New Detergent Mechanism Using Cellulase Revealed by Change in Physicochemical Properties of Cellulose

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Sebum in naturally soiled cotton undershirt and oleic acid in artificially soiled cotton cloth, which entered interfiber space in the interior of cotton fibers were easily removed by alkaline cellulase from *Bacillus* sp., but only with difficulty by commonly used detergent ingredients such as surfactant and protease. Adsorption isotherms and the rate of hydrolysis of alkaline cellulase against insoluble cellulose powders revealed that the lower the relative crystallinity of cellulose powder, the more adsorptive alkaline cellulase became and the more hydrolysis was promoted. With alkaline cellulase, cotton having the highest relative crystallinity was adsorbed at pH 9 and 5°C, liberated a negligible small amount of reducing sugar at pH 9 and 40°C, and produced no changes in the degree of polymerization of cotton cellulose and in the tensile strength of cotton fabric at pH 9 and 30°C. On the other hand, differential scanning calorimetric studies revealed that under similar conditions even a small quantity of alkaline cellulase drastically reduced the amount of water bound to cellulose in cotton. Because water was bound only with hydroxy groups of cellulose molecules in the amorphous region of cotton fibers, it can be understood that soil entering the interfiber space of amorphous interlamellae in the interior of cotton fibers, was easily removed as the hydrated cellulose in the interlamellae was slightly hydrolyzed by alkaline cellulase. A new detergent mechanism is proposed.

KEY WORDS: Alkaline cellulase, *Bacillus* sp., bound water, cellulase, cotton, detergent mechanism, insoluble cellulose, interlamellae, oleic acid, sebum soil.

In the preceding paper (1), microscopic studies showed that sebum, which is difficult to remove with ordinary detergents, entered interfiber space in the interior of the fibers (2) in cotton undershirts soiled while worn. Adsorption of alkaline cellulase onto parts in the interior of the cotton fibers and removal of sebum soil from there by the detergent incorporating alkaline cellulase were also shown by electron microscopic observation (1).

This paper deals with the detergency effect of alkaline cellulase confirmed both through bundle tests with naturally soiled cotton undershirts and washing tests with artificially soiled clothes, and estimates the detergent mechanism of alkaline cellulase based on measurement of the physicochemical action of alkaline cellulase against cotton.

EXPERIMENTAL PROCEDURES

Alkaline cellulase, like the one used in the experiment reported in the preceding paper (1), was obtained as purified enzyme from *Bacillus* sp. KSM-1001. The alkaline cellulase had an optimum pH of 9.5, an optimum temperature of 40°C and an activity of 1,500 units/g. One unit of activity of the alkaline cellulase was defined as the amount of enzyme that catalyzed the liberation of reducing sugar equivalent to 1.0 μmol of D-glucose per min from a 0.35% solution of sodium carboxymethyl cellulose (CMC, A01MC, DS = 0.68, DP = 200, Sanyo Kokusaku Pulp Co., Tokyo, Japan) at pH 9.0 and 40°C. As in the experiment reported in the last paper (1), protease (*Bacillus licheniformis*, Type VIII: Bacterial, Sigma Chemical Co., St. Louis, MO) with an activity of 32.5 Anson units/g (3) was also used.

The formulation of a heavy-duty detergent containing a variation of the above-mentioned enzymes was the same as in the last paper (1).

American cotton cloth (Daiwa Shizai Co., Tokyo, Japan) or yarn taken from it was used for the measurement of the amount of bound water, the degree of polymerization and tensile strength, and also as artificially soiled specimens.

Cellulose powders used for X-ray diffractography, the measurement of adsorption isotherms and the rate of hydrolysis were respectively: the powder of the above-mentioned American cotton, cotton linters of the same origin, and Avicel (microcrystalline cellulose powder, Art 2331, E. Merck Darmstadt, Darmstadt, Germany), which were cut into 100 or smaller mesh pieces. The three types of cellulose powders were swollen by treatment with phosphoric acid (4). These cellulose powders were defatted by 9-h extraction in a Soxhlet apparatus with chloroform.

The relative crystallinity index (CrI) of the cellulose powders was determined by measuring X-ray diffraction intensity [$I(\theta)$] at diffraction angle 2θ between 5° and 45° with an X-ray diffractometer (Rigaku Denki Co., Tokyo, Japan). X-ray diffractograms of the cotton, cotton linter and Avicel showed distinctive peaks at $2\theta = 14°$, 16° and 22.5°. This indicated that they had the typical structure of crystalline cellulose I (5). By contrast, X-ray diffractograms of the cotton, cotton linter and Avicel treated with phosphoric acid did not show peaks stemming from crystalline cellulose I or other crystalline lattices, indicating that they could be regarded as amorphous cellulose. From $I(\theta)$, CrI was calculated by the following equation (5):

$$\mathrm{CrI} = [I_s(\theta) - I_a(\theta)]/[I_c(\theta) - I_a(\theta)] \quad [1]$$

where I_c, I_a, and I_s are X-ray diffraction intensities of the most crystalline cotton, the least crystalline cotton linters treated with phosphoric acid and the other cellulose powders, respectively. The CrI values obtained from the above equation at 2θ at every 5° were as follows: cotton, 1; cotton linter, 0.910; Avicel, 0.568; cotton treated with phosphoric acid, 0.140; Avicel treated with phosphoric acid, 0.037; cotton linter treated with phosphoric acid, 0.

Adsorption isotherms of alkaline cellulase against different cellulose powders were determined by the following method: a pH 9 solution containing 1 wt% cellulose powder and alkaline cellulase, buffered by 0.05M sodium carbonate, was incubated in a sealed container at 5°C for 60 min, and then the cellulose powder was removed by centrifugation. The saccharifying power of cellulase in the solution against CMC before and after adsorption was measured by the PAHBAH method (6), and differences thus obtained were regarded as the amount of adsorbed

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cellulase (units per kg of cellulose powder). In this experiment, saturated adsorption was reached in 30 min. The adsorption behavior of cellulase inactivated by heating at 95°C for 20 min was also investigated. The amount of inactivated enzyme in the supernatant was measured as the amount of protein by the method of Lowry *et al.* (7).

The rate of hydrolysis of different cellulose powders by alkaline cellulase was measured at 40°C. A pH 9 solution containing 1 wt% cellulose powder and 6.22 units of alkaline cellulase/mL of solution, buffered by 0.05M sodium carbonate, was shaken for a certain period of time. Then, the alkaline cellulase in a solution obtained after removing cellulose powder by 10 min centrifugation at 5°C and 3,000 rpm was inactivated by 20-min heat treatment at 95°C. Water-soluble cellooligosaccharide, a hydrolyzate contained in the solution thus obtained, was analyzed as the amount of glucose by the PAHBAH method.

The amount of water bound to American cotton cloth (bound water) was measured in a differential scanning calorimeter (SSC580DS, Seiko Instrument and Electronics Co., Tokyo, Japan) by the method of Nakamura *et al.* (8). Cotton cloth (0.1 g) was soaked for 120 min at 30°C in 100 mL of purified enzyme solution buffered by 0.05 M sodium carbonate at pH 9. After reaction, the cotton cloth was washed thoroughly with pure water, cut into 5- × 10-mm pieces, and dried under reduced pressure while kept at 40°C in a silver cell. Pure water, equivalent to 20~250% of the dry weight of the cotton fabric, was added to the dried cotton cloth in the cell, and the cell was sealed. The cotton cloth in the cell was cooled from room temperature to −73°C at the rate of 8°C/min, and exothermic peaks due to the freezing of free water in the cotton fabric observed at around −10°C were measured with the differential scanning calorimeter. The peak area thus obtained was regarded as the amount of enthalpy change (J/g of dry cotton cloth) due to freezing, and the extrapolated value (amount of water when the enthalpy change is zero) based on the plotted line of five points for the added water was considered as the amount of bound water.

The tensile strength of American cotton cloth was examined with a Tensilon RTM-100 (Orientec Co., Tokyo, Japan) to measure the effect of the alkaline cellulase. Twenty 1- × 14-cm strips of cotton cloth were put into a 100-mL solution of the same alkaline cellulase (pH 9) as in the adsorption test, and they were incubated at 30°C for 120 min. Then, the cloths were rinsed with pure water and dried. The dried cloths were kept in a desiccator at 25°C and 65% relative humidity for 3 d, and after humidity adjustments of the cloths, their tensile strength was measured.

The degree of polymerization of cellulose ($\overline{DP}$) under the influence of alkaline cellulase was measured. The measurement was made with a mixed solution containing 1 wt% of yarn taken from American cotton cloth and the purified alkaline cellulase, buffered by 0.05 M sodium carbonate (pH 9). Before the measurement, the solution had been incubated in a sealed container at 30°C for 120 min. After incubation, yarn was rinsed well with pure water and acetone and dried in a desiccator with phosphorous pentoxide. A certain amount (approx. 10 mg) of dried yarn was dissolved at 5°C in 10 mL Cadoxen solution (9). The solution was then diluted with the same amount of water, and its viscosity was measured at 25°C with an Ubbelohde No. 1 type viscometer. The $\overline{DP}$ value was calculated from the molecular weight obtained from the Brown-Henley equation (10) adjusted for polydispersity on the basis of the Schultz-Zimm distribution equation:

$$[\eta] = 4.02 \times 10^{-4} \overline{M}_W{}^{0.76} \qquad [2]$$

where $[\eta]$ is the intrinsic viscosity, and $\overline{M}_W$ is the weight-average molecular weight.

A bundle test, repeating wear and wash ten times, was conducted as mentioned in the preceding paper (1). Undershirts for men that had been worn and washed in twenty households were cut into right and left halves, which were washed with either a protease-based detergent or an alkaline cellulase-based detergent. After washing, the split undershirts were sewn together, and were worn by their owners for 2 d. Then, the undershirts were cut in halves and washed again. This cycle was repeated 10 times. The washing was carried out under the following conditions: twenty sets of undershirts cut in halves (about 1.5 kg) were soaked in 6 L of 0.667% detergent solution dissolved in tap water (55 ppm of $CaCO_3$) at 30°C for 60 min. After that, the undershirts and the detergent solution were placed together in a washing machine (VH-1330, Toshiba Co., Tokyo, Japan). After tap water was added to adjust the volume of the solution to 30 L, the undershirts were washed in this washing machine at 20°C for 10 min. The concentration of enzyme was as follows: 50 units of alkaline cellulase/L or 1.1 Anson units of protease/L during soaking, and 10 units of alkaline cellulase/L or 0.22 Anson units of protease/L during washing. Measurement of the amount of residual sebum and visual judgment on relative cleanliness were conducted before the wear-and-wash cycles and after the fourth and tenth cycles. Two 2.5- × 2.5-cm pieces each were cut out of the upper front parts of the split undershirts for each measurement and were soaked in chloroform in a Soxhlet extractor for 9 h to extract residual sebum. Holes in the upper front parts of the undershirts were covered with new pieces of cloth. The cleanliness of the backs of a pair of split undershirts was compared by ten expert panelers in daylight shining in from a north window. The relative cleanliness obtained by the two detergents was evaluated by the Japanese Industrial Standard method (11) in terms of clearly superior (+2), slightly superior (+1), similar (0), slightly inferior (−1), and clearly inferior (−2). For instance, if the cleanliness obtained by one type of detergent was clearly superior to that obtained by the other, it was graded +2, while the other was graded −2 (11). The data were analyzed by Scheffe's paired comparison method (11). The cleanliness was compared after 0, 4 or 10 wear-and-wash cycles, independently. The relative cleanliness between different wear-and-wash cycles cannot be compared.

Artificially soiled cloth containing 20 mg of oleic acid/g of cotton cloth was prepared by soaking cotton cloth in perchloroethylene solution of oleic acid and evaporating the perchloroethylene. A mixed solution containing 1 wt% of this artificially soiled cloth, 0.1 wt% of heptaoxyethylene dodecyl ether (Nikko Chemicals Co., Tokyo, Japan) and the purified alkaline cellulase, buffered by 0.05 M sodium carbonate (pH 9), was prepared. For the purpose of comparison, a similar mixed solution was used containing the corresponding amount of the purified alkaline

cellulase that had been inactivated by 20-min heat treatment at 95°C. These solutions were incubated at 30°C for 120 min. After incubation, the activity of cellulase was measured by the PAHBAH method. Residual oleic acid was extracted with chloroform from artificially soiled cloth that had been washed and dried after incubation. A known amount of heptadecanoic acid was added as an internal standard to the extracted oleic acid. Then, carboxyl groups in the samples were fluorescent-labelled with 9-anthryl diazomethane reagent (Wako Chemicals Co., Tokyo, Japan). The amount of oleic acid was quantitated through measurement of the fluorescence intensity during high-performance liquid chromatography (HPLC; HLC-803D, Toyo Soda Co., Tokyo, Japan) (12).

A transmission electron microscope (JEL-100CX, JEOL Co., Tokyo, Japan) was used in the above experiment to observe ultrathin cross-sections of fibers that had been taken from artificially soiled cloth washed in a solution containing 400 units of the alkaline cellulase/L or the corresponding amount of inactivated alkaline cellulase, and from cotton cloths before and after soiling with oleic acid. Before preparing ultrathin sections, the fibers had been stained with 1% osmium tetroxide, to form a complex with oleic acid, by the same procedure as given in the preceding paper (1).

RESULTS AND DISCUSSION

The cleaning power of the alkaline cellulase-based detergent on naturally soiled cotton undershirts was compared with that of protease-based detergent in bundle tests by means of cotton undershirts with 33.5 ± 4.7 mg of residual sebum soil/g at zero wear-and-wash cycles in the test. These undershirts were collected from twenty households after home wearing and washing. Dependence of the relative cleanliness of the washed undershirts, based on visual judgments and the amount of residual sebum extracted from the washed undershirts by chloroform, on the number of wear-and-wash cycles is shown in Figures 1a and 1b, respectively. Differences between the cleanliness achieved by the alkaline cellulase-based detergent and that achieved by the protease-based detergent increased with the number of wear-and-wash cycles (Fig. 1a). With increasing number of wear-and-wash cycles, the amount of residual sebum soil in the cotton undershirts was clearly reduced by the alkaline cellulase-based detergent, whereas only a slight decrease of the amount of sebum by the protease-based detergent was observed (Fig. 1b). It appears that the alkaline cellulase-based detergent will remove more than the amount of sebum adhered newly by wearing. On the other hand, the amount of sebum removed by the protease-based detergent seems to be compensated by that of newly adhered sebum. It has been reported that the apparent cleanliness of cotton undershirts relates closely to the amount of residual sebum, rather than to that of other residual soils such as protein (13). Therefore, the relative cleanliness shown in Figure 1a is considered to depend mainly on the difference in the amount of residual sebum between the two types of detergent (Fig. 1b).

Cotton cloth artificially soiled with oleic acid was incubated at pH 9 and 30°C for 120 min in the alkaline cellulase solution in the presence of 0.1 wt% heptaoxyethylene dodecyl ether or in inactivated alkaline cellulase

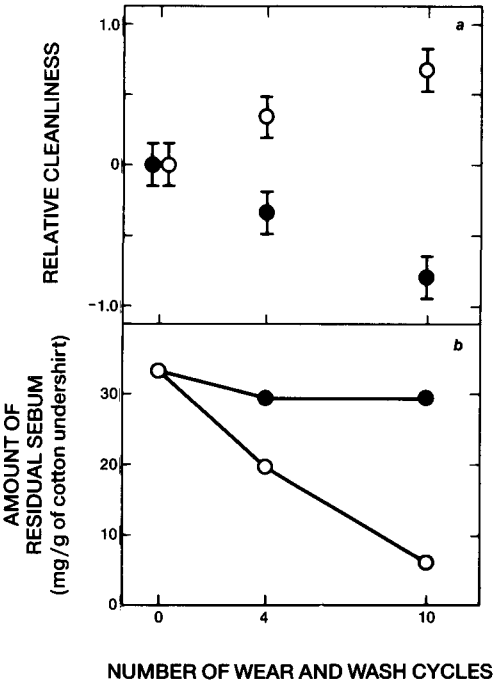

FIG. 1. Cleaning power of alkaline cellulase-based detergent on naturally soiled cotton undershirts in comparison with that of protease-based detergent in bundle tests: ○, alkaline cellulase-based detergent; ●, protease-based detergent. a, Relative cleanliness of the washed undershirts on visual judgments; b, amount of residual sebum extracted from the washed undershirts by chloroform. The washing was carried out at 20°C for 10 min after presoaking at 30°C for 60 min. The enzyme concentration in the washing liquor was 10 units of the alkaline cellulase/L or 0.22 Anson units of the protease/L.

solution in the presence of the same surfactant. Figure 2 shows the relationship between the amount of residual oleic acid in the cotton cloth and the concentration of the alkaline cellulase in the detergent solution. As the concentration of alkaline cellulase in the detergent solution increased, the amount of oleic acid remaining in the cotton cloth decreased. However, adding a corresponding amount of inactivated alkaline cellulase did not affect the cleaning power.

Thus, it was confirmed from the experiments on the naturally soiled cloth (Fig. 1) and the artificially soiled cloth (Fig. 2) that the soils, which were not natural substrates for cellulase, were removed from the cotton cloths under the indirect influence of the alkaline cellulase.

In an attempt to determine from what part of the microstructured cotton the soil was removed, ultrathin cross-sections of fibers, taken from the artificially soiled cloths shown in Figure 2, were observed with a transmission electron microscope. Figure 3 shows pictures obtained from this microscopy. As reported previously (1), when clean cotton fiber (Fig. 3a) was soiled with oleic acid, oleic acid stayed not only on the exterior, but also penetrated the interior of the fiber (Fig. 3b). When this fiber was washed with 0.1 wt% of heptaoxyethylene dodecyl ether containing inactivated alkaline cellulase, oleic acid on the exterior of the fiber was removed, but that in the interior remained almost intact (Fig. 3c). When washed with 0.1 wt% of heptaoxyethylene dodecyl ether containing 400 units of the alkaline cellulase/L, oleic acid both

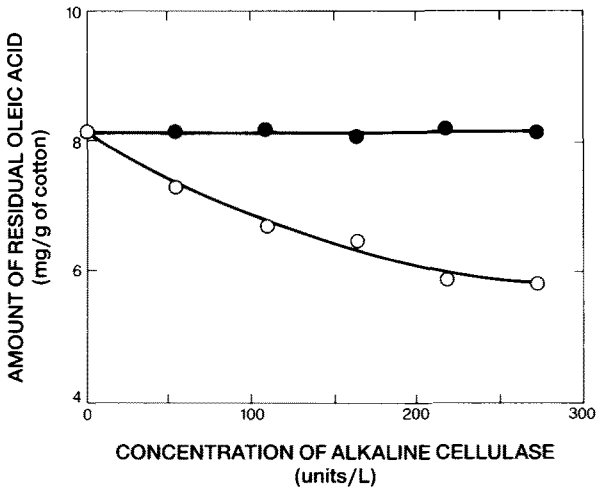

FIG. 2. Cleaning power of alkaline cellulase on cotton cloth artificially soiled with oleic acid in comparison with that of inactivated alkaline cellulase in the presence of 0.1 wt% of heptaoxyethylene dodecyl ether at pH 9 and 30°C, and 120 min washing time: ○, alkaline cellulase; ●, inactivated alkaline cellulase.

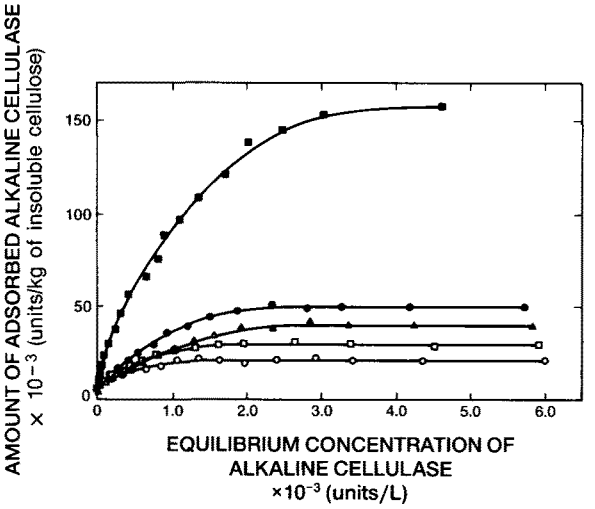

FIG. 4. Adsorption isotherm of alkaline cellulase against insoluble cellulose powders of different crystallinities at pH 9 and 5°C: ○, cotton; □, cotton linter; △, Avicel; ●, H_3PO_4-treated cotton; ■, H_3PO_4-treated cotton linter.

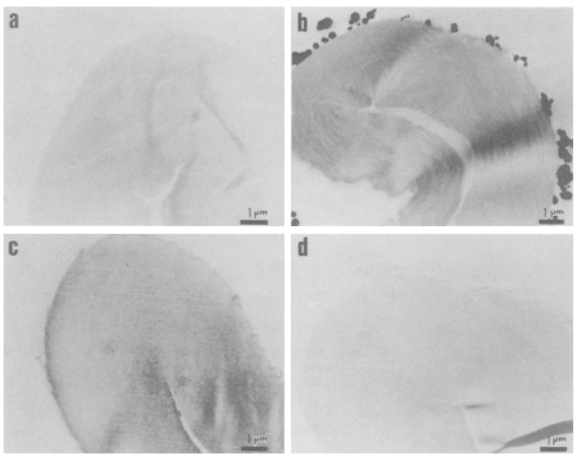

FIG. 3. Transmission electron micrographs of ultrathin cross-sections of fibers in cotton cloth soiled artificially with oleic acid: The cotton fibers were a, unsoiled; b, soiled with oleic acid; c, soiled with oleic acid and washed at pH 9 and 30°C with 0.1 wt% heptaoxyethylene dodecyl ether containing inactivated alkaline cellulase; d, soiled with oleic acid and washed at pH 9 and 30°C with 0.1 wt% heptaoxyethylene dodecyl ether containing 400 units of alkaline cellulase/L. These cotton fibers were stained with osmium tetroxide to produce a complex with oleic acid.

in the interior and on the exterior of the fiber was almost completely removed (Fig. 3d). This strongly indicates that by reacting with cellulose in the interior of the cotton fiber, the alkaline cellulase removed soil from the interior of the fiber, which was difficult to remove by the surfactant alone. This result agreed well with the results of the microscopic studies of naturally soiled cotton undershirts reported previously (1).

For the purpose of understanding these results of the washing experiments and those in the electron microscope, the adsorptivity and reactivity of the alkaline cellulase against insoluble cellulose were investigated. Figure 4 shows adsorption isotherms of the alkaline cellulase against insoluble cellulose powder with five different CrI values at pH 9 and 5°C. Even at the highest equilibrium concentration of the alkaline cellulase for each isotherm shown in Figure 4, no reducing sugar produced from cellulose was detected in the reaction mixture by the PAHBAH method, that is, no enzymatic hydrolysis of cellulose seemed to occur, because of the low temperature. Measurement of the amount of cellulase protein revealed that, under conditions similar to those shown in Figure 4, the alkaline cellulase inactivated by heating was not adsorbed to any type of insoluble cellulose powder. Therefore, the isotherms shown in Figure 4 reflected purely specific adsorption of the alkaline cellulase to insoluble cellulose. This was supported by the fact that data given in Figure 4 satisfied the Langmuir isotherm equation:

$$W = W_{max}\ KE/(1 + KE) \qquad [3]$$

where W and W_{max} are the nominal and the maximum amounts of adsorbed enzyme (units/kg of cellulose) at the adsorption equilibrium of the alkaline cellulase, respectively; K is an adsorption equilibrium constant, and E is the equilibrium concentration of the alkaline cellulase in the liquid phase (units/L). Figure 5 shows the dependence of the W_{max} value on the CrI values of insoluble cellulose powders as substrates at pH 9 and 5°C. As the CrI value of insoluble cellulose increased, W_{max} value decreased sharply. The more amorphous the substrate, the more the alkaline cellulase adsorbed.

Figure 6 illustrates the time courses in hydrolysis of five types of insoluble cellulose powders with different CrI values by alkaline cellulase at pH 9 and 40°C. Little liberation of reducing sugar from cotton and cotton linter was observed. On the other hand, a considerable amount of reducing sugar was liberated from insoluble cellulose powders made amorphous by phosphoric acid. From the linear section during the initial hydrolysis stage shown in Figure

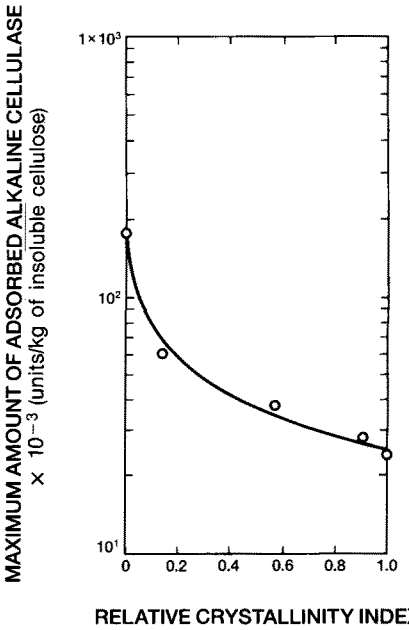

FIG. 5. Dependence of the maximum amount of adsorbed alkaline cellulase in the Langmuir isotherm equation at pH 9 and 5°C on the relative crystallinity of insoluble cellulose powder.

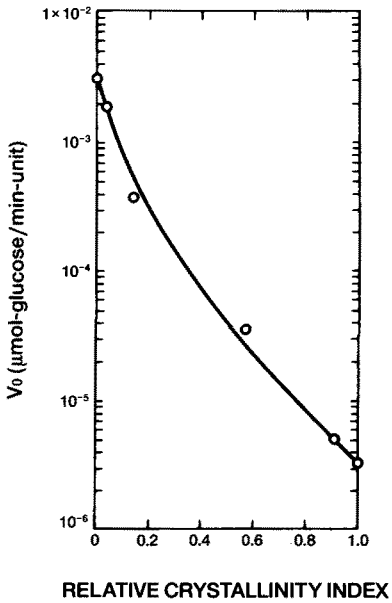

FIG. 7. Dependence of the initial rate (V_0) in the hydrolysis of insoluble cellulose powder by alkaline cellulase at pH 9 and 40°C on the relative crystallinity of insoluble cellulose powder.

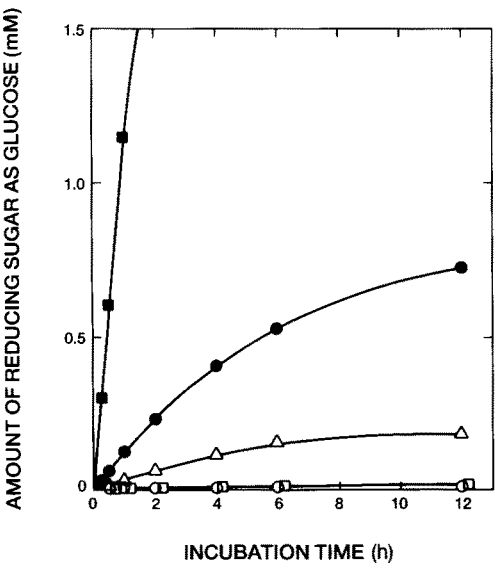

FIG. 6. Hydrolysis of insoluble cellulose powder with different crystallinities by alkaline cellulase at pH 9 and 40°C: The reaction mixture consisted of 6.22 units of alkaline cellulase/mL and 1 wt% insoluble cellulose powder. ○, cotton; □, cotton linter; △, Avicel; ●, H_3PO_4-treated cotton; ■, H_3PO_4-treated cotton linter.

6, the initial rate V_0 (μmol of glucose/min·unit of alkaline cellulase) of hydrolysis was calculated, and the V_0 value was plotted against the CrI value of the insoluble cellulose in Figure 7. The V_0 value for cotton linter made amorphous by phosphoric acid (CrI = 0) was approximately 10^3 times larger than that for cotton (CrI = 1). The hydrolysis reaction by the alkaline cellulase is thus extremely rapid against amorphous insoluble cellulose, while it is slow against crystalline insoluble cellulose. A similar result was reported in the reaction of endo-type cellulase from *Irpex lacteus* (14,15). Figures 5 and 7 suggest that the more cellulose is adsorbed by the alkaline cellulase, the more it is hydrolyzed.

The reactivity of alkaline cellulase adsorbed to cotton with high crystallinity was difficult to trace quantitatively because of the small amount of liberated reducing sugar (Fig. 6). Therefore, the changes in the physicochemical properties of cotton itself were observed. The effects of alkaline cellulase on the tensile strength of cotton cloth (American cotton 100%) and the $\overline{DP}$ value of cellulose that composes the cotton cloth were measured at pH 9 and 30°C, and the results are shown in Figure 8. The $\overline{DP}$ value of cellulose in the American cotton used was approximately 4,000. There were no changes in the tensile strength of the cotton cloth and the $\overline{DP}$ value of the cellulose until the activity of alkaline cellulase reached as high as 600 units/L. Although the alkaline cellulase is not very reactive against crystalline regions of cotton, it may be much more reactive against the amorphous regions of the cotton. The reason why the change in the apparent $\overline{DP}$ value of cotton cellulose is immeasurably low is probably because, even if the $\overline{DP}$ value of cellulose in the amorphous region becomes small due to the hydrolysis by alkaline cellulase, its effect is small and cellulose in the crystalline region of the cotton is not affected by alkaline cellulase at all. It can be easily understood that the effect of alkaline cellulase on more macroscopic properties of cotton, such as the tensile strength, should be more difficult to detect.

Under the conditions of pH 9 and 30°C, the effect of alkaline cellulase on bound water of American cotton cloth was measured with a differential scanning calorimeter. Results of the measurement are shown in Figure 9. The amounts of bound water measured were 18% for American cotton, 19% for Egyptian cotton, and 18% for Pakista-

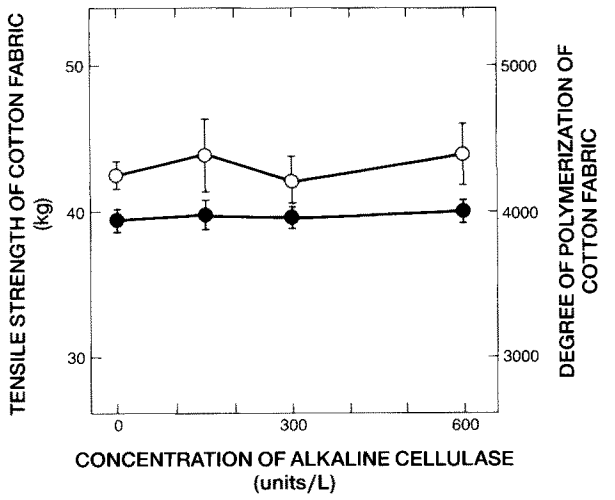

FIG. 8. Effects of alkaline cellulase on the tensile strength of cotton cloth and the degree of polymerization of cellulose from the cotton cloth at pH 9 and 30°C, and 120 min of reaction time: ○, average tensile strength of twenty cotton fabrics; ●, average degree of polymerization of five cotton fabrics. Standard deviation in the figure has 95% confidence level.

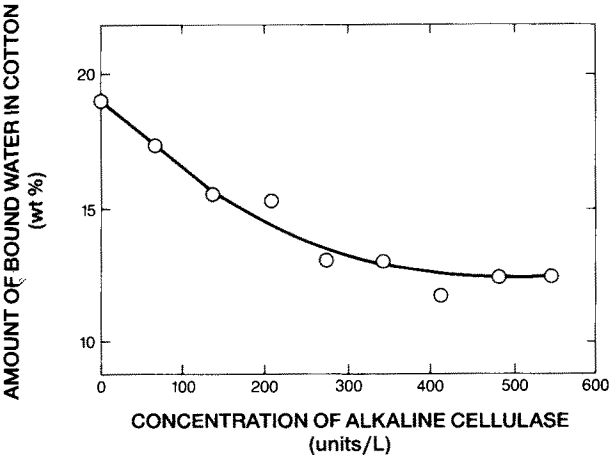

FIG. 9. Effect of alkaline cellulase on the amount of water bound to cotton at pH 9 and 30°C, and 120 min of reaction time in the presence of 0.1 wt% cotton cloth by means of differential scanning calorimeter.

nian cotton, which all agreed well with known values (8). With increasing concentration of alkaline cellulase, the bound water of American cotton decreased almost linearly up to about 100 units/L, and gradually reached a constant level of about 12.4% in the concentration range tested in the experiment. The reactivity of the cellulase against insoluble cellulose of high crystallinity such as natural cotton, could be observed by determining the amount of bound water in the insoluble cellulose, and the reaction mode obtained under the experimental conditions of Figure 9 was typical for homogeneous enzymatic reactions (16). Because bound water was found only in the hydroxy groups of cellulose molecules in the amorphous region of cotton fiber (8), the changes shown in Figure 9 indicate that, although the crystallinity of the cotton as a whole was high, distinct hydrolysis by the alkaline cellulase occurred in its amorphous regions. In view of the facts that a considerable amount of alkaline cellulase is adsorbed in the interior of cotton fibers, as observed in the double-labeling immunoelectron micrograph of ultrathin cross-sections of cotton fibers by alkaline cellulase (1), and that the alkaline cellulase selectively removes soil from interfiber space in the interior of cotton fibers [Fig. 3 in this paper, and Figs. 1 and 2 in the preceding paper (1)], it is clear that in highly crystalline cotton fabrics the alkaline cellulase reacts only with amorphous regions of the interfiber space in the interior of the cotton fiber. The possibility that alkaline cellulase hydrolyzes cellulose in crystalline regions is very low (Fig. 7). The similarity of the effects shown in Figures 2 and 9 suggest that the reduction of bound water of cotton fibers by alkaline cellulase favorably correlated with the removal of oleic acid from artificially soiled cotton cloth.

From the above, a new detergent mechanism can now be presented: oily soil, which enters interfiber space in the interior of cotton fiber [probably in the amorphous interlamellae between crystalline lamellae (17)] during the actual wearing or artificial soiling of cotton clothes, is easily removed by alkaline cellulase, which partially hydrolyzes hydrated cellulose in the interlamellae without reacting with the oily soil itself.

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