Direct Imaging of Single Crystals of Regioselectively Substituted Cellulose Heteroesters by Atomic Force Microscopy

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Introduction. Recently, the folded surfaces of crystals of polyethylene,[1] cyclic alkanes,[2] poly(ethylene oxide),[3] and poly(hydroxybutyrate)[4,5] have been reported using a relatively new technique of atomic force microscopy (AFM). In the field of polysaccharides, however, there exist only a few papers about surface images of cellulose microfibrils, xanthan, acetan, etc.[6−8]

With respect to the single crystals of cellulose ester derivatives and cellulose itself, the argument, as to whether chain-foldings occur on the crystal surfaces or not, has been previously reported by the relationship between lamellar thickness determined by the shadow length method and degree of polymerization (DP).[9,10] Manley has prepared single crystals of cellulose triacetate (CTA) ranging in DP from 35 to 450 from mixtures of nitromethane and *n*-butanol, which assumed the folded configurations of CTA molecules in the lamellae.[9] However, the crystals obtained were in fact a CTA−nitromethane complex.[11,12] On the other hand, Buléon and Chanzy investigated the influence of the DP of cellulose against the growth of cellulose IV_{II} lamellar crystals.[10] They reported that well-developed lamellar crystals were obtained from a DP of 22−24 whereas higher-DP materials gave polycrystalline aggregates. And, they concluded that chain-folded crystals with high-DP cellulose samples had not been occurred.

During a past few years, we have been investigating the crystal and molecular structures of two kinds of regioselectively substituted cellulose heteroesters, cellulose propanoate diacetate (CPDA, 2,3-di-*O*-acetyl-6-*O*-propanoyl cellulose)[13] and cellulose acetate dipropanoate (CADP, 6-*O*-acetyl-2,3-di-*O*-propanoyl cellulose)[14] through combined X-ray fiber diagrams and electron diffraction patterns of their single crystals. The CPDA single crystal was lozenge-shaped with {110} as growth planes as in cellulose triacetate II,[15] while CADP had the ribbon-like single crystal that belonged to the $P2_1$ space group with *b* as the unique axis as in cellulose tripropionate II.[16] These two cellulose heteroesters have been organized with antiparallel-chains packing fashion. In this paper, we report direct images of the surfaces of CPDA and CADP single crystals by AFM, and discuss whether chain-foldings can be demonstrated on the crystal surfaces or not.

Experimental Section. Sample Preparation. Regioselectively substituted cellulose heteroesters, cellulose propanoate diacetate (CPDA, 2,3-di-*O*-acetyl-6-*O*-propanoyl cellulose) and cellulose acetate dipropanoate (CADP, 6-*O*-acetyl-2,3-di-*O*-propanoyl cellulose), were prepared either from low-molecular-weight cellulose (DPw = 30−50) or from a microcrystalline cellulose (Avicel SF, Asahi Chemical Co., Ltd., DPv = 133) following the method described previously.[17] Lamellar single crystals of CPDA and CADP have been prepared at high temperature in a mixture of dibenzyl ether and *n*-tetradecane according to the method described previously.[18] Preparation of single crystals from higher molecular weight cellulose heteroesters (DPw > 210), however, did not succeed.

Atomic Force Microscopy. The thicknesses of regioselectively substituted cellulose heteroesters single crystals were investigated on the basis of atomic force microscopy (AFM). AFM was performed with a SPI3700/SPA300 (Seiko Instruments Inc.). Pyramid-like Si_3N_4 tips, mounted on 100 μm long micro cantilevers with spring constants of 0.09 N/m, were used for the contact mode experiments. Simultaneous registrations were performed in the contact mode for height and deflection images. Crystal suspensions were dropped on mica and allowed to dry. All images were recorded at room temperature.

Molecular Weight Measurement. Molecular weights of single crystals were obtained by gel-permeation chromatography (GPC) at 40 °C, using a Shimadzu 10A GPC system and 6A refractive index detector with joint columns of Shodex K-80M and K-802 (each 4.6 × 300 mm). Chloroform was used as an eluent at the flow rate of 0.8 mL/min, and a sample concentration of 1.0 mg/mL was applied. The number-average and weight-average molecular weights (M_n and M_w) were calculated by using a Shimadzu Chromatopac C-R7A plus equipped with a GPC program. Polystyrene standards with a low polydispersity (Shodex Standard SM-105, 1.3×10^3 to 3.1×10^6) were used to generate a calibration curve.

Results and Discussion. The CPDA crystals were confirmed by using transmission electron microscopy (TEM). Crystals occurred as platelets of lozenge-like appearance, and each crystal yielded a sharp electron diffraction pattern defined by the two orthogonal axes *a** and *b** as reported previously.[18] On the basis of CPDA having an orthogonal crystal system,[13] the polymer chains align perpendicular to the lamellar base of the crystal. Number-average molecular weight (M_n), degree of polymerization (DPn), and polydispersity (DPI) of CPDA single crystals measured by GPC are 1.7×10^4, 56, and 1.9, respectively. Figure 1 shows a 2 μm × 2 μm AFM image of CPDA single crystals and line profile data. Even at the resolution of the picture, AFM has the advantage over TEM of giving direct information about the height of the crystals. The typical measurements of the monolamellar thickness of crystals yield values of around 30 nm. CPDA has a 2-fold screw symmetry along the molecular axis, and its fiber repeat distance is 1.044 nm.[13] On the basis of the DPn and the fiber repeat distance with 2-fold screw symmetry, the thickness of single crystal is calculated to be 29 nm, which perfectly agrees with the experimental value. This indicates that the chain-folding at lamellar surface may not occur in the CPDA single crystals.

In the case of CADP crystals, long narrow ribbon-like CADP single crystals radiating from the same nucleation center also yielded a spot electron diffractogram as described previously.[18] The 6 μm × 6 μm AFM image of CADP single crystals and line profile data are shown in Figure 2. The typical lamellar thicknesses of CADP single crystals are almost 45 nm. M_n, DPn, and DPI of

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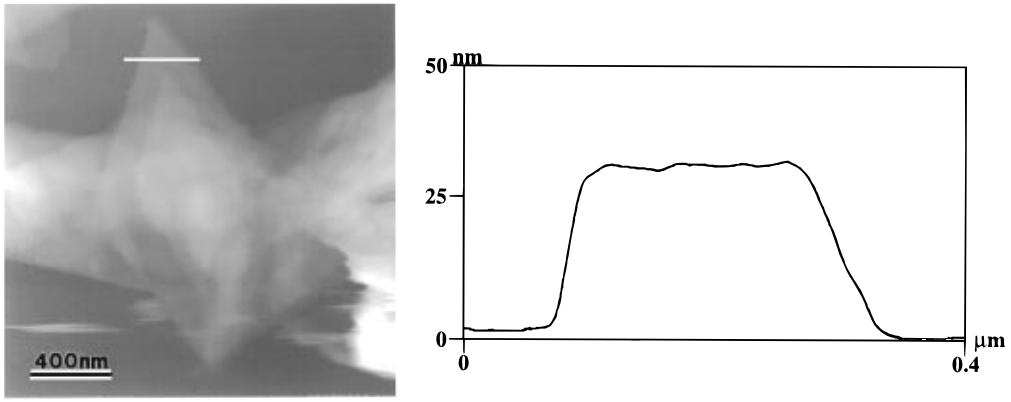

Figure 1. AFM images and line profile data of CPDA single crystals grown from a mixture of dibenzyl ether and *n*-tetradecane (25:75, v/v). The white line corresponds to the profile data.

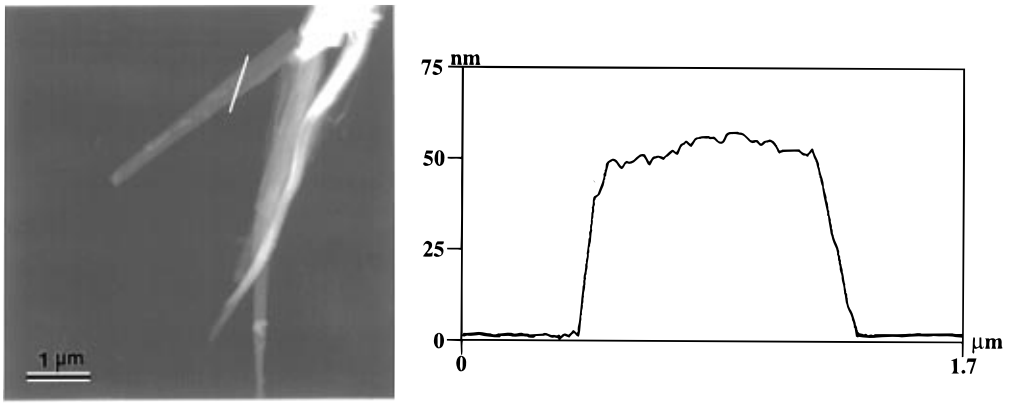

Figure 2. AFM images and line profile data of CADP single crystals grown from a mixture of dibenzyl ether and *n*-tetradecane (30:70, v/v). The white line corresponds to the profile data.

CADP single crystals are 1.6×10^4, 51, 1.7, respectively. CADP has a 3-fold screw symmetry along the molecular axis, and its fiber repeat is 1.509 nm.[14] On the basis of the DPn and the fiber repeat distance with 3-fold screw symmetry, the thickness of single crystal is calculated to be 25 nm which closely corresponds to half of the experimental value. This result suggests that CADP single crystals are organized multilamellarly, or that the hydrogen bondings are generated between CADP chains to permit "head-to-tail" alignment in CADP crystals. In both cases, CADP crystals do not have a chain-folding surface.

In crystallization processes of single crystals grown from dilute solution at high temperature, the esterified cellulose chains should possess sufficient mobility in order for occur chain-folding to occur. However, it is concluded that CPDA and CADP single crystals do not have the chain-foldings internally, based on their thicknesses and molecular weights. This might be due to the repulsions between esterified side-chains. Furthermore, it was not possible to prepare well-defined single crystals from high-molecular-weight materials. This fact suggests that CPDA and CADP crystals are organized with extended chains under this experimental condition. Further studies concerning the other cellulose esters are now in progress.

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