Thermally Induced Crystal Transformation from Cellulose I_α to I_β

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ABSTRACT: It is known that cellulose I_α is metastable and mostly converted into I_β phase by a heat treatment of 280 °C in an inert gas, helium. To elucidate the mechanism of this heat-induced crystal transformation from cellulose I_α to I_β, we measured X-Ray diffraction of the highly crystalline cellulose (I_α rich type) samples as a function of temperatures. In the heating process, d-spacings of equatorial reflections increased in line with the temperature, and furthermore the rate of change of the d-spacings increased considerably above 200 °C. This result indicated that 200 °C was the critical temperature for breaking intermolecular hydrogen bonds. Above that temperature, cellulose molecular chains became more flexible, inducing a thermal expansion of the crystal lattice, and it formed a transformation intermediate, a "high-temperature" structure. In the cooling process, new types of hydrogen bonds may form at 200 °C as a result of the transformation from I_α to I_β phase. This indicates that I_α transformed into I_β *via* the above expanded intermediate. The heat-induced expansion of the crystal lattice may be a trigger for the rearrangement of the hydrogen bonds, which may enhance the transformation from I_α to I_β.

KEY WORDS Cellulose I_α / Cellulose I_β / X-Ray Diffraction / Fourier Transform Infrared Spectroscopy (FT-IR) / *Cladophora* /

Cellulose is one of the most abundant crystalline biomacromolecules on earth. Despite a long history of research on the crystal structure, native cellulose was believed to be composed of a unique structure called cellulose I.[1] Recently the structure was revealed to be a composite of two crystalline phases, I_α and I_β, by using solid-state nuclear magnetic resonance (NMR).[2–4] Afterwards structures of cellulose I_α and I_β were assigned to the triclinic and monoclinic systems, respectively.[5] The two crystalline phase system has been extensively studied from the point of view of physicochemical and biological interests as well as crystallographic studies.[6] From the physicochemical standpoint of the two crystalline phases, the most striking phenomenon to emerge was that cellulose I_α is transformed into I_β by a hydrothermal treatment in an alkaline solution.[7,8] This result indicates that the I_β phase is thermodynamically more stable than the I_α phase. Furthermore, Debzi *et al.*[9] reported that most I_α phases could be easily converted into I_β in a helium gas atmosphere, whereas some residuals remained intact. As an explanation for the transformation,[10] a *break-slip* model is proposed based on the molecular dynamics simulation. However, experimental evidence to clarify the phenomenon is still required. In the present article, we tried to monitor the transformation from I_α to I_β in an inert atmosphere of helium, using X-Ray diffraction techniques. The results lead to the conclusion that a heat-induced expansion of the crystal lattice may be a trigger for the rearrangement of the hydrogen bonds, which may, in turn, enhance the transformation from I_α to I_β.

EXPERIMENTAL

Materials

The cellulose sample was prepared from the cell wall of a marine green alga *Cladophora* collected at a depth of about 2 m in the sea of Chiba, Japan. The sample was treated repeatedly in 5% KOH solution at room temperature and then in 0.3% $NaClO_2$ solution at 80 °C until it became perfectly white.[11] A purified sample was disintegrated into small fragments using a double cylinder-type homogenizer, and treated in 4N HCl solution at 80 °C for 4 h. The sample suspended in the solution was repeatedly washed by centrifugation with deionized water until the supernatant in the tube became turbid.[12] The turbid supernatant, in which cellulose microcrystals were suspended, was thoroughly dialyzed against deionized water, and freeze-dried. The dried sample was further vacuum-dried at 60 °C for 3 weeks.

X-Ray Diffraction under Heat Treatments[13]

X-Ray diffraction profiles were obtained using Ni-filtered Cu-$K\alpha$ radiation (λ = 0.15418 nm) produced by a Rigaku RINT 2200 X-Ray generator, equipped

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with a one dimensional Position Sensitive Proportional Counter (PSPC) as a detector. The sample, which was randomly oriented on thin film to avoid heterogeneous distribution of heat through surface to core, was irradiated at the desired temperature in the heating and cooling scans in an atmosphere of helium. After the diffraction pattern was recorded for the initial sample at room temperature, the sample was heated to the desired temperature at a rate of $5\,°C\,min^{-1}$ and kept there for 5 min to record the diffraction pattern. The sample in a heating scan was measured at each desired temperature from $100\,°C$ to $280\,°C$. After holding the final temperature for 15 min, the sample was then cooled down to follow the same measurement process as the above in the cooling scan.

The accurate d-spacings were extracted by fitting the X-Ray diffraction profiles into background and peak functions.[14, 15] The fifth-degree polynomial function was used for the amorphous background, and pseudo-Voigt function for crystalline reflections. The Levenberg–Marquardt algorithm of the nonlinear least squares method was used for the fitting.

FT-IR Spectroscopy

The sample was heated in an atmosphere of nitrogen from $150\,°C$ up to the desired temperature (from $250\,°C$ to $320\,°C$) at a rate of $10\,°C\,min^{-1}$. After holding the temperature for 5 min, the sample was cooled down to $150\,°C$ at a rate of $10\,°C\,min^{-1}$.

The samples before and after heat treatment were embedded in KBr pellets, and subjected to FT-IR measurements using a PerkinElmer Spectrum 2000 FT-IR spectroscopy. All spectra were recorded with an accumulation of 32 scans, resolution of $2\,cm^{-1}$, in the range from $4000\,cm^{-1}$ to $400\,cm^{-1}$, normalized using the COC band at $2900\,cm^{-1}$. The I_β fraction of the samples $f_{\beta IR}$ were calculated by the following equations, modified from the method reported by Yamamoto *et al.*:[16]

$f_{\beta IR} = 0.753 - 2.43\,A_\alpha\,/\,(A_\alpha + A_\beta)$,

$A_\alpha = A_{750\,cm^{-1}} - A_{800\,cm^{-1}}$,

$A_\beta = A_{710\,cm^{-1}} - A_{800\,cm^{-1}}$,

where A_n is absorbance at a corresponding wavenumber ($n\,cm^{-1}$ in the IR spectrum).

RESULTS AND DISCUSSION

X-Ray diffraction profiles of *Cladophora* cellulose under the heat treatment were recorded. In the X-Ray profiles of 6 crystalline reflections (Figure 1), equatorial reflections (peak 1, 2, and 4) shifted to smaller angles with increasing temperature, and then returned to the almost original position by cooling. They were
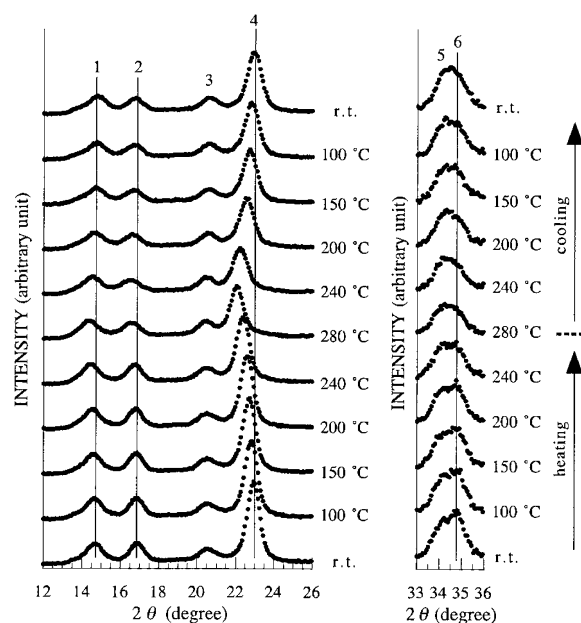

Figure 1. X-Ray diffraction profiles of *Cladophora* cellulose microcrystals at heating and cooling temperatures in an atmosphere of helium.

indexed from 2 crystalline phases I_α and I_β: peak 1, 010_{I_α} and $1\bar{1}0_{I_\beta}$; peak 2, 110_{I_β} and 100_{I_α}; peak 4, 110_{I_α} and 200_{I_β}. However, peak 6, which is assigned to the fiber repeat ($\bar{1}14_{I_\alpha}$ and 004_{I_β}, was not significantly changed by heating or cooling. These results indicate anisotropic thermal expansion behavior of cellulose crystals between normal and parallel to the fiber axis. The expansion was especially marked in the normal direction to the fiber axis, depending on changes in peaks 1, 2, and 4, but much less marked in the parallel direction with regard to changes in peak 6. d-Spacings d_1, d_2, and d_4,[17] which were calculated on the basis of positions for peaks 1, 2, and 4, increased with increasing temperature (Figure 2). In particular, the changes at above $200\,°C$ were considerably larger than those below. This phenomenon may be attributed to the breakdown of the intermolecular hydrogen bonding. In the cooling scan, the d-spacings decreased rapidly until the temperature reached about $200\,°C$, and then gradually decreased. When the sample was cooled down to room temperature, the d_4 returned to the same value as the initial one. In contrast, d_1 became smaller whereas d_2 was larger than the initial value of each. These changes in d-spacings by the thermal treatment are due to the transformation from I_α to I_β. Recently, a method to estimate the I_α/I_β ratio in a sample from the d-spacings, d_1 and d_2, was reported.[18] By modifying the method, we calculated the I_β fraction of the sample $f_{\beta X-Ray}$ using the following function:

$f_{\beta X-Ray} = -70.542\,d_1 + 37.583\,d_2 + 23.360$,

where d_1 and d_2 are nm in unit. The I_α/I_β ratio of the initial sample was $0.78/0.22$ ($d_1 = 0.611$ nm and $d_2 = $
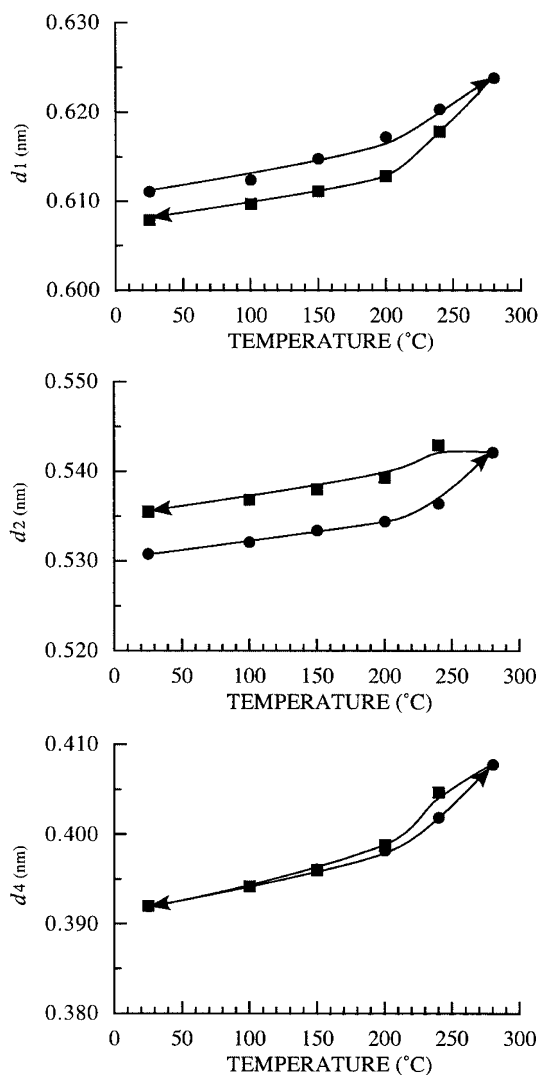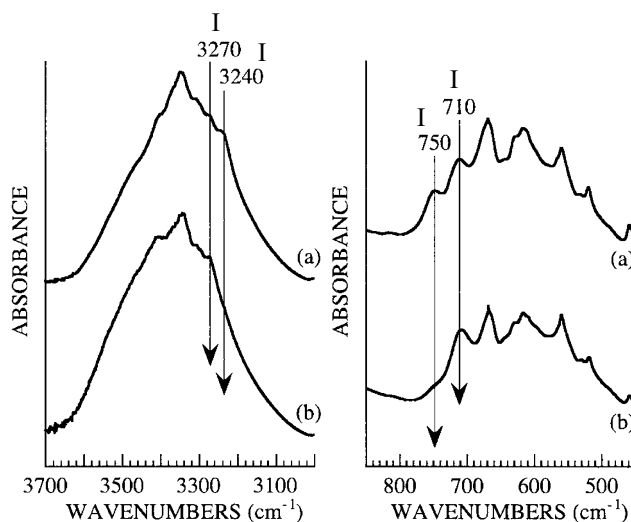
Figure 2. Variation of d-spacings for *Cladophora* cellulose microcrystals in the heating (●) and cooling (■) scans.

Figure 3. FT-IR spectra in the region 3700–3000 cm^{-1} and 900–400 cm^{-1} of *Cladophora* cellulose before (a) and after (b) heat treatment at 300 °C.

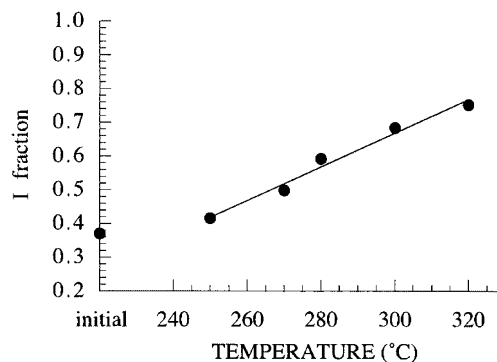**Figure 4.** The I_β fraction in the samples after the heat treatment.

0.531 nm), and it became 0.39/0.61 after the thermal treatment (d_1 = 0.608 nm and d_2 = 0.536 nm).

The selected regions of FT-IR spectra before (a) and after (b) heat treatment were shown in Figure 3. According to Sugiyama et al.,[11] bands at 3240 and 750 cm^{-1} were assigned to the I_α phase, whereas bands at 3270 and 710 cm^{-1} were assigned to the I_β phase. The bands at 3240 and 750 cm^{-1} and the bands at 3270 and 710 cm^{-1} respectively decreased and increased after heat treatment. This result indicated that the I_α/I_β ratio decreased by the thermal treatment in agreement with the result from X-Ray diffraction.

To obtain the converting ratio from I_α to I_β through the thermal history, we measured the I_β fraction in the samples produced after the heat treatments using the FT-IR method (Figure 4). The initial fractional ratio between I_α and I_β was 0.63/0.37 (I_α/I_β). When the sample was treated above 250 °C, transformed I_β fraction exhibited a linear relationship to the treated temperature. Namely, converting the ratio from I_α to I_β increased

with the elevating temperature. Even if the sample was treated at 320 °C, which is just below the thermal decomposition temperature of the highly crystalline celluloses, the I_α/I_β ratio was 0.25/0.75. Some I_α phase still remained intact. Thus repeated or longer treatments would be required to achieve complete transformation from I_α to I_β. To the best of our knowledge, the annealing on the sample in dilute NaOH solution is the most efficient treatment so far reported.[7–9] Polar mediums such as dilute alkali solution may interact with cellulose chains and make them flexible. However, the chains are not fully flexible in the thermally expanded state. Due to this rigidity of the chains, complete transformation from I_α to I_β would not be induced only by the thermal expansion in an inert gas atmosphere. Further investigation is required to elucidate the relationship between conditions of annealing and converting ratio from I_α to I_β.

How does the transformation from I_α to I_β occur? Considering the above results, we want to propose the "high-temperature" structure[19] as an intermediate for
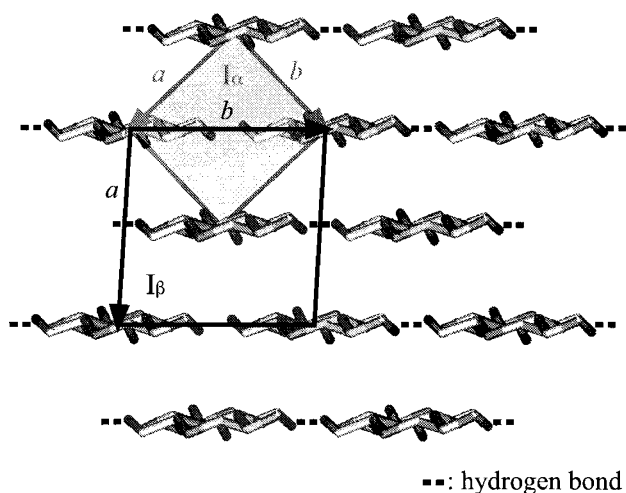
Figure 5. Schematic drawing of arrangements for cellulose chains in the plane perpendicular to chain axis. The gray and the black lines indicate the unit cell of I_α and I_β, respectively.[5] The c-axis of both unit cells corresponds upwards.
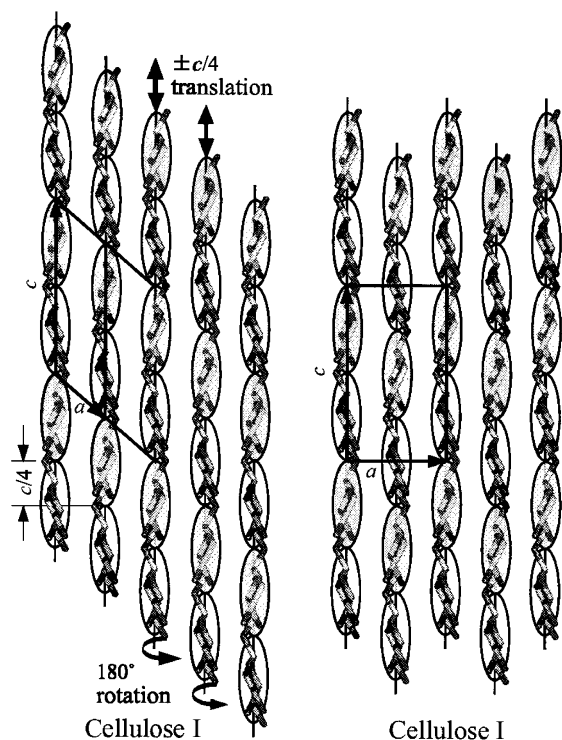
Figure 6. Two possible models for the transformation from cellulose I_α to I_β: rotation and translation models.

the transformation, where the crystal lattice is fully expanded by thermal treatments. We consider this phase transformation mechanism, using schematic drawing of molecule chains of the plane perpendicular to the chain axis (Figure 5). While the structure expanded slightly in the parallel direction to the glucose ring (b-axis of I_β lattice in Figure 5), 6% of the expansion in the perpendicular direction (a^*-axis of I_β lattice) compared with the structure at room temperature occurred. As cellulose I has intermolecular hydrogen bonds in the parallel direction to the glucose ring (b-axis of I_β lattice), but does not have them in the perpendicular direction where weak van der Waals forces exist,[20] these intermolecular hydrogen bonds should restrain the expansion in the parallel direction. There are two possible mechanisms for transformation from I_α to I_β; one is due to the rotation of 2 chains in Figure 6 (half of all chains) by 180 degrees along their chain axis, and the other is due to translation of these chains up or down by one glucose residue. In this transformation process, rearrangements of intermolecular hydrogen bonds are inevitable due to the different intermolecular hydrogen bonding patterns of I_α and I_β phases.[11] In other words the intermolecular hydrogen bonds should be canceled once in the expanded "high-temperature" structure. Based on the computer simulations, the *break-slip* model corresponding to the translation model was proposed for the transformation mechanism from I_α to I_β. Our experimental results also suggest the translation model because the small lattice expansion does not allow a 180 degrees rotation of the molecular chains. Further studies are still required, but here we want to insist that the thermally induced lattice expansion and attendant thermal vibration of molecular chains should be a trigger for the transformation from I_α to I_β.

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17. Throughout this study we used d_1, d_2, and d_4 to express d-spacings, because there are two indices for a diffraction peak corresponding to I_α and I_β phases: d_1: $(100)_{I_\alpha}$ and $(1\bar{1}0)_{I_\beta}$, d_2: $(010)_{I_\alpha}$ and $(110)_{I_\beta}$, and d_4: $(110)_{I_\alpha}$ and $(200)_{I_\beta}$.[5]

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