Resistance to Alkali of the Nascent Fibril Produced by *Acetobacter xylinum*

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Synopsis. The resistance of the nascent fibril produced by *Acetobacter xylinum* to alkali is lower than that of the crystalline cellulose I and higher than that of the amorphous cellulose. In the nascent fibril the cellulose chains are thougth to be in the form of cellulose sheets corresponding to the (1$\bar{1}$0) plane of cellulose I.

Investigation of the structure of the nascent fibril produced by *Acetobacter xylinum (A. xylinum)* is very important for the clarification of the biogenesis and the structure of bacterial cellulose. We have studied the structure of cellulose fibril after production by *A. xylinum,* in particular its staining behavior toward heavy metal salts[1] and its resistance to alkali.[2,3] However, the structure of nascent fibril is not yet clear enough. In this paper, therefore, the structure of nascent fibril produced by *A. xylinum* was examined from the aspects of the resistance to alkali of the never-air-dried fibrils. These were formed by glucose incubation for 3 h at 28 °C; they did not contain any crystalline microscopic fibril but were composed of 15 wt% of the amorphous fibril and 85 wt% of the fibril having the uneven staining behavior.[2]

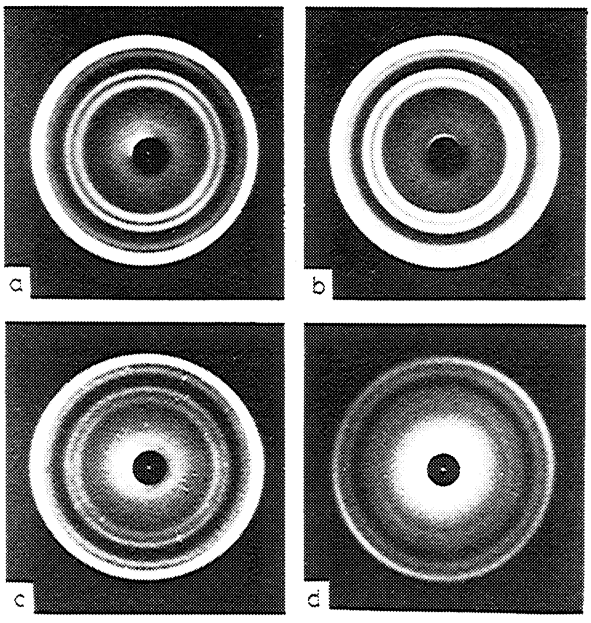

Fig. 1. X-Ray photographs of alkali-treated samples. a: Purified and dried sapmle obtained from the complex incubation for 2d at 28 °C, b: 10.8 wt% alkali-treated sample made from purified and dried sample a, c: 9.0 wt% alkali-treated never-air-dried cellulose obtained from the glucose incubation for 3 h at 28 °C, d: 10.8 wt% alkali-treated never air-dried cellulose obtained from the incubation of the same conditions as sample c.

The never-air-dried cellulose samples from the 3 h incubation were treated with 9.0 or 10.8 wt% aqueous NaOH solution. The X-ray diffraction diagram of 9.0 wt% alkali-treated sample showed only the diffraction of cellulose I, but not that of cellulose II (Fig. 1c). On the other hand, very little diffraction of the (1$\bar{1}$0) plane of cellulose II was found in the X-ray diffraction diagram of 10.8 wt% alkali-treated sample (Fig. 1d). In the diffraction diagram of the 10.8 wt% alkali-treated purified and dried sample, of course, the diffraction of cellulose II does not occur (Fig. 1b).

As described in the previous paper,[2] from the relationship between the change in the morphology of an amorphous fibril after production and the increase in the yield of cellulose with the incubation time, it is presumed that in the never-air-dried cellulose from the 3 h incubation some amorphous fibrils (about 15 wt%) are contained. However, it is considered that such amorphous fibril (15 wt%) will not shown up on the X-ray diffraction diagram of the 10.8 wt% alkali-treated sample. If the cellulose chains are arranged at random in the cross-section of an amorphous fibril, some cellulose chains in the low order region may be dissolved by alkali-treatment with 9.0—10.8 wt% aqueous NaOH solution. But, as shown in Table 1, the values for each weight of alkali-treated samples coincides with the values of each untreated sample, within the experimental errors. This result shows that the cellulose chains are not rejected by alkali-treatment. Accordingly, the X-ray test shows that the resistance of an amorphous fibril to alkali is close to that of the crystalline cellulose I.

The results shown above suggest that in the cross-section of amorphous fibril, as one of the authors described previously,[3] the cellulose chains are in the form of monomolecular layers corresponding to the (1$\bar{1}$0) plane of cellulose I. The amorphous fibril may include staining agent, water and culture substrates between (1$\bar{1}$0) planes.[3] This idea is supported by the studies on the alkali-treatment of cotton,[4,5] which conclude that the structure of a cellulose sheet formed by hydrophobic bond does not easily change by alkali-treatment.

TABLE 1. COMPARISON OF WEIGHTS OF ALKALI-TREATED AND UNTREATED NEVER-AIR-DRIED CELLULOSE FROM THE 3 h GLUCOSE INCUBATION

Concn of NaOH/wt%	Untreated	9.0	Untreated	10.8
Weight (mg/720 ml)	0.18	0.19	0.15	0.17

a) 720 ml is the volume of the culture medium.

Experimental

The culture of *A. xylinum* and the alkali-treatment of the never-air-dried cellulose were as described previously.[2,3] 40 ml of 3 wt% glucose medium (pH 6.8) was added to 200 ml of cellulose-free cell suspension and the mixture was then incubated at 28 °C for 3 h. To the incubated medium was gradually added dropwise an equal volume of aqueous NaOH, two times as dense as the given concentration. After gently making a homogeneous alkali solution, we kept the solution at 20 °C for 1 h and then added 30 vol% aqueous acetic acid solution to neutralize it. The solution was dialyzed through Cellulose Dialyzer Tubing (Nakarai Chemical Ltd, through M. W. cut-off: 8000, diameter: 90 mm, thickness: 0.09 mm) until it became sodium acetate free. The requisite quantity of cellulose for the X-ray photographing was obtained from preparing many times over (1 cycle/week). The dialyzed solution from many times preparations was boiled to collect cellulose. This collected cellulose was boiled in 1 wt% aqueous NaOH solution for 10 h under a nitrogen atmosphere, then neutralized with 1 vol% aqueous acetic acid solution and washed with distilled water until sodium acetate free. The wet cellulose was formed into a disk with the diameter of 1.5 mm and the thickness of about 1 mm for the X-ray photographing. The X-ray diffraction diagram of cellulose was taken with a flat-film camera with Ni filtered Cu $K\alpha$ radiation (35 kV, 15 mA, time of exposure: about 35 min) at specimen film distance of 50 mm.

References

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