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Biosynthesis of Cellulose by Acetobacter Xylinum IV

—The Morphology of Growth Tips of Bacterial Cellulose Microfibrils—

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Abstract

It has been observed that the shape of growth tips of bacterial cellulose microfibrils are tapered or frayed under an electron microscope. A reasonable interpretation for the shape of the microfibrils is obtained if one imagines a spiral growth mechanism in the formation of cellulose microfibrils. Three suitable models for the mechanism have been obtained by assuming three types of chains; folded chain, short chain, and extended chain. It seems that bacterial cellulose microfibrils consist of fully extended chain crystals formed by a simultaneous polymerization and crystallization with a spiral growth.

Introduction

Although cellulose is a compound of qualitative importance as a skeletal material, the most abundant and widely used natural plant product, the next five questions of importance in such structures remain open; (1) whether cellulose chains are arranged in parallel or antiparallel, (2) whether chain foldings exist or not, (3) a staining periodicity is reflected by any fine structure, (4) the discrepancy between crystallite widths from x-ray line broadness of wide-angle interferences and form electron microscopy, (5) why and how the preferential orientation of the crystallites is produced in a membrane of bacterial or Valonia cellulose. The investigations for the above questions are made difficult in green plants by the fact that cellulose is produced within the celles of the plants. It appears, therefore, to be more profitable to choose an organism which synthesizes cellulose extra-cellularly.

In recent years a number of models for the chain folding[1-4] of cellulose molecules have been proposed. None of them can account for all chemical, physical and morphological data from cellulosic materials. The pronounced swelling and the mechanical anisotropy, the low extensibility, the high strength, the behavior of birefringence of cellulosic materials indicate that the chains are aligned in the direction of the fibrillar axis. A number of observations suggest that the chains are intially extended. Chain folding, of course, automatically implies an antiparallel molecular arrangement and a less stadle thermodynamic situation. Muggli[5] carried out a rather simple experiment which basically, involves the determination of the degrees of polymerization by gel permeation chromatography (GPC) before and after the transversal sectioning of ramie fibers. The observed derease in the molecular weight of cellulose in the 2 micron sections from that in the original fiber

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was fully explained assuming completely extended chains. In the previous papers, we suggested the possibility of a moleculcular chain folding using a molecular model of cellulose[4]. It is not impossible to fold the cellulose chain in the (101) plane. Two anhydroglucose units of a boat (B 3) and chair (C 1) configurations are sufficient for the fold to be realized. There should be intramolecular and intermolecula hydrogen-bonds in this scheme. However, we assume that there is no chain folding at least in native cellulose, because native cellulose is entirely different from the regenerated cellulose in their crystallization process. Regenerated cellulose (rayons) are reproduced from the alkaline solution of cellulose xanthate in which the molecules may molecularly disperse. Acording to the study of crystallization of cellulose from dilute solutions[6], the cellulose chain is highly rigid under the condition in which the intramolecular hydrogen bonding is formed between the O(3) and O(5) position, but it can be more flexible and takes random coil conformations when the formation of the intramolecular hydrogen bonding is not achieved. It appears, therefore, that the possibility of a chain folding is present in the regenerated cellulose molecules under the latter condition. On the other hand, if the crystallization of native cellulose accompanies not only the formation of intermolecular hydrogen bonds but also that of intramolecular hydrogen bonds, the chains will never be folded.

Meterials and Methods

Preparation of Bacterial Cells

Strain AHU-1595 of Acetobacter Xylinum was obtained from the Applied Microbiological Laboratory, Departmet of Agriculture Chemistry, Faculty of Agriculture, Hokkaido University, through the courtesy of Drs. T. Sasaki and S. Takao. At first a suspension of whole cells free from cellulose in sterilized substrate was prepared. One ml of this solution was inoculated into 200 ml of liquid medium (0.5% yeast extract and 2% glucose ; pH 5.9) in a smooth wall 500 ml shaken flask, and then incubated in both static and shaken cultures at 30°C and harvested after various periods of time.

Preparation of Specimens for Electron Microscopy

In order to observe the elongation process of cellulose microfibrils, specimens were prepared by the following procedure. One volume of standard cell suspension (about 10^9 viable cells per ml) was filtered through gossypium cotton, and then added to 1 volume of 2% glucose and 0.5% yeast extract. The mixture was incubated with gentle shaking for 30 min, after which growth was stopped by adding a half volume of glacial acetic acid. The aqueous suspension was then diluted with 35 volumes of phosphate buffer solution. One ml of the suspension was further diluted with 35 ml of water. Drops of this diluted suspension were placed on grids with collodion films thinly coated with carbon. They were washed three times by flotating over distilled water kept on parafilm and the water was removed by absorbing it with a strip of filter paper. After drying, the material on the grids were shadowed with Pt–C. All specimens were photographed using Hitachi model HS–8 and HS–11–DS electron microscopes.

Results and Discussion

In the molecular mechanism of the formation of the cellulose microfibril, two hypotheses have been proposed. One is a continued, longitudinal crystalline

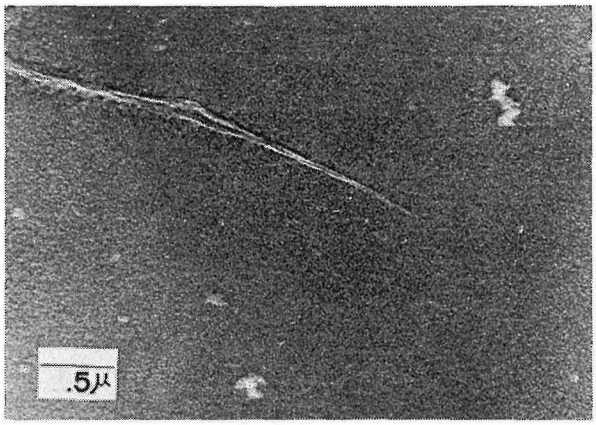

Fig. 1 Tapered tips of unextracted bacterial cellulose microfibrils shadowed with Pt-C

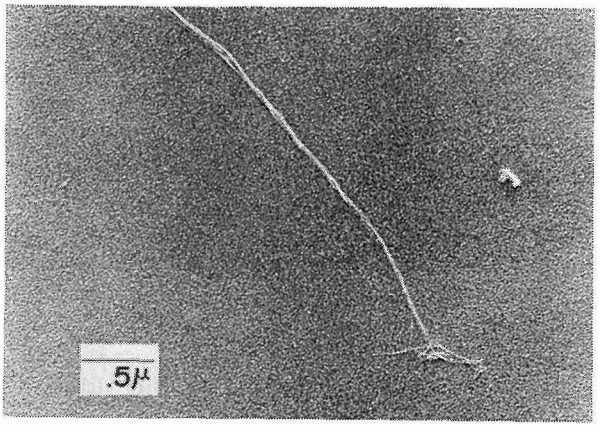

Fig. 2 Frayed tips of unextracted bacterial cellulose shadowed with Pt-C

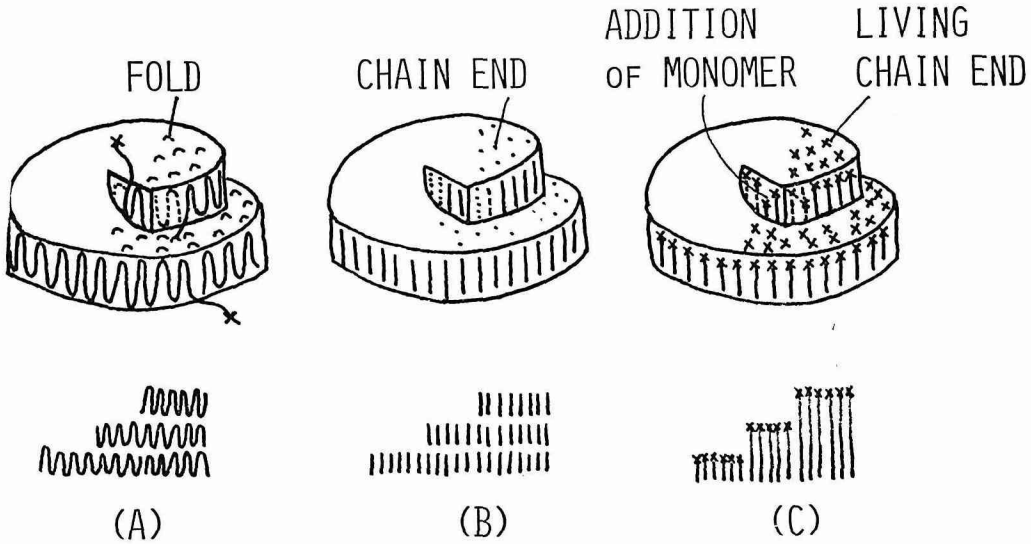

Fig. 3 Schematic models of the crystallization accompanying polymerization in a spiral growth: (A) A pile of lamellae of folded chain; (B) A pile of lamellae of short chain; (C) An extended chain crystal

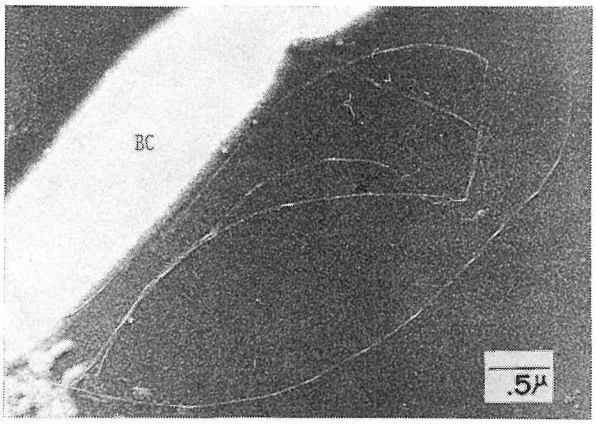

Fig. 4 A typical electron microphotograph of twisting in bacterial cellulose microfibrils. BC: bacteria cell

growth of the microfibril from a previously formed, soluble, polymeric intermediate[7-9]. The other is a simultaneous polymerization and crystallization through the growth of insoluble cellulose microfibrils from their tips[10,11]. It has been recognized that the former hydrothesis requires the slow tapering or frayed tips of the fiberous crystals, in order to provide the continous growth of the chain molecules along a given direction. According to the latter hypothesis, on the other hand, the shape of the microfibrils should be blunt. Many experimental evidences have shown that the ends of the microfibrils are quite blunt and not tapered of frayed. However, we have recently obseved, by the Pt–C shadowing method under an electron microscope, microfibrils of bacterial cellulose with tips tapered or frayed shapes as shown in Fig. 1 and 2. If one imagines a spiral growth mechanism in the formation of celluloce microfibrils by a simultaneous polymerization and crystallization, a tapered shape of the microfibrils in their tips will result. Fig. 3 shows three suitable models of the crystallization accompanying polymerization based on a spiral growth. If polymers, which are produced in a homogeneous system prior to crystallization, were crystallized, the products would have lamellar structure with folded chains (Fig. 3 (a)), such as observed in the case of solution grown crystals. The propagation of the chains may also be achieved by an addition of monomers to the free ends of the molecules crystallized. As can be seen in Fig. 3 (b), short chains may form lamellar crystals composed of extended chains as in the case of parafine crystals. The third one (Fig. 3 (c)) is a mechanism of a simultaneous polymerization and crystallization with the spiral polymerization and crystallization with the spiral growth. The nucleaction and the growth of the nucleus are the important steps as an initiation of this mechanism. If a screw dislocation results from this type of nuleation and growth, and the propagation further proceeds by an addition of monomers only to the activated tips of the crystals formed, and simultaneously by the crystllization of them on the side surface of the crystals, the shape of the tips of the crystals will be tapered as shown in Fig. 3 (c). In this mechanism, it may be assumed that the crystals consist of fully extended chains. The existence of the spiral growth in the cellulose microfibrils[12] has been observed as twistings at 0.5–1 μ intervals along the fibrillar axis (Fig. 4). They may be interpreted as a typical Eshelby twist[13] due to the uncompensated torque induced in the microfibrils by the dislocation. They gave the same appearance as seen in many metallic or inorganic "whiskers." In

the ideal case of a simultaneous polymerization and crystallization of type Fig. 3 (c), the product should be a perfect single crystal without defects. However, the cellulose microfibrils actually have defects and are only pertially crystalline, and not uniform either along or across the microfibril. Therefore, the process of the formation of the microfibrils may not be resulted from an ideal "living" polymerization, but may involve termination and reinitiation by the chain transfer in the activated tips. When the cellulose molecules are fully extended chains, their length estimated from the degree of polymerization (DP) would not coincide with that of the microfibrils observed under the electron microscope. This discrepancy may be due to the termination mentioned above or to the DP measurements made after the cupro-ammonium processes.

Wunderlich[14] has discussed the general two paths of A and B from monomers to crystalline polymers. Path A is crystallization during polymerization (simultaneous or successively) such as polyoxymethylene obtained by the solid state polymerization of trioxane. Path B is separate polymerization and crystallization as observed in a general solution or melt grown polymer crystals. X-ray wide-angle scatteing from the resultant crystalline polymers exhibits well orientated fiber diagrams, irrespective of the polymerization pathways A or B. However, on small-angle scattering of the samples of the path A, no long period would be observed. Electron micrographs show small fibers with smooth surfaces whose fiber axis is in parallel with the original molecular axis of the monomer. From these results it is safe to assume that the polymerization through path A leads to the crystals with extended polymer chains. If the formation of the cellulose microfibrils proceeded in the same manner as observed in simultaneous polymerization and crystallization, no long period would be observed in the meridional direction. In fact, native cellulose shows no long period. On annealing the polymers obtained through path A, long periods come to be detected by the small-angle scattering[15]. This observation may be due to back-folding of the polymer chains. In the solution or melt grown crystals, similar long period scatterings are also observed, corresponding to the folded pitches. In view of the similarity mentioned above, the native cellulose microfibrils may consist of crystals with extented chains formed by a simultaneous polymerization and crystallization with the spiral growth. The microfibrils consist of a comparatively continuous but partially imperfect crystal, in which the end groups of the molecules would have local distortions. Thus, there can be no sharp distinction between crystalline and non-crystlline regions.

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