Electron Diffraction Study of Enzyme-Hydrolyzed Cotton Samples

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Synopsis

An electron diffraction study on continuously and repeatedly enzyme-hydrolyzed cotton samples was carried out. The electron diffraction pattern on repeatedly enzyme-treated sample reveals a cellulose I pattern with the absence of the 101 plane. This confirms that enzymatic hydrolysis of cotton fibers proceeds preferentially along the 101 plane, being a clevage plane.

INTRODUCTION

The effect of cellulase enzyme on the percent crystallinity, crystallite size and the influence of crystallinity on the susceptibility of cellulosic material to enzymatic hydrolysis was studied by various investigators.[1-7]

The high degree of lateral and longituinal order makes the cotton fibers less susceptible to enzymatic attack by the cellulase enzyme,[4] even if the treatment is prolonged. However, in a recent publication[8] we have shown that the action can be enhanced considerably by subjecting the fibers to repeated enzymic action. Further, the residue obtained after repeated enzymatic hydrolysis followed by mechanical disintegiation consists of almost crystalline particles of cellulose having length about 350 Å.[8]

In the present note electron diffraction study on both continuously and repeatedly enzyme-hydrolyzed cotton sample has been reported.

EXPERIMENTAL

The production of cellulase enzyme from the organism *Penicillium funiculosum* (CTRL isolate F4) and treatment of cotton samples with cellulase enzyme remains the same as described in an earlier publication.[8] The enzymic hydrolysis of purified cotton samples was carried out using the following system; 100 mg cotton; 5 ml enzyme filtrate; 10 ml acetate buffer (PH. 4.8), 5 ml distilled water.

In one set of experiments the system was incubated at 50°C in water bath with constant shaking for different time intervals varying from 1 day to 7 days continuously. In another set of experiments, the enzyme solution was replaced by fresh enzyme solution after 3 days of incubation time. Considering the 3 days-continuous treatment as one cycle of enzyme hydrolysis the sample was subjected to 6 cycles of enzyme hydrolysis.

The dried cellulase enzyme treated samples were suspended in double distilled water and put in an ultrasonicator for 5 min. to form uniform

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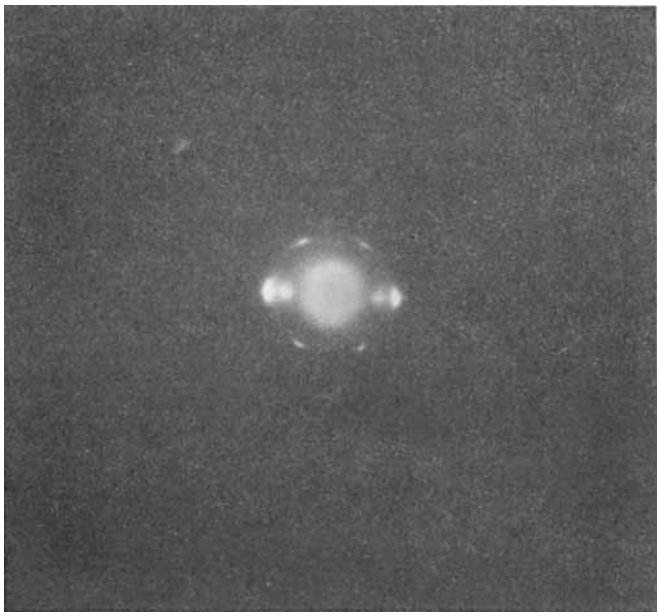

Fig. 1. Electron diffraction pattern of cotton cellulose enzymatically hydrolyzed for 3 days.

suspension. A drop of diluted suspension was placed on carbon coated copper grids and dried at room temperature. The electron diffraction (ED) patterns of selected area of specimen were recorded using Hitachi HUIIE electron Microscope as described earlier.[9]

RESULTS AND DISCUSSION

The ED pattern recorded on cotton sample treated with cellulase enzyme continously for 1 to 7 days revealed the typical cellulose I pattern with all predominant reflections viz., (101), ($10\bar{1}$), (002), (021) and (040). The typical ED pattern of cotton cellulose treated with cellulase enzyme for 3 days is shown in Fig. 1. It exhibits the cellulose I lattice structure with all the prominant reflections mentioned above. The ED pattern of cotton sample after second cycle of enzyme treatment revealed only two reflections (Fig. 2) having 'd' spacings of 3.98 Å and 4.28 Å corresponding to (002) and (021) reflections respectively of cellulose I lattice. All the other prominant reflections expected of cellulose I lattice particularly the equatorial (101) and ($10\bar{1}$) reflections are absent in the pattern. Similar ED patterns were also recorded for samples corresponding to higher cycles of enzyme treatment up to the sixth cycle.

The striking absence of the equatorial (101) reflection in the ED pattern of drastically acid hydrolyzed cotton sample was also observed by Dobb *et al.*[10] This is explained on the basis that (101) plane of cellulose crystals is a cleavage plane and the acid hydrolysis progresses preferentially along this plane. Hence the particles in the substrate exhibit uniplanar orientation with their 101 lying flat on the grid.

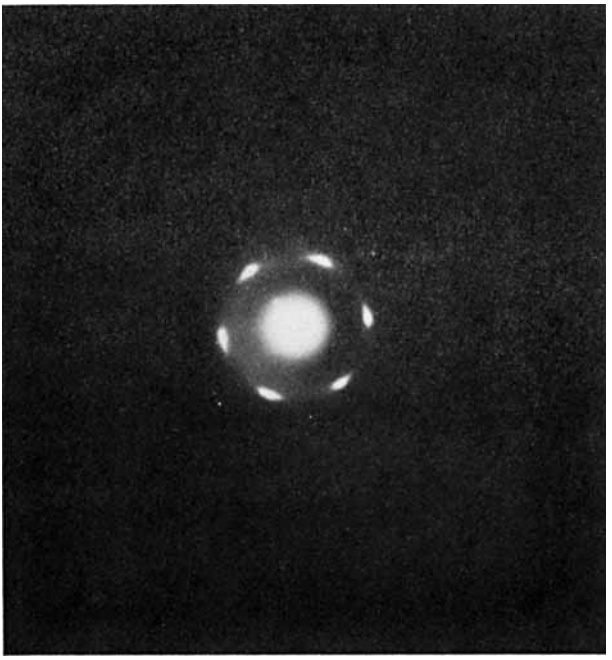

Fig. 2. Electron diffraction pattern of cotton cellulose repeatedly treated with enzyme for the 2nd cycle.

In the enzyme hydrolysis the absence of (101) reflection could be explained as follows. The cellulase enzyme is constituted of three components c_1, c_x and cellobiase. According to Siu[12] c_1 is responsible for weakening mutual cohesion between chains of cellulose by breaking hydrogen bonds and thus facilitating the entry and final breakdown of cellulose chains by c_x. In continuous enzyme treatment, the c_1 component of cellulase enzyme may not be able to break the hydrogen bonds because of its inactivation due to formation of reducing sugar.[11] Hence the enzyme action is limited although the duration is sufficiently long. Therefore, in continuous enzyme treated samples the ED pattern is exactly the same as that for pure cellulose I lattice with all predominant reflections until the end of 7 days.

On the other hand, in the case of repeated treatment, the residue is once again exposed to fresh hydrogen bond breaking action by the c_1 component of cellulase enzyme. Thus, at the end of the second cycle of treatment the action appears to be quite drastic and resulting in the cleavage of cellulose crystallites along the 101 plane. Therefore the ED pattern recorded on cellulose sample after the second cycle of enzyme treatment is marked by the absence of (101) reflection and the presence of only (002) and (021) reflections. The same action continues in the successive cycles of enzyme treatment and the ED patterns from samples belonging to higher cycles of treatment remained invariant as mentioned earlier. The only change found is in particle length which progressively reduces at the later cycles of repeated enzyme treatment. At the end of the 6th cycle the particle length is ultimately reduced to about 350 Å which corresponds to the length of crystallites in the cotton fibers.[8] It

may be mentioned that Battista[13] also obtained uniform microcrystall particles of "diameter" ranging from 100 Å to 300 Å by severe mechanical shear to cellulose from wood pulp to disperse its microcrystals.

In conclusion, it may be said that although the mechanism of degradation of cellulose by acid hydrolysis and cellulose enzyme are quite different, the cellulose particles obtained at the end of drastic treatment in both the cases show identical structure and morphology.

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