CP/MAS ^{13}C NMR Analysis of the Crystal Transformation Induced for *Valonia* Cellulose by Annealing at High Temperatures

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ABSTRACT: The crystal transformation of *Valonia* cellulose induced in a dilute alkaline solution at high temperatures has been examined in detail by high-resolution solid-state ^{13}C NMR spectroscopy. The C1 and C4 resonance lines, which are characteristic triplets for the celluloses from primitive organisms, markedly undergo changes in relative intensities of the constituent lines with increasing annealing temperature, and those lines are finally converted to doublets with almost equivalent intensities. Such spectral changes have been successfully analyzed in terms of the composite crystal model in which native cellulose crystals are assumed to be composites of two allomorphs, celluloses I_α and I_β, resulting in confirmation of the validity of the composite crystal model. Moreover, the fraction of cellulose I_α is found to be reduced from 0.64 for intact *Valonia* cellulose to about 0.12 by annealing as a result of the crystal transformation from cellulose I_α to I_β.

Introduction

It is well-known that native celluloses such as cotton, bacterial, and *Valonia* celluloses have the crystal structure named cellulose I on the basis of X-ray diffraction analyses. However, the detailed structure of cellulose I is not well characterized as yet. Many models of the crystal structure have been proposed according to the hypothesis that native cellulose has a homogeneous crystal phase, for example, a two-chain monoclinic unit cell of Meyer-Misch type,[1] an eight-chain unit cell,[2] and a two-chain triclinic unit cell.[3] In contrast, Atalla and VanderHart[4,5] have made the proposal, as a result of cross polarization/magic angle spinning (CP/MAS) ^{13}C NMR measurements, that each native cellulose is a composite of two different allomorphs, celluloses I_α and I_β. Cael et al.[6] have also proposed that CP/MAS ^{13}C spectra of native celluloses are linear combinations of two types of spectra, but these spectra are assumed to correspond to the resonances from the carbons in the Meyer-Misch type two-chain and the eight-chain unit cells.

We have also studied the crystal structure of cellulose I in detail by selectively measuring CP/MAS ^{13}C NMR spectra of the crystalline components using the difference in ^{13}C spin–lattice relaxation time T_{1C} between crystalline and noncrystalline components.[7,8] As a result, native celluloses are classified into two groups, cotton–ramie and bacterial–*Valonia* groups,[7-9] which seem significantly rich in celluloses I_β and I_α, respectively, in accord with the previous proposal.[4,5] Furthermore, we have examined the possibility of the interconversion between celluloses I_α and I_β by employing a physical or chemical method. It has become apparent that the two different groups of native cellulose are transformed into a cellulose more definitely rich in cellulose I_β by annealing with saturated steam or in an aqueous alkaline solution at high temperatures.[10,11] Although the same crystal transformation has also been induced in the cases of solid-state regenerations to cellulose I from cellulose triacetate I[12] and cellulose III_I,[12,13] the degree of crystallinity significantly decreases and the microfibrils seem to undergo appreciable changes in shape and diameter in these two cases. In contrast, the annealing with saturated steam or in the alkaline solution induces only minor changes in crystallinity[10,11] as well as in the morphological structure of microfibrils, as revealed by our preliminary electron microscopic observation.[14]

In this paper we have examined the $I_\alpha \rightarrow I_\beta$ crystal transformation induced for *Valonia* cellulose in a 0.1 N NaOH aqueous solution at 220–280 °C in detail by CP/MAS ^{13}C NMR spectroscopy. The total CP/MAS ^{13}C NMR spectra of the annealed samples have also been analyzed in terms of the composite crystal model in which each spectrum is assumed to be a linear combination of celluloses I_α and I_β.

Experimental Section

Sample. *Valonia* cellulose was obtained from the cell walls of *Valonia macrophysa* vesicles, which were naturally grown at 19–20 °C in the sunshine in a water tank for fish in Kushimoto Marine Park. Here, 6 tons of water in the tank is circulated with the filtration with sand and the partial addition of 20–30 L of fresh saline water per min. The *Valonia* cell walls were purified by boiling in a 1% NaOH aqueous solution for 8 h. After well rinsing with deionized water, the cellulose sample was subjected to annealing in a 0.1 N NaOH aqueous solution at 220–280 °C for 30 min. Each sample was washed with deionized water and packed in a MAS rotor with an O-ring seal[15] together with an appropriate amount of deionized water (g of H_2O/g of cellulose = 0.7–1.0) without drying.

CP/MAS ^{13}C NMR Measurements. CP/MAS ^{13}C NMR spectra were recorded at room temperature on a JEOL JNM-FX200 spectrometer equipped with a JEOL variable-temperature (VT)/MAS system operating at 50 MHz under a static magnetic field of 4.7 T. A cylinder-type MAS rotor with an O-ring seal, which was modified from a commercial rotor made of aluminum oxide and poly(amide–imide) resins,[15] was used for hydrated cellulose samples. ^1H and ^{13}C radio-frequency field strengths $\gamma B_1/2\pi$ were 65.8 kHz for the CP process, while the ^1H field strength was reduced to 54.3 kHz in the dipolar decoupling process. The MAS rate was 3.5 kHz, and the contact time for the CP process was 1 ms throughout this work. No digital resolution enhancement was employed. The chemical shift relative to tetramethylsilane (Me_4Si) was determined by using the crystalline peak at 32.89 ppm of linear polyethylene as an internal or external standard.

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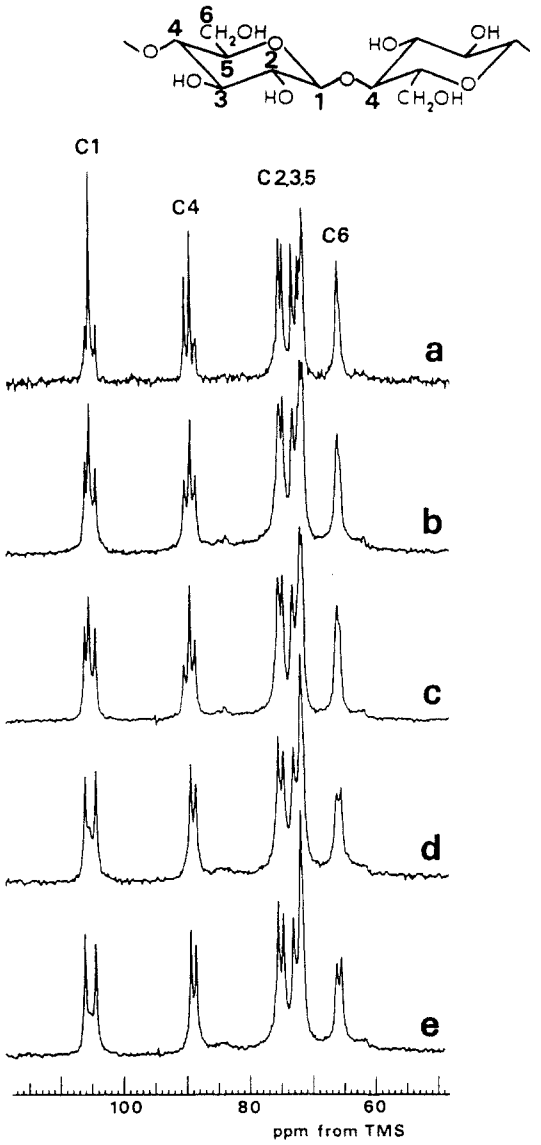

Figure 1. 50-MHz CP/MAS ^{13}C NMR spectra of *Valonia* cellulose annealed at different temperatures in the 0.1 N NaOH solution: (a) original; (b) 220 °C; (c) 240 °C; (d) 260 °C; (e) 280 °C.

Results and Discussion

Figure 1 shows 50-MHz CP/MAS ^{13}C NMR spectra of *Valonia* cellulose and the samples annealed at 220–280 °C for 30 min in a 0.1 N NaOH solution, which were measured at room temperature in the hydrated state. Some of these spectra are the same as previously reported.[11] The C1 resonance line of *Valonia* cellulose is composed of an enhanced central line and a considerably small doublet beside the central line. This type of C1 triplet is characteristic of the spectrum for the celluloses from primitive organisms.[7,8] Such a triplet drastically changes in shape with increasing annealing temperature; the central line markedly reduces in intensity with the increase of temperature, whereas the two lines at both sides concomitantly increase in intensity. As a result, the C1 line becomes a doublet with the almost identical intensity at 280 °C. The C4 triplet is also transformed into a doublet as a result of the reduction of the downfield line and the increment of the most upfield line. In contrast, the intensity of the central line of the C4 triplet seems to stay constant by the annealing. This result will be explained in terms of a composite crystal model described later. The C6 line seems to be originally a triplet, although it is not well recognized because of the low magnification of the figure. However, the high-temperature annealing also induces the decrease in intensity of the central line for C6 carbon and the concomitant increase in intensity of the side lines like the change in the C1 line. Such spectral changes also result in the appearance of the doublet for the C6 carbons above 260 °C.

These results are in good accord with those obtained by the annealing with saturated steam using glass fiber sheets.[10] In that annealing we kept each sample in the vapor phase by fixing it with glass fiber sheets and stainless steel nets without contact with the liquid phase. However, those sheets have been found to prevent the decomposition of cellulose at high temperatures like the 0.1 N NaOH aqueous solution used as an annealing medium in this work.[11] Similar annealing effects on spectra were also observed for bacterial cellulose as well as cotton and ramie cellulose, although the latter two samples originally reveal spectra similar to the spectrum shown in Figure 1d. As has already been pointed out, the spectra of the samples annealed at 280 °C are very close to the spectrum assigned to cellulose I_β by Atalla and VanderHart.[4,5] Therefore, we try to explain the spectral changes shown in Figure 1 in terms of their composite crystal model. First, we resolve the C1 and C4 triplets into several constituent lines, assuming each line to be a Lorentzian as in the cases[7,10] previously reported, by a nonlinear least-squares method aided by a computer.

Figures 2 and 3 show the results of line-shape analyses for the C1 and C4 triplets of different *Valonia* celluloses, respectively. Here, it is assumed that each triplet is described as a superposition of three Lorentzians, although some minor Lorentzians are also introduced in some cases to obtain better fitting. Lines IX and VIII should be ascribed to additional contributions of lines VI and VII, while lines IV and X are assigned to the noncrystalline components of the C1 and C4 resonances, respectively.[7,8] Table I files chemical shifts, line widths, and integrated fractions of the respective constituent lines of C1 and C4 resonance lines for different samples, which have been obtained by the line-shape analysis shown in Figures 2 and 3.

On the basis of the composite crystal model proposed by Atalla and VanderHart,[4,5] ^{13}C NMR spectra of native cellulose are assumed to be described in terms of a linear combination of the spectra of celluloses I_α and I_β as are shown in Figure 4. Here, C1 and C4 resonance lines of cellulose I_β are doublets, whereas these resonance lines of cellulose I_α are a singlet and doublet, respectively. According to this model, the integrated fractions of lines I–III in the C1 resonance line are given by $(1 - f_\alpha)/2$, f_α, and $(1 - f_\alpha)/2$, respectively, where f_α is the mass fraction of cellulose I_α. Accordingly, f_α is expressed by the least-squares method as

$$f_\alpha = (1 - f_I + 2f_{II} - f_{III})/3 \quad (1)$$

where f_I, f_{II}, and f_{III} are the fractions of lines I–III that are experimentally obtained as a result of the line-shape analysis. In the C4 resonance line the integrated fractions of lines V–VII should be expressed as $f_\alpha/2$, $1/2$, and $(1 - f_\alpha)/2$, respectively. This leads to the following equation for f_α in the C4 resonance line:

$$f_\alpha = 0.5 + f_V - f_{VII} \quad (2)$$

Here, f_V and f_{VII} are the integrated fractions of lines V and VII obtained by the line-shape analysis, respectively. It should be here noted that the integrated fraction of line VI does not depend on f_α but stays constant as $1/2$. This

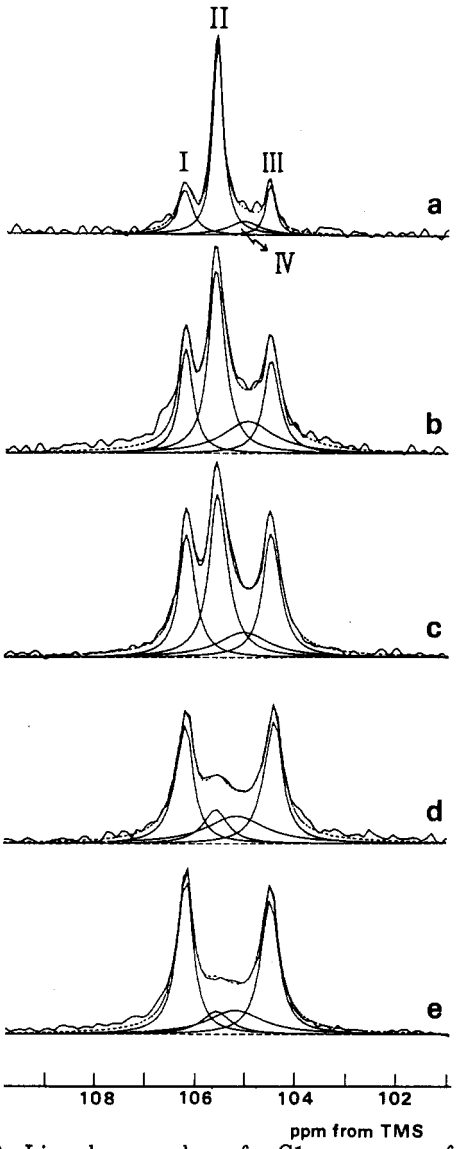

Figure 2. Line-shape analyses for C1 resonances of *Valonia* cellulose annealed at different temperatures in the 0.1 N NaOH solution: (a) original; (b) 220 °C; (c) 240 °C; (d) 260 °C; (e) 280 °C.

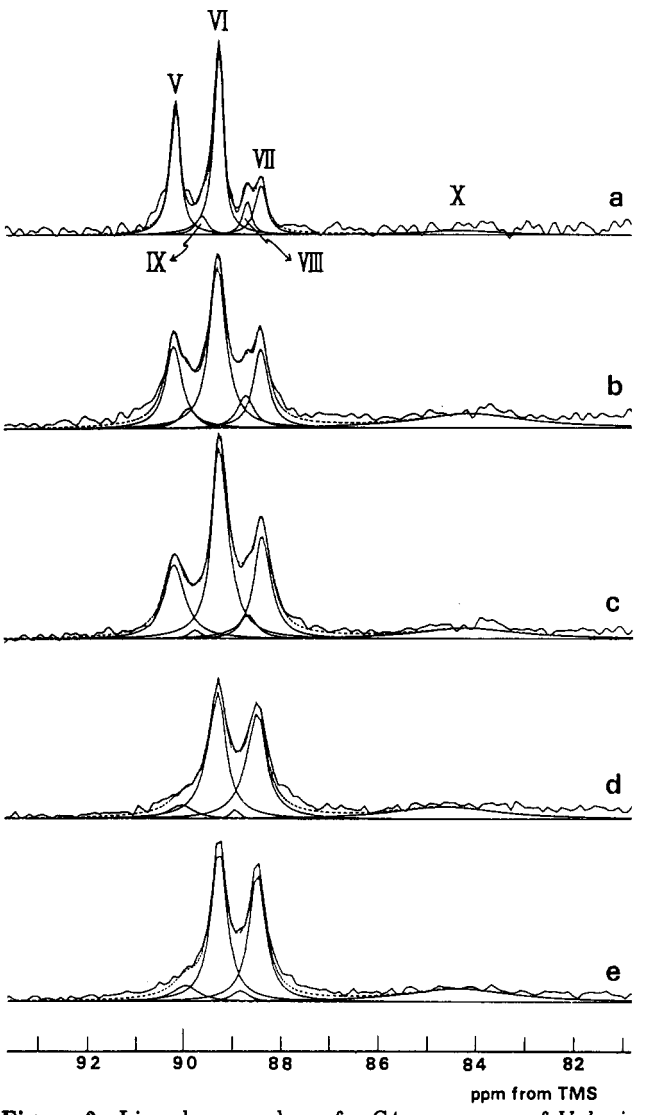

Figure 3. Line-shape analyses for C4 resonances of *Valonia* cellulose annealed at different temperatures in the 0.1 N NaOH solution: (a) original; (b) 220 °C; (c) 240 °C; (d) 260 °C; (e) 280 °C.

fact seems to be associated with no change in intensity for the central line of the C4 triplet by the annealing (see Figure 1).

Table II shows fractions f_α for the C1 and C4 lines calculated by using eqs 1 and 2 together with the fractions of the respective constituent lines of C1 and C4 triplets. The f_α values determined from the C1 and C4 triplets are in good accord with each other. This indicates that the C1 and C4 triplets can be well interpreted in terms of the composite crystal model for annealed samples as well as for intact *Valonia* cellulose, and thus it has also been confirmed that these samples are composites of celluloses I_α and I_β. According to this result, intact *Valonia* cellulose contains 64% cellulose I_α, supporting numerically the previous conclusion that this sample is rich in cellulose I_α.[7] However, the fraction of cellulose I_α markedly decreases with increasing annealing temperature as a result of the crystal transformation from cellulose I_α to I_β. Finally, by annealing at 280 °C, the fraction of cellulose I_β attains to 0.88. Since a temperature of 280 °C is a limiting annealing temperature without significant degradation, it may be impossible to obtain a sample with pure cellulose I_β by this kind of annealing. Similar annealing in some organic solvents can also induce the same crystal transformation but the fraction of cellulose I_β finally obtained is the same level.[16]

As already reported,[17] electron diffraction patterns have also been measured for delaminated specimens of *Valonia* cellulose annealed under the same experimental condition as in this work. All diffraction spots of the specimen annealed at 260 °C are indexed with the Meyer-Misch type monoclinic unit cell[18] with a = 0.792 nm, b = 0.822 nm, c = 1.036 nm, and γ = 97.3°. In contrast, it has been suggested that some diffraction spots for the original *Valonia* cellulose should be indexed with the two-chain triclinic unit cell with a = 0.954 nm, b = 0.825 nm, c = 1.036 nm, α = 90.0°, β = 57.0°, and γ = 96.9° proposed by Sarko et al.,[3] while the other spots can be indexed with the monoclinic unit cell. A recent, preliminary electron diffraction pattern analysis[14] has also pointed out that the diffraction intensity of triclinic crystals drastically decreases with increasing annealing temperature, with the concomitant increase in the diffraction intensity of monoclinic crystals. The comparison of these results with the NMR results mentioned above leads to the conclusion that celluloses I_α and I_β should be assigned to triclinic and monoclinic crystals, respectively. Sugiyama et al.[19] have recently confirmed this conclusion more definitely on the

Table I
Chemical Shifts (δ), Line Widths ($\Delta\nu$), and Integrated Fractions (I) of the Respective Lines of C1 and C4 Triplets for *Valonia* Cellulose Annealed at Different Temperatures

sample	annealing temp/°C		C1 I	C1 II	C1 III	C1 IV	C4 V	C4 VI	C4 VII	C4 VIII	C4 IX	C4 X
V1		δ/ppm	106.3	105.6	104.5	105.0	90.3	89.5	88.6	88.9	89.8	84.4
		$\Delta\nu$/Hz	18	13	12	39	13	12	15	11	14	98
		I	0.18	0.56	0.14	0.12	0.29	0.39	0.13	0.07	0.05	0.07
V2	220	δ/ppm	106.2	105.6	104.5	105.0	90.3	89.4	88.5	88.8	90.0	84.2
		$\Delta\nu$/Hz	16	20	19	61	21	21	18	21	21	133
		I	0.19	0.40	0.20	0.21	0.18	0.35	0.15	0.07	0.05	0.20
V3	240	δ/ppm	106.4	105.7	104.7	105.3	90.5	89.5	88.7	89.0	90.0	84.4
		$\Delta\nu$/Hz	19	22	22	63	26	20	21	21	15	130
		I	0.23	0.35	0.26	0.16	0.19	0.38	0.22	0.05	0.02	0.14
V4	260	δ/ppm	106.3	105.7	104.6	105.3	90.0	89.3	88.5	88.9		84.6
		$\Delta\nu$/Hz	20	29	21	70	31	22	24	10		134
		I	0.30	0.12	0.34	0.24	0.06	0.37	0.34	0.01	0	0.22
V5	280	δ/ppm	106.2	105.6	104.5	105.2	90.0	89.3	88.5	88.8		84.3
		$\Delta\nu$/Hz	19	35	21	68	31	19	20	20		132
		I	0.35	0.10	0.35	0.20	0.06	0.36	0.33	0.03	0	0.22

Table II
Fractions of the Respective Lines of C1 and C4 Triplets for *Valonia* Cellulose Annealed at Different Temperatures

sample	annealing temp/°C	C1 f_{I}	C1 f_{II}	C1 f_{III}	C1 f_α[b]	C4 f_{V}	C4 f_{VI}	C4 f_{VII}	C4 f_α[c]
V1[a]		0.20	0.64	0.16	0.64	0.36	0.42	0.22	0.64
V2	220	0.24	0.51	0.25	0.51	0.29	0.44	0.27	0.52
V3	240	0.27	0.42	0.31	0.42	0.25	0.44	0.31	0.44
V4	260	0.39	0.16	0.45	0.16	0.08	0.47	0.45	0.13
V5	280	0.44	0.12	0.44	0.12	0.08	0.46	0.46	0.12

[a] Original. [b] $f_\alpha = (1 - f_{\mathrm{I}} + 2f_{\mathrm{II}} - f_{\mathrm{III}})/3$. [c] $f_\alpha = 0.5 + f_{\mathrm{V}} - f_{\mathrm{VII}}$.

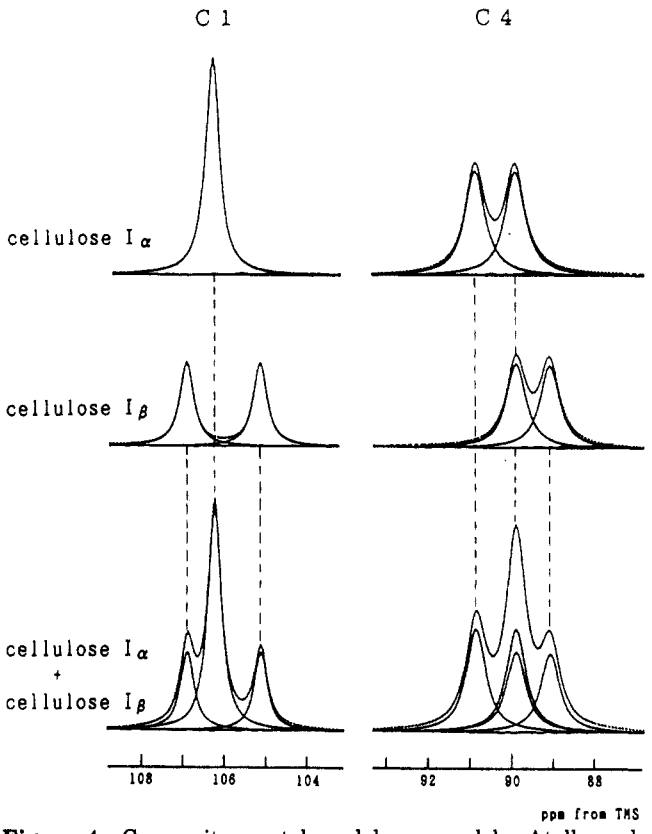

Figure 4. Composite crystal model proposed by Atalla and VanderHart.[4,5]

basis of the electron diffraction analysis for cellulose from the green alga *Microdictyon tenuius*, although the one-chain triclinic crystals are assigned to cellulose I_α instead of the two-chain triclinic crystals described above. Moreover, they have also found that the triclinic and monoclinic phases are alternatively distributed with a period of about 100 nm along the long axis of the microfibrils with the width of 25–30 nm. This suggests that both phases may be produced in the same cellulose assembly at different stages in the process of crystallization. Since the triclinic crystals are transformed into the monoclinic crystals by annealing as mentioned above, the former may be crystallized in a higher energy state.

In order to understand the cause of the formation of the two crystal phases, we have started to examine the crystallization process of bacterial cellulose in vivo as a model system. A preliminary experiment[20] has revealed that xyloglucan and (carboxymethyl)cellulose sodium salt, which are assumed to control or reduce the size of fibrils of cellulose,[21,22] can significantly increase the fraction of the monoclinic crystals, when they are added in the media for the culture of *Acetobacter xylinum*. On the other hand, Kai et al.[23,24] have recently found that some kind of fluorescent brightener inhibits the crystallization of cellulose as a result of the formation of the cellulose–brightener complex, when the bacterial cellulose is cultured in the presence of the brightener. Moreover, cellulose I_β is preferentially regenerated from this complex by the selective extraction of the brightener without any drying. These results suggest that the crystallization of the two forms may occur in a certain period in the growing process of microfibrils or fibrils. More detailed discussion will be made somewhere after obtaining more information about the crystallization of bacterial cellulose in vivo.

Finally, the annealing effect on crystallinity should be briefly noted according to the result of the line-shape analysis shown in Table I. The crystallinity, which is estimated as $1 - f_{\mathrm{a}}$ using the averaged value f_{a} of the fractions of lines IV and X, is 0.90 for original *Valonia* cellulose, whereas this value slightly decreases to about 0.80 almost irrespective of annealing temperatures. This suggests that smaller crystallites may be disordered by the annealing, although the apparent shape of microfibrils has almost no change as confirmed by the electron microscopic observation.[14]

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