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The Effect of a Direct Dye on the Formation Process of the Structure of Bacterial Cellulose

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It was found that the structure of cellulose obtained from the Acetobacter-culture in the presence of the direct dye of cellulose is fixed in a priori by the structure of a dye. The product from the culture with a direct red 80 formed the cellulose II, and it, from culture with a congo red formed the cellulose IV.

The bacterial cellulose is extruded from the cell in a noncrystalline state.[1,2] The cellulose obtained from the Acetobacter-culture in the presence of a fluorescent brightener of a direct dye for cellulose forms a crystalline complex of a brightener and cellulose.[3-5] What a brightener is included between the $(1\bar{1}0)$ planes of the complex, signifies that the cellulose chains are extruded from the cell in a monomolecular cellulose sheet corresponding to the $(1\bar{1}0)$ plane.[4] Recently, Haigler et al. refuted Kai's model from their investigation concerning the product from the Acetobacter-culture with various direct dyes by the electron microscopy and X-ray method.[6] They suggested that a direct dye adhered to the surface of a cellulose fibril with diameter about 17 Å in a stack state, therefore, the product easily forms cellulose I with dye-extraction. We also found that the product from the culture with a brightener forms cellulose I with extraction.[3]

In this paper, we report the finding that the structure of cellulose from the Acetobacter-culture in the presence of a direct dye is fixed in a priori by the structure of the dye.

The strains of Acetobacter xylinum used were ATCC 23769 and IFO 13693. The direct dyes used were a congo red (Kodak made) and a direct red 80 (Ciba-Geigy made). A congo red (C. R.) was refined by repeating the operation of 1) - 3): 1) soluting it in distilled water, 2) precipitation by

sodium acetate, 3) washing of a sediment with 70 vol% aqueous ethanol solution. A direct red 80 (R. 80) was refined by repeating the operation of 1) - 2): 1) soluting in 68 vol% aqueous ethanol at 70 °C, 2) precipitation by cooling the solution in ice water. All regent used were an article of superior quality except for peptone and yeast extract.

The dyed sample from the culture with a dye was obtained as follows; 60 ml of cell suspension[3] was added to 140 ml of the complex medium[3] (pH of the medium adjusted to 7.0) with a dye of given concentration (ranging from 0.005 to 0.1 wt%), and it was incubated at 28.0°C for 24 h. The product from the incubated medium with C. R. was washed with 0.1 wt% aqueous NaOH solution for about 1 week, then with distilled water until alkali-free, and then dried. The product from the incubated medium with an R. 80 was washed with dilute alkaline (NaOH) water of pH 9.0 for 48 h and with distilled water until alkali-free, and then dried.

The extracted sample was obtained as follows; The never-dried product from the above culture was extracted by boiling in a solution of 70 vol% aqueous ethanol for 18 h (aqueous ethanol was exchanged for fresh every 3 h). The extracted sample was refined in a boiling 1 wt% aqueous NaOH solution for 10 h under N_2 atmosphere. X-Ray diagrams from the dyed sample and the extracted sample were obtained after previous paper.[3]

The dyed sample from the culture in the presence of a C. R. or an R. 80 showed the characteristic X-ray diffraction diagram as shown in Fig. 1, respectively. As described the above, the sample from the culture with a brightener also shows the characteristic X-ray diffraction diagram of the crystalline complex of a brightener and cellulose.[3] 2θ values of $(1\bar{1}0)$ and (110) planes of all dyed samples clearly shift to the lower angle side than that of cellulose I as shown in Table 1. The extension of space of $(1\bar{1}0)$ and (110) planes shows to be due to each dye.

When the dyed samples were extracted, as shown in Fig. 2, the sample from the incubated medium with a C. R. showed a diffraction diagram of cellulose IV, and the sample from the culture with an R. 80 showed that of cellulose II (Table 1). The X-ray diffraction diagrams of the dyed and extracted samples were found to be reproducible. Also, these results were not changed by the strain or the dye concentration.

The space of $(1\bar{1}0)$ and (110) planes of the complex from the incubated medium with a brightener extends similar to that of the sample from the culture with C. R. or R. 80. In the case of the extraction of the complex of a brightener and cellulose, however, it forms cellulose I crystals although its crystallinity is lower as described above.

Although these results are judged from the necessary condition for a

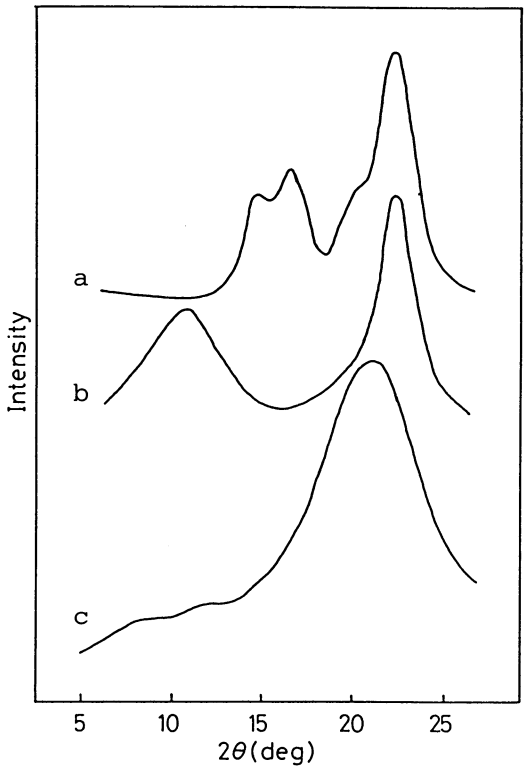

Fig. 1. X-Ray profiles of the dyed samples from the Acetobacter-culture in the presence of a congo red or a direct red 80 a: sample from the incubated medium without a dye, b: congo red, c: direct red 80.

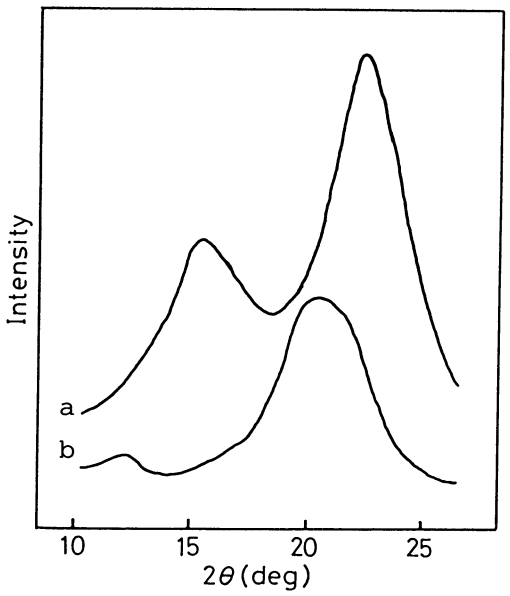

Fig. 2. X-Ray profiles of the extracted samples a: congo red, b: direct red 80.

Table 1. 2θ values of $(1\bar{1}0)$, (110) and (020) planes of cellulose-dye complexs and its extracted samples

sample	$(1\bar{1}0)$	(110)	(020)
Cellulose I	14.6°	16.6°	22.6°
C. R. complex	10.9	10.9(?)	22.8
extracted sample	15.3	15.3	22.1
Cellulose IV	15.4	15.4	22.2
R. 80 complex	8.2	12.5	20.9
extracted sample	12.3	19.9	22.1
Cellulose II	12.1	20.2	22.4

direct dye such as the planarity of a dye molecule, its straightness, length of the conjugated double bond and the relation of the proton donor or acceptor and sulfonate, and so on, the difference of the effect of these dyes on the nascent structure of bacterial cellulose cannot satisfactorily be explained at present. The molecular weight of these dyes is C. R.: 696.7, R. 80: 1373.1, respectively. The effect of these dyes on the nascent structure also cannot satisfactorily be explained from viewpoint of its molecular weight. It is difficult to systematically explain which parts of the structure of a dye produce what effect on the nascent structure of bacterial cellulose. Therefore, the solution will be the subject for a future study.

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