Hydrolysis of Cuprammonium Rayon by Endocellulases

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ABSTRACT: The first step in the hydrolysis of cuprammonium rayon with endocellulases was examined and the results were compared with those of hydrolysis with a cellulase complex. Mechanical agitation during endocellulase treatment was effective for the separation of the oligomers from the substrate. The endocellulases removed the intermediately ordered regions in the disordered matrix. The crystalline regions were negligibly affected by the endocellulases, while the cellulase complex considerably deaggregated the surface of the crystallite. The endocellulases enhanced the brittleness of the fabric more than did the cellulase complex. It was assumed that the hydrolysis with the endocellulases proceeded without molecular deaggregation of the fiber. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 79: 2543–2547, 2001

Key words: cellulase; endocellulase; cuprammonium rayon; crystallinity; water sorption; molecular deaggregation

INTRODUCTION

Crude cellulase (cellulase) secreted by fungi and bacteria contains major hydrolytic enzymes of endocellulases, exocellulases, and β-glucosidases.[1] Endocellulases randomly hydrolyze cellulose molecules to produce cellulose oligomers of various lengths. Exocellulases successively cleave cellobiose from the ends of cellulose molecules. β-Glucosidases hydrolyze cellobiose to form two glucose molecules. Endocellulases presumably attack disordered regions of cellulose material, because a high degree of accessibility of the substrate to the enzyme is required to hydrolyze cellulose molecules randomly.

Molecular deaggregation of cotton fiber has been observed during cellulase treatment.[2] It is assumed that the cooperation of enzymes in cellulase causes molecular deaggregation of highly crystalline materials. Recently, we found that the crystallite surface of cotton fiber was activated and water sorption was considerably increased due to cellulase treatment.[3]

The development of gene-cloning procedures has made it possible to produce a cellulase which attacks hydrolytic points in disordered regions[4] and which has endocellulase activity. The endocellulases can eliminate fabric fuzz, which probably includes a large number of disordered regions, to improve the appearance of fabrics.[4] The endocellulases produced are less active with respect to highly crystalline cellulose materials. Cavaco-Paulo et al.[5] noted that mechanical agitation during endocellulase treatment enhanced the separation of hydrolyzed fragments from the substrate. They also suggested that a combined treatment with endocellulases and cellulase enabled the desired fabric product to be achieved.[5]

The initial hydrolysis of cuprammonium rayon by the endocellulases was investigated in this study and the results were compared with those of the hydrolysis by cellulase. Cuprammonium rayon has a large number of disordered regions and is useful for examining the action of the endocellulases to crystalline cellulose materials.

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EXPERIMENTAL

Materials

The cuprammonium rayon (cupra) fabrics used were of the plain weave type and were interwoven with filament yarns. Counts in warp and weft directions were 7 and 14 tex, respectively. The numbers of threads per centimeter in the warp and weft directions were 50 and 37, respectively. The fabric was rinsed in an aqueous 0.2% (v/v) solution of a nonionic surfactant at 40°C before enzyme treatment.

The endocellulases used were a developing sample supplied by Novo Nordisk Bioindustry Co. (manufacturer's code number sp640) and were produced by a genetically modified *Aspergillus* microorganism. The cellulase, supplied by the Meiji Seika Co. (Tokyo, Japan) and produced by *Trichoderma viride,* was the same as used previously.[6]

The carboxylmethylcellulase activities of the endocellulases and the cellulase used were 509 and 233,000 units/g, respectively. One unit was defined as the amount of enzyme that liberates 10 μg of glucose equivalents in 10 min in an aqueous carboxylmethylcellulose solution. The cotton fiber, which is a highly crystalline cellulose material, was treated for 6 h with the endocellulases. The result was that the amount of glucose equivalents liberated was negligible.

Cellulase Treatment

Hydrolysis with the endocellulases was performed with an enzyme concentration of 0.3% (v/v) at 60°C and pH 7. Cellulase hydrolysis was carried out with an enzyme concentration of 0.2% (w/v), at 40°C and pH 4.5. The liquor-to-sample ratio was 1 : 100. The treatment solution containing the substrate was inactivated by immersion in boiling water after the treatment. During enzyme treatments, the 3.5-cm-diameter agitation rotor was rotated at 82 rpm. The endocellulase solution was replaced every 24 h with a fresh solution to reduce the extent of enzyme inactivation and obtain a relatively high value of weight loss. Weight loss values were obtained by comparing fabric weights before and after treatment.

Measurement of Total Sugars and Reducing Sugars

The total number of glucose equivalents of the cellulose oligomers and the number of reducing sugars in the treatment solution were measured by the sulfuric acid–phenol method[7] and the Nelson–Somogyi method,[8] respectively. The average degree of polymerization (DP) was obtained by dividing the total number of glucose equivalents by the number of reducing sugars measured within the same sample. The treatment solution examined was separated from the substrate through decantation.

IR Spectroscopic Measurements

The crystallinity index was obtained using an IR spectrophotometer (FTS-30, Bio-Rad Co.). The absorptivity ratio of 1372–2900 cm^{-1} was obtained as a crystallinity index.[9] Measurement was performed by the diffusion reflection method.[10]

X-ray Diffraction Measurements

An X-ray diffractometer (SRA M18XHF, MAC Science Co.) was used to determine crystallinity in the 2θ range of 8° to 40°. Measurements were obtained using pellets made of cut fiber segments and an Ni-filtered Cuα radiation source under the conditions of 45 kV and 200 mA. After the background based on air scattering was eliminated, the percent crystallinity was estimated from the ratio of integrated intensities of crystalline scattering to total scattering.

Water Adsorption

Equilibrium sorptions were obtained at a relative humidity (RH) of 95% and a temperature of 20°C. The equilibrium sorption obtained under 95% RH was an indication of the internal pore volume of the substrate.

Measurement of Mechanical Properties

The low-stress mechanical properties were measured using a KES-FB apparatus (Kato Tech Co.), based on a method of KES.[11] The changes in the properties were represented as percentage changes in the properties of the treated fabrics compared to those of the unprocessed fabrics.

RESULTS AND DISCUSSION

Hydrolyses by the Endocellulases

Weight loss of the cupra after endocellulase treatment was much less than that after cellulase treatment for a given treatment time, as shown in Figure 1. We previously noted that the cellulase

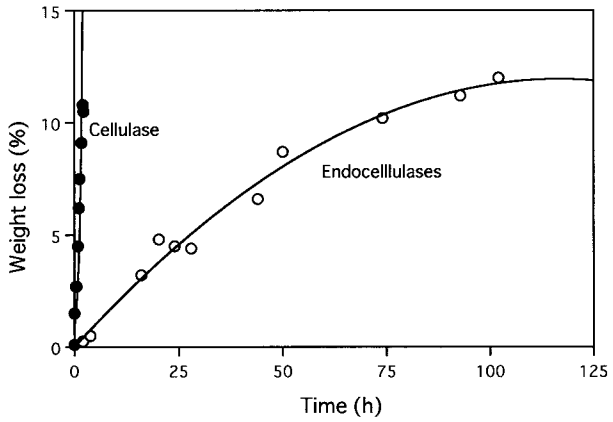

Figure 1 Treatment times with endocellulases and cellulase complex plotted against weight loss of cuprammonium rayon.

used here caused marked deaggregation of cotton substrates.[2] It is clear that the cellulase used deaggregated the cellulose chains of the cupra and markedly enhanced the rate of hydrolysis.

The DP values of the cellulose oligomers by endocellulase and cellulase treatments are compared with respect to weight loss, as shown in Figure 2. The endocellulases provided the relatively low DP value of 6 in the first step of the hydrolysis. This presumably reflected the difficulty for the polymeric enzyme to penetrate even the disordered regions. The DP increased to approximately 20 at a 12.8% weight loss.

The maximum DP value of cellulose oligomers, oligosaccharides, which are soluble in water, is approximately 6.[12] The larger DP value of more than seven of the oligomers in the treatment solution was due to the presence of insoluble oligomers which were separated from the substrate by agitation during the treatment. Figure 2 shows that the further the hydrolysis proceeded, the longer the molecular length of the insoluble fragments removed from the substrate.

The DP dependence on weight loss for the cellulase is also shown in Figure 2. The DP was initially approximately 20 and decreased with increasing weight loss. This trend was completely opposite to that observed upon endocellulase treatment. Adsorption of component enzymes in the cellulase occurred prior to the actual hydrolysis of the cellulose.[13,14] The cellulase-based hydrolytic sites were spaced sufficiently far apart by the deaggregation of cellulose chains, so that the DP of the removed oligomers was high after cellulase treatment. With the hydrolysis by exocellulases and β-glucosidases following the hydrolysis by endocellulase, the DP of the oligomers in the cellulase treatment solution decreased gradually with treatment time and increasing weight loss.

Structural Change of Fiber in Response to Endocellulases

The changes in the crystallinity index and crystallinity of the fibers during endocellulase and cellulase treatments are shown in Figure 3. The crystallinity index of the endocellulase-treated fibers decreased up to approximately 5% weight loss and increased at higher weight-loss values. The ordered regions detected using IR as the crystallinity index presumably reflect the restricted freedom of the atomic group vibration in the molecules and are based on small units.[9] These regions are not only the intermediately ordered regions in the disordered matrix but also build up large crystalline units detected by X-ray diffraction.

The crystallinity of the fiber increased continuously with increasing weight loss upon endocellulase treatment. This suggested that the crystalline regions detectable through crystallinity evaluation were negligibly hydrolyzed by the endocellulases. The change in crystallinity index and crystallinity during endocellulase treatment

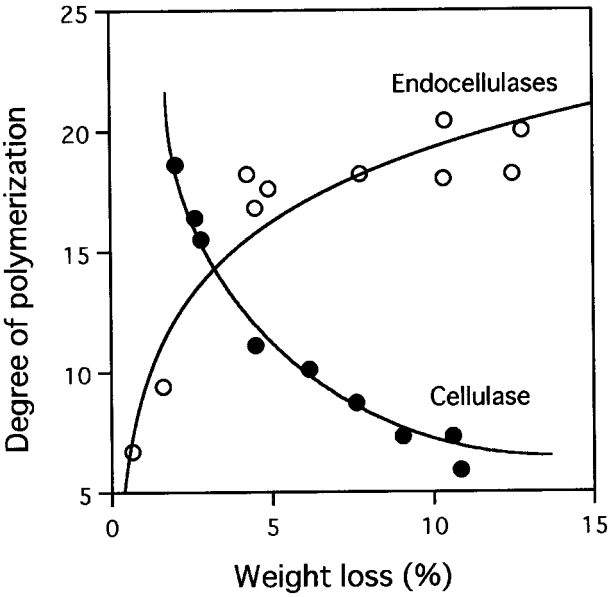

Figure 2 Average DP of cellulose oligomers plotted against weight loss. The oligomers were separated from cuprammonium rayon during endocellulase and cellulase complex treatments.

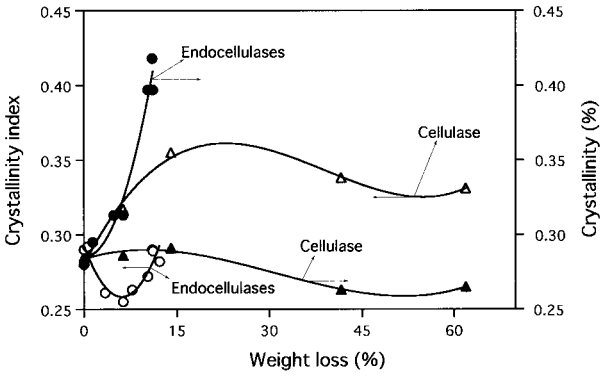

Figure 3 (Outlined symbols) Crystallinity index measured by infrared spectroscopy and (filled symbols) crystallinity measured by X-ray diffraction plotted against treatment time of cuprammonium rayon. The treatments were performed with (circles) endocellulases and (triangles) cellulase.

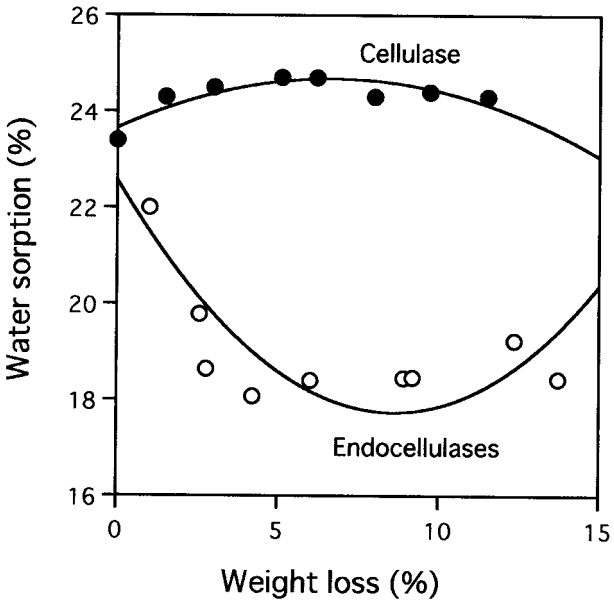

Figure 4 Change in water sorption of cuprammonium rayon due to endocellulase and cellulase complex treatments.

indicated that the intermediately ordered regions in the disordered matrix were removed.

The crystallinity index of the fiber treated with the cellulase was significantly higher than that of the unprocessed fiber, irrespective of weight loss. The crystallinity of the cellulase-treated fiber was lower than that of the unprocessed fiber, except for that at less than approximately 20% weight loss. In addition, the crystallinity of the endocellulase-treated fiber was considerably higher than that of the cellulase-treated fiber at a given weight loss.

These results suggested that cellulase treatment hydrolyzed the crystalline regions even at a low weight loss of 5% and that the endocellulases negligibly affect the crystalline regions. It was assumed that the degradation of the crystalline regions during cellulase treatment caused the increase of the intermediately ordered regions.

Water-sorption versus weight-loss plots for the endocellulase-treated and cellulase-treated fibers are shown in Figure 4. Water sorption upon endocellulase treatment decreased with increasing weight loss, exhibited a minimum at an approximately 5% weight loss, and then increased with increasing weight loss. On the other hand, water sorption upon cellulase treatment increased, exhibited a maximum at approximately 5% weight loss, and then decreased with increasing weight loss. Water sorption at a given weight loss was less after endocellulase treatment than after cellulase treatment.

The water sorption of cotton fibers increased in the first step of hydrolysis with the cellulase, even though the disordered regions were preferably hydrolyzed.[3] This was because the crystallite surface was deaggregated by the cellulase complex and rendered greatly accessible to water molecules.[3] Water sorption of the fiber first decreased

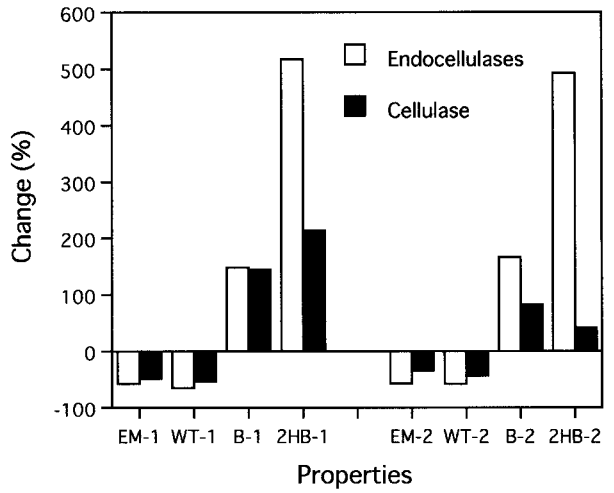

Figure 5 Mechanical properties of endocellulase-treated and cellulase-complex-treated cuprammonium rayon fabrics. Weight losses of fabrics were 5.4 and 4.9% for endocellulase and cellulase treatments, respectively. Property abbreviations 1 and 2 denote warp and weft measurements, respectively. Property abbreviations are explained in the text.

upon endocellulase treatment, as shown in Figure 4. This suggested the preferable hydrolysis of the disordered regions without the deaggregation of the crystallite surface, different from the hydrolysis with the cellulase.

Enhancement of Fabric Brittleness

The changes in elongation EM, tensile energy WT, bending rigidity B, and bending hysteresis $2HB$ caused by the enzyme treatments are shown in Figure 5. These results were obtained using samples with an approximately 5% weight loss. Both the endocellulase- and cellulase-treated cupra exhibited a reduction in EM and WT and an increase in B and $2HB$. The extent of change was larger after endocellulase treatment than after cellulase treatment.

Both treatments increased the brittleness of the cupra fabrics. This was because the hydrolysis involved the disordered regions, which are associated with the flexibility of the fiber. Furthermore, the preceding molecular deaggregation induced during cellulase treatment was thought to enhance the flexibility of the fiber. It was, therefore, reasonable that the endocellulase-treated cupra was more brittle than was the cellulase-treated cupra.

CONCLUSIONS

The initial hydrolysis of cuprammonium rayon fiber with endocellulases was studied. The average degree of polymerization of the separated cellulose oligomers indicated that mechanical agitation during enzyme treatment was effective to separate hydrolyzed oligomers. Water sorption and mechanical properties of the fabrics indicated that the structural deterioration of the fiber by the endocellulases proceeded without a preceding deaggregation of cellulose chains during the hydrolytic process.

The endocellulases hydrolyzed the intermediately ordered regions in the disordered matrix, even though there was no preceding molecular deaggregation of the substrate. The crystalline regions of the fiber were not affected by the endocellulases.

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