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# Cellulose Assemblies Produced by *Acetobacter Xylinum*

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Structures of cellulose assemblies produced by *Acetobacter xylinum* under various conditions have been studied mainly by transmission electron microscopy. Native cellulose crystals are composites of cellulose  $I_{\alpha}$  and  $I_{\beta}$ . Twisted-ribbon cellulose assemblies produced in the HS medium at 28 °C were rich in cellulose  $I_{\alpha}$ . On the contrary, splayed microfibrils produced in the presence of CMC at 28 °C were rich in  $I_{\beta}$ . Not only the ribbon assembly but also the bundle of splayed microfibrils was determined to twist in the right-handed manner. When the bacteria were incubated at 4 °C, two kinds of band-like assemblies, “dense” and “coarse”, were extruded perpendicularly to the long axis of bacterial cells. The number of cellulose chains produced by one bacterium was different between “dense” and “coarse” assemblies. The “dense” assembly gave the electron diffraction pattern of cellulose II. In certain cases the transition region from dense to coarse portions was observed in one assembly. Initially a “dense” portion was produced and thereafter a “coarse” portion was produced. The number of cellulose synthesis sites seems to decrease, because a bacterium becomes less active after a certain period of time at 4 °C.

**Keywords:** Bacterial cellulose/ Ribbon assembly/ Band-like assembly/ Cellulose  $I_{\alpha}$  and  $I_{\beta}$ / Cellulose II / CP/MAS  $^{13}\text{C}$  NMR

It is known that native cellulose crystals are composites of two allomorphs, cellulose  $I_{\alpha}$  and  $I_{\beta}$ . The ratio of cellulose  $I_{\alpha}$  to  $I_{\beta}$  greatly differs from species to species[1]. Why do two allomorphs exist and why does the ratio vary, in nature? To answer these questions, we have studied the crystallization process for a cellulose-producing bacterium, *Acetobacter xylinum*, as a model system, because the biosynthesis of cellulose with the bacterium has been relatively well studied and the ratio is changed depending on the culture conditions, e.g. additives and temperature[2-4]. Recently we have found that two kinds of band-like cellulose assemblies, “dense” and “coarse”, are produced when incubated at 4 °C [5,6]. The “dense” assembly gives the electron diffraction pattern of cellulose II.

This paper reports structures of cellulose assemblies produced by *Acetobacter xylinum* under various conditions, as revealed by transmission electron microscopy (TEM).

Cell suspensions prepared from smooth colonies isolated from *Acetobacter xylinum* ATCC 23769 were stored at 4 °C in the Hestrin-Schramm (HS) medium or in the phosphate buffer (pH7) until use. A drop of cell suspension at 4 °C was put on a formvar/carbon-coated Cu grid for TEM (TEM grid). After incubation for a desired period of time at 28 °C or 4 °C, the specimen on the TEM grid was washed with water and was negatively stained with 1% aqueous uranyl acetate containing bacitracin of 0.5 mg/ml. Some specimens on the grids were not stained but shadowed with Pt-Pd. All observations and electron diffraction (ED) experiments were carried out with a JEOL JEM-200CS transmission electron microscope operated at 200 kV. For ED, specimens were mounted on carbon-coated Cu grids. In order to suppress electron irradiation damage, appropriate specimen areas were searched with the help of a TV system, Gatan Model 622SC+ 663, and

## FUNDAMENTAL MATERIAL PROPERTIES — Molecular Dynamic Characteristics —

The research activities in this subdivision cover structural studies and molecular motion analyses of polymers and related low molecular weight compounds in the crystalline, glassy, liquid crystalline, solution, and frozen solution states by high-resolution solid-state NMR, dynamic light scattering, electron microscopy, X-ray diffractometry, and so on, in order to obtain basic theories for the development of high-performance polymer materials. The processes of biosynthesis, crystallization, and higher-ordered structure formation are also studied for bacterial cellulose.



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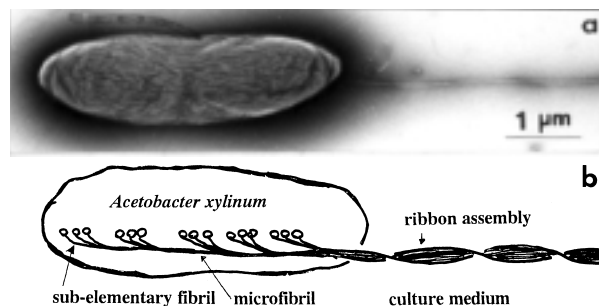
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selected-area ED patterns were recorded onto photo-films. CP/MAS  $^{13}\text{C}$  NMR spectra were measured on a JEOL GSX-200 spectrometer operating under 4.7 T.

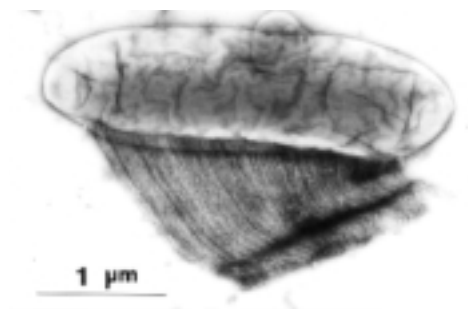
Figure 1a is a typical TEM photograph of a negatively stained ribbon assembly produced from a bacterial cell when grown in the HS medium at 28 °C. Figure 1b shows a model of ribbon assembly. The ribbon assembly is 40-60 nm wide and has a twist with a periodicity of 0.6-1.0  $\mu\text{m}$ . The twist sense was determined to be right-handed, judging from ribbon assemblies shadowed with Pt-Pd [7]. Ribbon assemblies were found by CP/MAS  $^{13}\text{C}$  NMR to contain both  $I_\alpha$  and  $I_\beta$  crystals, with  $I_\alpha$  being dominant (64%) [1]. On the contrary, splayed microfibrils were rich in  $I_\beta$  (80%) when produced in the presence of 1% CMC with DP=80 and DS=0.57 at 28 °C [3]. Compared to the twisted ribbon assembly, the splayed microfibrils formed a rather loose bundle and the bundle itself exhibited a right-handed twist sense. From these results we assume that the twisted ribbon assembly is produced while the bacterium travels translationally along its longitudinal axis with rotating around its axis.

When the bacteria are incubated at 4 °C, two kinds of band-like cellulose assemblies, “dense” and “coarse”, are produced [5,6]. Figure 2 shows a “dense” assembly which is extruded directly from a bacterium during 20 min incubation at 4 °C. For comparison, a “coarse” assembly produced during 3 h incubation is shown in Figure 3. Both of the band-like assemblies are extruded perpendicularly to the long axis of the cell and appear to be wavy and coiled. It seems that at 4 °C the translational and rotational movements of the cells are suppressed. The number of cellulose chains produced by one bacterium is different between “dense” and “coarse” assemblies. The “dense” assembly gave the ED pattern of cellulose II. In contrast, the “coarse” one gave diffuse scattering. Even though a folded-chain “antiparallel” structure has been proposed for the band-like assembly [8], how to arrange “parallel” cellulose chains, when produced from a bacterium, into cellulose II with so-called “antiparallel” packing is a problem to be solved. In certain cases, the transition region from “dense” to “coarse” portions was observed in one assembly. Initially a “dense” portion was produced and thereafter a “coarse” portion was produced. It is assumed that the number of cellulose synthesis sites decreases, because a bacterium becomes less active after a certain period of time at 4 °C.

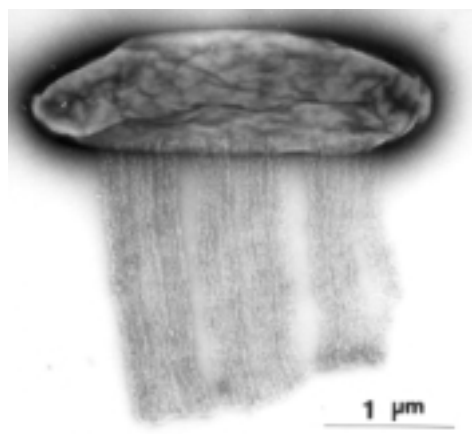
When a cell producing a “dense” band-like assembly at 4 °C was transferred into an incubator thermostated at 28 °C, a ribbon assembly (cellulose I) was produced after formation of the “dense” band-like assembly (cellulose II). When a cell producing a ribbon assembly at 28 °C was transferred into an incubator thermostated at 4 °C, a “coarse” band-like assembly began to form and the ribbon was pushed away from the cell. These results suggest that the movements of the bacterial cell are responsible for the crystallization of cellulose I or cellulose II in the bacterial system.



**Figure 1.** (a) Ribbon assembly (negatively stained). (b) A model of ribbon assembly in *A. xylinum*.



**Figure 2.** “Dense” band-like assembly (negatively stained).



**Figure 3.** “Coarse” band-like assembly (negatively stained).

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