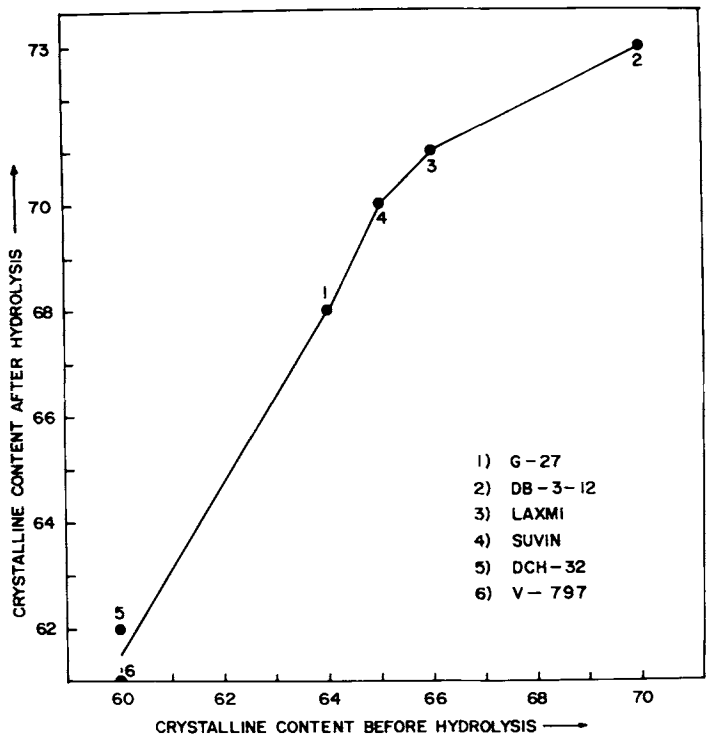

Figure 3 Plot depicting influence of initial crystallinity of different cottons on the final crystallinity obtained after acid hydrolysis.

et al.[9,10] has shown that the pore structure and crystallite dimensions are different in cottons belonging to *G. herbaceum* as compared to those of *G. hirsutum*. The pore size and its distribution, as well as crystallite dimensions, could also be expected to influence profoundly the enzymatic hydrolysis.

In an earlier study on the factors affecting the enzymatic degradation of cellulose, Rowland et al.[11] have shown that, in addition to other things, the enzymatic sacharification is highly influenced by the pore structure of the cotton fiber. Cowling and Brown[12] have also shown that, upon adsorption of enzymes onto the cotton fiber, considering the size and shape of the enzyme molecule, these molecules could be expected to readily diffuse within the gross capillaries present on the surface of the fibers. These large molecules would later gain access to the smaller pores existing on the interior regions of the fiber by a process of enlarging the available capillaries. In this manner, the pore size and its variety of distribution would influence the rate of enzymolysis.

It is well known that never-dried cotton has a much larger pore volume than nature-dried fiber and that the pore size and its distribution could be expected to be different than that of the nature-dried cotton since some small pores are expected to be "sealed off" during drying. In addition, the pore size distribution could be different in different varieties, even in their never-dried state, and the distribution would thus lead to the different susceptibilities for enzyme action.

Another factor that could be responsible for the differences in the enzymatic hydrolysis among cottons is the structural organization of the cellulose in the secondary wall. It is assumed that the never-dried fibers are made up of unassociated Protofibrils[13] with complete accessibility to the differently ordered regions because of the water present in them. These fibers possess a lateral order distribution even before drying.[14] However, the lateral order distribution could be marginally different from the distribution present in dried fibers, due to the fact that the crystallinity only slightly improves upon drying. The disordering that took place during enzymolysis has been explained earlier,[1] based upon the lateral order distribution in cotton. The different distributions envisaged here for the different cottons used probably explains the different extents of disordering encountered during enzymolysis. Studies in our laboratory on the lateral order distribution of cottons[15] have shown that there is a greater amount of higher order fraction in *G. arboreum* cottons.

A recent work by Peters et al.[16] has demonstrated

that the mass of cellulose per unit volume of secondary wall is identical for all cottons in their never-dried state. However, an identical density of cellulose does not necessarily mean identical packing of the fine structural–fibrillar elements in the secondary wall for all the cottons, especially when the fibrillar dimensions have been reported to depend on fineness of the fiber.[17] Such packing differences could be expected strongly to influence the enzymolysis, particularly in the never-dried state.

The degree of polymerization of cotton fibers is another parameter, which is capable of influencing the course of enzymolysis. As noted earlier, the fact that the average DP is different in different cottons suggests that the chain length distribution could be different in them as well. Timpa et al.[18] have shown that the molecular weight distributions were different even among cottons that seemingly were similar in their physical properties, such as fiber length, micronaire, and strength. Hence it is logical to assume different chain length distributions for these cottons since it is well known that the growth and environmental conditions greatly influence the chain length.[19,20]

A preliminary experiment conducted to separate molecular fractions having DP above 10,000, based on the method suggested by a group of Russian workers[21] using cadoxen and propyl alcohol–water systems, indicated that this fraction was higher in Suvin and G–27 cottons as compared with other cottons. The method was suitably modified using our standardization procedures. However, a detailed study should be conducted before any conclusions can be drawn from the above experiment. Interestingly, the percentage increase in disorder after enzymolysis (6 h) was found to be the lowest in these two cottons (8% for Suvin and 10% for G–27). This result, although preliminary, suggests the influence of chain length on the disordering pattern obtained during enzyme attack on never-dried fibers.

CONCLUSION

The present study on the enzymolysis of never-dried cotton fibers belonging to different species reveals between cottons certain significant differences in the molecular and structural organization of the cellulose present in the secondary cell wall.

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