Characterization of Individual Hydrogen Bonds in Crystalline Regenerated Cellulose Using Resolved Polarized FTIR Spectra

Yukako Hishikawa,† Eiji Togawa,‡ and Tetsuo Kondo*†‡

†Forestry and Forest Products Research Institute (FFPRI), 1 Matsunosato, Tsukuba 305-8687, Ibaraki, Japan
‡Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, 6-10-1 Hakozaki, Fukuoka 812-8581, Japan

ABSTRACT: Cellulose nanofibers (CNFs), which are directly isolated as a native form, have drawn considerable attention as eco-friendly and distinctive material to be partly substituted for fossil products. In addition to the increasing attention to the native CNFs, conventional regenerated cellulose having cellulose II crystals also attracts more attention because of its cost-effective method of production in a moderately easy and repeatable fashion. Inter- and intramolecular hydrogen bonds are, in particular, thought to contribute greatly to the physical properties of cellulosic commercial products. More than half century ago, Marchessault et al. attempted to directly assign the hydroxyl (OH) group vibrations related to hydrogen bonding in infrared (IR) spectra. The assignment, however, has not been significantly updated. One reason for the delayed assignments is the difficulty in preparing pure cellulose II. Here, we show successful IR assignments of the interacted OH groups in cellulose II by using the nematic ordered cellulose to prepare a highly oriented regenerated film. The film had anisotropic crystalline domains, which provided a clearly resolved component in the IR spectra. The OH bands were well assigned, and this IR assignment becomes an effective tool to understand the structure–property relationship for engineering advanced regenerated cellulose materials.

INTRODUCTION

In the past decade, cellulose nanofibers (CNFs), which are less than 50 nm wide and over 100 in the aspect ratio, have drawn considerable attention as eco-friendly, novel, and distinctive material to be partly substituted for a variety of fossil products. CNFs are directly isolated as a native form from various widely available sustainable biomass resources, such as wood fibers and bacterial cellulose, without dissolving after chemical treatments. The character of CNFs leads to their use as reinforcement in composites, components for electronic displays, and CNF–polymer hybrid materials.1,2 In addition to the increasing attention to the native CNFs, regenerated cellulose, which has another crystalline phase and is prepared from the solution, has also a long history as commonly available film and fiber materials.3 Electrospun CNFs as one of the regenerated cellulosics has recently attracted more attention, as well as native CNFs, because of their cost-effective method of production in a moderately easy, repeatable, and simple fashion.4–6 Thus, it becomes further important to understand more detailed basic information on the structure–property relationship for engineering advanced regenerated cellulose materials.7,8

Inter- and intramolecular hydrogen-bonding interactions are, in particular, thought to contribute greatly to the physical properties of cellulosic commercial products. Many researchers have investigated the formation of hydrogen bonds in the crystalline phases of regenerated cellulose, termed cellulose II, using analytical methods, such as X-ray diffraction (XRD),9–17 neutron diffraction,16 and infrared (IR) spectroscopy.19–22 More than half century ago, Marchessault et al. attempted to directly assign hydroxyl (OH) group vibrations related to hydrogen bonding in IR spectra using polarized IR spectroscopy, mercerized ramie, and Fortisan 36. They interpreted OH bands at 3175, 3305, and 3350 cm−1 as intramolecular hydrogen bonds and OH bands at 3447 and 3488 cm−1 as intramolecular hydrogen bonds.20 The assignment established by Marchessault et al. has not been significantly changed since then. That is, the OH group vibrations have not been sufficiently elucidated, particularly when compared with IR vibrations of native cellulose crystals (cellulose I).21 One reason for the delayed assignments is the difficulty in preparing highly ordered cellulose II.

Our recent research proposed a new supramolecular structure of cellulose, which is noncrystalline but contains highly ordered β-glucan cellulose chains.24–27 We termed this structure nematic ordered cellulose (NOC). An NOC film could be transformed into uniaxially ordered cellulose II while maintaining the initial molecular orientation of NOC, when the film was mercerized in 17.5% aqueous sodium hydroxide (NaOH).28

Received: November 5, 2016
Accepted: March 30, 2017
Published: April 17, 2017

DOI: 10.1021/acsomega.6b00364
ACS Omega 2017, 2, 1469–1476
In the current study, we assign hydrogen-bonding vibrations in cellulose II crystalline phases from resolved polarized Fourier transform infrared (FTIR) spectra of the mercerized NOC film. The main difficulty in analyzing OH bands arises from the overlapping crystalline and noncrystalline spectral regions of cellulose samples. The use of vapor-phase deuteration and FTIR measurements enables the separation of molecular packing domains depending on the engaged states of OH groups to be investigated.\(^2^9\)\(^{−}\)\(^3^1\) We propose another method using vapor-phase deuteration and polarized FTIR to examine OH group orientation in the NOC film.\(^3^2\) These two vapor-phase deuteration methods allow the separation of OH group absorptions of crystalline regions from those of noncrystalline regions, in the IR spectra of cellulose samples. The IR spectrum reveals the OH band of cellulose II crystalline phases of the mercerized NOC film. The deconvolution of OH bands upon deuteration yields resolved OH bands of individual hydrogen bonds. Nondestructive IR spectroscopy is commonly used because of its easy handling of sampling and easy measurements. IR spectroscopy, moreover, has potential to become a powerful tool to provide specific information about the assignment of hydrogen-bond vibrations of crystalline cellulose II when it combines with a polarizer, mercerized NOC films, vapor-phase deuteration, and deconvolution of OH bands after vapor-phase deuteration.

**RESULTS**

Wide-angle XRD (WAXD) patterns (Figure 1) of the mercerized film in the equatorial and meridional directions show that the ordered supramolecular NOC structure was converted to the oriented cellulose II allomorph upon alkaline treatment. The equatorial and meridional profiles of the NOC film are typical of those expected for a cellulose II crystalline structure.\(^2^8\),\(^3^3\) The degree of crystallinity of the treated NOC film was 44.3%, which indicated that the film contained more than 50% noncrystalline regions. The film had been deuterated for 10 days, and the exchange reaction did not almost proceed in the last 72 h. Then, the hydrogen bonds were assigned using the remained OH band due to OH groups in cellulose II in IR spectra. Therefore, the interpretation of the hydrogen bonds in cellulose II was not probably affected by the noncrystalline regions. The crystalline orientation factor was estimated at 92% from the azimuthal WAXD pattern at the (004) plane.

Figure 2 shows that the intensity of the OH absorption band in the nonpolarized IR spectrum decreased and became less saturated, following vapor-phase deuteration (cf. Figure 2a,b). This suggests that the available OH groups in the noncrystalline regions of the mercerized NOC were converted to OD groups using vapor-phase deuteration,\(^2^9\),\(^3^2\) as stated in the Experimental Section, accounting for the decreased absorption of OH band (Figure 2b). Figure 2b shows the OH band obtained after deuteration and derived from the OH groups in the crystalline regions of the mercerized NOC. The polarized IR spectrum after deuteration indicates that the β-1,4-glucan chains of cellulose were highly oriented because of the profound difference between the parallel (∥) and perpendicular (⊥) spectra in Figure 2c. Moreover, the dichroic ratio, which indicates the orientation of the β-1,4-glucan chains, was calculated in the same manner as our previous report.\(^3^2\) The dichroic ratio (R) is a relative value of the absorbance of the

![Figure 1. WAXD data of the mercerized NOC film. Upper left: WAXD image; upper right: crystallinity and crystalline orientation factors; lower left: equatorial WAXD profiles before and after mercerization; lower right: meridional WAXD profile before and after mercerization.](image-url)
perpendicular spectra to the absorbance of the parallel spectra. A value giving $R < 1.0$ represents a preferentially parallel orientation, whereas a value showing $R > 1.0$ indicates a favorable perpendicular orientation. The calculated $R$ was 0.17, indicating that $\beta$-1,4-glucan chains contained in the mercerized NOC film were highly oriented to the stretching direction.

Parallel and perpendicular OH bands obtained after vapor-phase deuteration were deconvoluted into nine individual OH bands in total (Figures 3 and 4) using GRAMS 386 “CurveFit” analysis, as described in the Experimental Section and the same manner of our previous report.

The OH band parallel to the stretching direction was deconvoluted into four OH bands (Figure 3). The two OH stretching vibrations at 3491 and 3447 cm$^{-1}$ were likely due to intramolecular hydrogen bonds, preferentially oriented along the long axis of the $\beta$-1,4-glucan chains. The small band at 3329 cm$^{-1}$ in Figure 3 is possibly attributed to an intermolecular hydrogen bond slightly parallel to the $\beta$-1,4-glucan chains. Details of small bands are described later.

The OH band perpendicular to the stretching direction was deconvoluted into five OH bands (Figure 4). Stretching vibrations at 3353, 3276, and 3162 cm$^{-1}$ are supposed to correspond to the intermolecular hydrogen bonds engaged in $\beta$-1,4-glucan chains. The small perpendicular bands centered at 3496 and 3448 cm$^{-1}$ may be due to the perpendicular components of the transition moments of the two intramolecular hydrogen bonds at 3491 and 3447 cm$^{-1}$ in the parallel spectrum of Figure 3. These two hydrogen bonds were largely (but not exactly) aligned along the long axis of the $\beta$-1,4-glucan chains. A more detailed study is necessary to further investigate these two small bands.

Force constants of OH groups related to the magnitude of intra- and intermolecular hydrogen bonds were calculated from the obtained wavenumbers, assuming the OH group to be a...
diatomic molecule. Details are available in our previous report. Force constants of OH groups engaged in intramolecular hydrogen bonds were calculated to be 680 and 663 N/m for the 3491 and 3447 cm\(^{-1}\) bands, respectively. OH force constants for intermolecular hydrogen bonds were 627, 599, and 558 N/m for bands at 3353, 3276, and 3162 cm\(^{-1}\), respectively. The magnitude of force constants represents the strength of bonding between the hydroxyl O and H atoms. A stronger O\(\cdots\)H covalent bond corresponds to a weaker hydrogen bonding due to the longer distance between O and H atoms in the engagement. Thus, a larger force constant indicates a longer hydrogen bond. The calculated force constants suggest that the length of the above two intramolecular hydrogen bonds was higher than that of the intermolecular hydrogen bonds.

**DISCUSSION**

The assignment of the five predominant OH absorption bands in the resolved parallel and perpendicular spectra is similar to recent analyses of the regenerated cellulose II crystalline allomorph using X-ray and neutron diffraction. The proposed hydrogen-bonding scheme differs from that of earlier studies based on XRD. The scheme does not contain intramolecular hydrogen bonds between the C-2 OH group (OH group at the C-2 position) and the C-6 OH group of the adjacent glucose ring, as is typical for cellulose I. The C-3 OH group is involved in a three-centered intramolecular hydrogen bond. Predominant bonding occurs between the C-3\(_{oc}\), OH group and the C-5\(_{oc}\), O atom of the adjacent glucose ring. Here, “origin” and “center” chains are denoted as “o” and “c”, respectively. Conventional “up” and “down” chains are referred to as origin and center chains, respectively. Minor bonding arises between the C-3\(_{oc}\), OH group and the C-6\(_{oc}\), OH group of the adjacent glucose ring. The O\(\cdots\)H distance of the latter is longer than that of the former. Four major intermolecular bonds are formed: (1) between the C-2, OH group and the neighboring C-2, O atom; (2) between the C-6, OH group and the C-6, O atom of the glucose ring of the adjacent \(\beta\)-1,4-glucan chain; (3) between the C-2, OH group and the neighboring C-6, O atom; and (4) between the C-6, OH group and the neighboring C-2, O atom. The lengths of these four bonds are in the range expected for intermolecular hydrogen bonding. Specifically, the distances between donor and acceptor atoms for each of the above intermolecular bonds (from Langan et al.) are 2.783, 2.643, 2.713, and 2.682 Å, respectively, and the distances between the hydrogen and the acceptor atom are 2.212, 2.015, 1.817, and 1.784 Å, respectively.

Deconvoluted bands were assigned on the basis of three factors: OH group force constant, cellulose II hydrogen-bonding scheme, and O\(\cdots\)H distance of the above-mentioned intra- and intermolecular hydrogen bonds. Of the two bands in the spectrum recorded parallel to the stretching direction (Figure 3), the band at 3491 cm\(^{-1}\) is probably assigned to the intramolecular bonding between the C-3\(_{oc}\), OH group and the C-6\(_{oc}\), OH group of the adjacent glucose ring (between D3 and O6 in Figures 5–7). The larger force constant indicates a longer bond length. The band at 3447 cm\(^{-1}\) is likely attributed to the intramolecular bonding between the C-3\(_{oc}\), OH group and the C-5\(_{oc}\), O atom of the adjacent glucose ring (between D3 and O5 in Figures 5–7). A further investigation, however, is necessary to assign appropriately one of the two bands to one of the two types of intramolecular bonding because the C-3 OH group is involved in a three-centered intramolecular hydrogen bond and one cannot separate definitely in IR spectra the bond between the C-3\(_{oc}\), OH group and the C-5\(_{oc}\), O atom and the bond between the C-3\(_{oc}\), OH group and the C-6\(_{oc}\), OH group.

The three predominant OH bands in the spectrum recorded perpendicular to the stretching direction (Figure 4) at (1) 3353, (2) 3276, and (3) 3162 cm\(^{-1}\) are attributed to the intermolecular bonding (1) between the C-2, OH group and the C-2, O atom (between D2 and O2 in Figure 5) and/or between the C-6, OH group and the C-6, O atom (between D6 and O6 in Figure 5), (2) between the C-2, OH group and the C-6, O atom (between D2 and O6 in Figure 7), and (3) between the C-6, OH group and the C-2, O atom (between D6 and O2 in Figure 6), respectively. Intermolecular hydrogen...
bonds were assigned on the basis of larger force constants indicating a longer hydrogen bond. Of these three bands, those at 3353 and 3162 cm$^{-1}$ were calculated to have the largest and smallest force constants, respectively, and thus were attributed to the longest and shortest bonds, respectively. Accurate hydrogen bond lengths were obtained from Langan et al., as already described.$^{18}$

Concerning the OH band at 3353 cm$^{-1}$ in the perpendicular spectrum, it is hard to assign it appropriately because the formation of hydrogen bonds is intricate in the sheet containing both origin and center chains. In the sheet, there are two major types of intermolecular hydrogen bonding between the C-2$_c$ OH group and the C-2$_o$ O atom and between the C-6$_o$ OH group and the C-6$_c$ O atom,$^{18}$ as mentioned above. The C-6$_o$ OH groups are engaged in four-centered intermolecular hydrogen bonding,$^{18}$ which produces possibly, except for the major bonding, two minor bonding between the C-6$_o$ OH group and the C-5$_c$ O atom and between the C-6$_o$ OH group and the C-3$_c$ O atom.$^{18,35}$ An explanation for this arrangement is that bonding between the C-6$_o$ OH group of origin chains and the neighboring C-6$_o$ O atom of center chains forms when the latter O atom is in a gauche–trans (gt) conformation.$^{35}$ Hydrogen bonding between the C-6$_o$ OH group and the C-3$_c$ O atom forms when the neighboring C-6$_c$ O atom is in a trans–gauche (tg) conformation.$^{35}$ The C-6$_c$ O atom of center chains in regenerated cellulose was reported to be up to 30% in a tg conformation and 70% in a gt conformation.$^{35}$ On the other hand, neither the C-2$_c$ OH group nor the C-2$_o$ OH group is involved in the four-centered intermolecular hydrogen bonding, which possibly indicates that more numbers of hydrogen bonds between the C-2$_c$ OH group and C-2$_o$ O atom are involved in our samples. Therefore, the OH band at 3353 cm$^{-1}$ could be due to the bonding between the C-2$_c$ OH group and the C-2$_o$ O atom. As to the parallel bands at 3450 and 3329 cm$^{-1}$ (Figure 3), it might be attributed to the complicated formation

Table 1. Predominant Hydrogen Bonds IR Absorption Assignments for the Cellulose II Allomorph

| Wave-numbers (cm$^{-1}$) | Type of H. B.$^{a,b}$ | Distance of H. B.$^{a,b}$ (Å) | The F. C.$^{a}$ of the OH group (N/m) |
|-------------------------|----------------------|-------------------------------|----------------------------------|
| The parallel bands ($l$) | C-3OH – C-6O$^c$ (intramolecular H. B.$^c$) | 2.8030$^d$, 2.500$^d$ | Large 680 |
|                         | C-3OH – C-5O$^c$ (intramolecular H. B.$^c$) | 1.9180$^d$, 1.848$^d$ | Large 663 |
| The perpendicular bands ($l$) | C-2OH – C-2O$^c$ (intramolecular H. B.$^c$ AND/OR) | 2.212 | 627 |
|                         | C-6OH – C-6O$^c$ (intramolecular H. B.$^c$) | 1.817 | 599 |
|                         | C-2OH – C-6O$^c$ (intramolecular H. B.$^c$) | 1.817 | 599 |
|                         | C-6OH – C-2O$^c$ (intramolecular H. B.$^c$) | 1.784 | 558 |

$^a$H. B. represents “hydrogen bond” and F. C. is “force constant”. $^b$According to Langan et al.$^{18}$ $^c$A further investigation is necessary to assign each intramolecular hydrogen bond accurately. $^d$Origin and center chains are denoted as o and c, respectively. Conventional up and down chains are referred to as origin and center chains, respectively.$^{18}$
of the hydrogen bonding between center and origin chains. A more detailed study is needed to obtain further information on the hydrogen bonding in the center—origin sheet and the corresponding IR bands.

Table 1 shows the five major OH absorption bands obtained by deconvolution, assigned to specific hydrogen-bonding engagements. Assignments are made according to the band type (parallel or perpendicular) and ordered by bond length evaluated from the magnitude of the OH force constant. For example, the OH band at 3491 cm$^{-1}$, which was assigned to the intramolecular hydrogen bond, is listed on the top of the table because the hydrogen bond is the longest$^{18}$ and its force constant has to be the largest of the five predominant OH bands.

The length of the hydrogen bond of the 3447 cm$^{-1}$ band is shorter than that of the 3353 cm$^{-1}$ band, although the force constant of the former is larger than that of the latter. The OH band at 3353 cm$^{-1}$ corresponds to the intermolecular hydrogen bonds between origin chains and center chains in this study. Therefore, this phenomenon occurs specifically in the sheet containing both origin and center chains. In the sheet, there are two three-centered (bifurcated)-type intramolecular hydrogen bonds and three four-centered-type and one two-centered (normal)-type intermolecular hydrogen bonds, as illustrated in Figure 5. The C-6 OH group of the origin chain is especially related to the intramolecular hydrogen bonds as well as the intermolecular hydrogen bonds. The O atom is involved in one of the two intramolecular hydrogen bonds, and the H atom is involved in three intermolecular hydrogen bonds. The complicated hydrogen bonding, including one two-centered (normal)-type intermolecular hydrogen bond, could affect the value of the force constant of the 3353 cm$^{-1}$ band, which caused the phenomenon that a smaller value of the force constant corresponds to longer hydrogen bonds.

### CONCLUSIONS

A pure cellulose molecule consists of only three kinds of atoms, carbon, oxygen, and hydrogen, which form a straight-chain composed of six-membered glucose rings having OH groups. The structure of the molecule is simple enough because the molecule is not associated with long branched chains or bulky functional groups. IR bands of most OH groups in cellulose I crystals have been assigned by Marčêch and Chanzy.$^{23}$ Such assignments are of interest, as these bands are thought to control the physical properties of native cellulose materials. An entire assignment of IR bands of the cellulose polymorph has not been reported although the molecular structure of cellulose is simple. More information is required especially on OH absorption bands and hydrogen bonding in cellulose II because of its widespread use in eco-friendly regenerated cellulose films and fibers. A regenerated cellulose allomorph, cellulose II, has been recently classified in terms of three-dimensional coordinates, including those of hydrogen-bond patterns. The current study characterizes the hydrogen bonding of an oriented cellulose II film, prepared by facile mercerization of NOC$^{24-28}$ that is highly noncrystalline but has highly ordered β-glucan chains. Vapor-phase deuteration of the available hydroxyl groups of cellulose II allowed the corresponding functional groups of crystalline domains to be investigated by polarized FTIR measurements. IR absorption bands due to stretching vibrations were deconvoluted and clearly resolved into individual component vibrations. Accordingly, absorption bands of OH groups in cellulose II were capable of being assigned, and this technique is an effective tool for characterizing hydrogen bonding in regenerated cellulose materials that are widely used as commodity chemicals as well as novel cellulosic products.

Our study assigns hydrogen bonds of ordered cellulose II with highly ordered β-glucan chains across the entire film, using vapor-phase deuteration and polarized FTIR as follows: Polarized infrared OH absorption bands of crystalline phases after the vapor-phase deuteration were deconvoluted into five predominant bands. The bands at 3491 and 3447 cm$^{-1}$ in the spectrum recorded parallel to stretching were assigned to intramolecular hydrogen bonds because these bonds preferentially form along the direction of the long axis of β-1,4-glucan chains. The remaining bands at 3353, 3276, and 3162 cm$^{-1}$ in the spectrum recorded perpendicular to stretching were assigned to the intermolecular hydrogen bonds between β-1,4-glucan chains. The interpretation of the OH absorption bands was almost similar to that reported by Marchessault et al.$^{20}$ They demonstrated that there were two types of OH bands related to intramolecular hydrogen bonds and three types of OH bands related to intermolecular hydrogen bonds$^{20}$ in cellulose II, as stated above. The distinguished point from our present claim is that they assigned the two OH bands in parallel spectra to the intramolecular hydrogen bonds formed only between the C-3 OH group and the C-5 O atom of the adjacent glucose ring because they predicted two different parallel bands derived from two types of the C-3 O atom: (1) the C-3 O atom involved in both intra- and intermolecular hydrogen bonds and (2) the C-3 O atom of the adjacent glucose ring not involved in the intermolecular hydrogen bond.$^{20}$ The obtained bands were, moreover, assigned by considering the force constants of OH groups forming intra- and intermolecular hydrogen bonds and the hydrogen bond lengths from a reported proposed scheme for cellulose II.$^{18}$ Combining vapor-phase deuteration and polarized FTIR spectroscopy allows the characterization of hydrogen bonds in regenerated cellulose II or crystalline phases in regenerated cellulose. Thus, our IR data in this study compensate for the assignment to cellulose II, which contributes with the published cellulose I and II IR data$^{19-23}$ to analyze cellulosic products that are widely used as commodity materials or produce novel cellulosic materials such as derivatives for medical use and nanofibers for additive substances from biomass.

### EXPERIMENTAL SECTION

#### Materials.

The starting material was bleached cotton linters with a degree of polymerization of 1300, which was dried under vacuum at 40 °C before use. N,N-Dimethylacetamide (DMAC) of >99% purity (Katayama Chemicals Co. Ltd., Osaka, Japan) was dehydrated with 3 Å molecular sieves and used without further purification. Lithium chloride (LiCl) powder (Katayama Chemicals Co. Ltd.) was oven-dried for 3 days at 105 °C. NaOH and deuterium oxide of purity >99.75% were purchased from Wako Pure Chemical Industries Ltd. (Tokyo, Japan).

#### Mercerization of the NOC Film.

The NOC film was prepared as previously described$^{24,25,26,28,29}$ and then dried under vacuum at 40 °C. The crystallinity index of this film was estimated to be 16.3% from density measurements. The NOC film was mercerized as described previously.$^{26}$ In brief, the fixed-state NOC film mounted on a stretching device was soaked in 17.5% aqueous NaOH at room temperature for several hours, followed by washing with water. The sample was air-dried while retaining sample tension and then dried under vacuum at 40 °C.
Measurements. The mercerized NOC film was mounted in a purpose-made IR-measurement cell suitable for vapor-phase deuteration.\textsuperscript{29–32} Polared FTIR spectra after deuteration were collected using a PerkinElmer Spectrum 2000 FTIR spectrometer, with the light electric vector parallel and perpendicular to the stretching direction. These spectra were obtained over the 4000–850 cm\textsuperscript{-1} spectral region from 32 scans at 2 cm\textsuperscript{-1} resolution using a DTGS detector. The film sample was thoroughly dried under vacuum at 50 °C before vapor-phase deuteration to remove residual water.

Spectra were recorded before and after the vapor-phase deuteration process, that is, when the OH−OD exchange reaction with D\textsubscript{2}O molecules reached an equilibrium state. As the exchange reaction proceeded, OH groups contained in the noncrystalline regions of the film were gradually exchanged to OD groups. Accordingly, the intensity of the OH absorption band was decreased, and instead the OD band in spectra appeared and increased, similar to the phenomenon we reported previously.\textsuperscript{29–32} The IR spectra following the vapor-phase deuteration were then capable of providing the OH band remained in the crystalline regions of the mercerized NOC film, which allowed to analyze the hydrogen bonding in cellulose II crystalline phases.

Deconvolution of OH Bands Parallel and Perpendicular to the Stretching Direction Obtained after Vapor-Phase Deuteration. Curve fitting for the peak deconvolution was performed by GRAMS 386 CurveFit analysis (Galactic Industries Corp., Salem, NH). The true shape of the peak involved was assumed to be Lorentzian. The number of peaks involved was reported previously.\textsuperscript{29–32} The IR spectra following the vapor-phase deuteration were then capable of providing the OH band remained in the crystalline regions of the mercerized NOC film, which allowed to analyze the hydrogen bonding in cellulose II crystalline phases.

Schematic Representation of the Hydrogen Bonding of β-1,4-Glucan Chains in Cellulose II. We used data on the hydrogen bonding of β-1,4-glucan chains in cellulose II reported by Langan et al.\textsuperscript{18} They deuterated cellulose II crystalline samples to "replace the six independent H atoms involved in hydrogen bonding to six deuterium atoms, without any loss of crystalline perfection" for high-resolution neutron fiber diffraction patterns.\textsuperscript{18} It should be noted that our aim, by using vapor-phase deuteration, focusing on the removal of OH band contained in noncrystalline regions is totally different from that of Langan et al.

Schematic representation of the hydrogen bonding in cellulose II based on the data by Langan et al.\textsuperscript{18} was made using VESTA: a three-dimensional visualization program for electronic and structural analysis.\textsuperscript{36} To interpret hydrogen bonding in cellulose, a three-dimensional visualization program for structural analysis.\textsuperscript{36} The number of peaks involved was reported previously.\textsuperscript{29–32} They deuterated cellulose II crystalline samples to replace the six independent H atoms involved in hydrogen bonding to six deuterium atoms, without any loss of crystalline perfection for high-resolution neutron fiber diffraction patterns.\textsuperscript{18} It should be noted that our aim, by using vapor-phase deuteration, focusing on the removal of OH band contained in noncrystalline regions is totally different from that of Langan et al.

Schematic representation of the hydrogen bonding in cellulose II based on the data by Langan et al.\textsuperscript{18} was made using VESTA: a three-dimensional visualization program for electronic and structural analysis.\textsuperscript{36} To interpret hydrogen bonding in cellulose II we consulted the report by Langan et al.\textsuperscript{18} which discussed hydrogen bonding in regenerated cellulose II crystalline structures. There are two distinct processes (regeneration and mercerization) for converting crystalline cellulose I to cellulose II.\textsuperscript{18} The molecular assembly states in regenerated cellulose are similar to those in mercerized cellulose; however, Langan et al.\textsuperscript{18} suggested the conformation of center-chain hydroxymethyl groups (defined as down chains) in regenerated cellulose differs from that in mercerized cellulose. Mercurization involves swelling cellulose I crystals, whereas regenerated cellulose II is prepared from either cellulose solution or its derivatives.\textsuperscript{35} Our cellulose film basically obtained from a DMAC/LiCl cellulose solution\textsuperscript{4,25,28,32} is considered to be a regenerated cellulose. Therefore, the conformation of hydroxymethyl groups in regenerated cellulose II\textsuperscript{18} is adopted in the current study to assign hydrogen bonds, referring to the distance between oxygen (O) and hydrogen (H) atoms in hydrogen-bonding interactions.
(14) Blackwell, J.; Kolpak, F. J.; Gardner, K. H. The Structures of Celluloses I and II. *Tappi* 1978, 61, 71–72.

(15) Geßler, K.; Krauß, N.; Steiner, T.; Betzel, C.; Sandmann, C.; Saenger, W. Crystal Structure of β-D-Cellotetraose Hemihydrate with Implications for the Structure of Cellulose II. *Science* 1994, 266, 1027–1029.

(16) Geßler, K.; Krauss, N.; Steiner, T.; Betzel, C.; Sarko, A.; Saenger, W. β-D-Cellotetraose Hemihydrate as a Structural Model for Cellulose II. An X-ray Diffraction Study. *J. Am. Chem. Soc.* 1995, 117, 11397–11406.

(17) Raymond, S.; Heyraud, A.; Tran Qui, D.; Krick, À.; Chanzy, H. Crystal and Molecular Structure of β-D-Cellotetraose Hemihydrate as a Model of Cellulose II. *Macromolecules* 1995, 28, 2096–2100.

(18) Langan, P.; Nishiyama, Y.; Chanzy, H. A Revised Structure and Hydrogen-Bonding System in Cellulose II from a Neutron Fiber Diffraction Analysis. *J. Am. Chem. Soc.* 1999, 121, 9940–9946.

(19) Brown, R. M., Jr., Saxena, I. M., Eds.; Springer: Dordrecht, The Netherlands, 2007; pp 285–339.

(20) Marchessault, R. H.; Liang, C. Y. Infrared Spectra of Crystalline Polysaccharides. III. Mercerized Cellulose. *J. Polym. Sci., Part B: Polym. Phys.* 1995, 33, 173–183.

(21) Blackwell, J.; Marchessault, R. H. *Cellulose and Cellulose Derivatives Part IV*, 2nd ed.; Bikales, N. M., Segal, L., Eds.; Wiley-Interscience: New York, 1971; pp 1–37.

(22) Šturcová, A.; His, I.; Wess, T. J.; Cameron, G.; Jarvis, M. C. Polarized Vibrational Spectroscopy of Fiber Polymers: Hydrogen Bonding in Cellulose II. *Biomacromolecules* 2003, 4, 1589–1595.

(23) Maréchal, Y.; Chanzy, H. The Hydrogen Bond Network in Iα Cellulose as Observed by Infrared Spectrometry. *J. Mol. Struct.* 2000, 523, 183–196.

(24) Togawa, E.; Kondo, T. Change of Morphological Properties in Drawing Water-Swollen Cellulose Films Prepared from Organic Solutions. A View of Molecular Orientation in the Drawing Process. *J. Polym. Sci., Part B: Polym. Phys.* 1999, 37, 451–459.

(25) Togawa, E.; Brown, R. M., Jr.; Togawa, E. "Nematic Ordered Cellulose": A Concept of Glucan Chain Association. *Biomacromolecules* 2001, 2, 1324–1330.

(26) Togawa, E.; Kondo, T. *Cellulose: Molecular and Structural Biology Selected Articles on the Synthesis, Structure, and Applications of Cellulose*, 1st ed.; Brown, R. M., Jr., Saxena, I. M., Eds.; Springer: Dordrecht, The Netherlands, 2007; pp 285–305.

(27) Togawa, E.; Kondo, T. *Bacterial Cellulose: A Sophisticated Multifunctional Material*; Gama, M., Klemm, D., Gatenholm, P., Eds.; CRC Press: Florida, 2012; pp 113–142.

(28) Togawa, E.; Kondo, T. Unique Structural Characteristics of Nematic Ordered Cellulose - Stability in Water and Its Facile Transformation. *J. Polym. Sci., Part B: Polym. Phys.* 2007, 45, 2850–2859.

(29) Hishikawa, Y.; Togawa, E.; Kataoka, Y.; Kondo, T. Characterization of Amorphous Domains in Cellulosic Materials Using a FTIR Deuteration Monitoring Analysis. *Polymer* 1999, 40, 7117–7124.

(30) Hishikawa, Y.; Inoue, S.-I.; Magoshi, J.; Togawa, E.; Kondo, T. A Novel Tool for Characterization of Noncrystalline Regions in Cellulose: A FTIR Deuteration Monitoring and Generalized Two-Dimensional Correlation Spectroscopy. *Biomacromolecules* 2005, 6, 2468–2473.

(31) Kondo, T.; Kataoka, Y.; Hishikawa, Y. *Cellulose Derivatives*, 1st ed.; Heinz, T. J., Glasser, W. G., Eds.; ACS Symposium Series; American Chemical Society: Washington, DC, 1998; Vol. 688, pp 173–183.

(32) Hishikawa, Y.; Togawa, E.; Kondo, T. Molecular Orientation in the Nematic Ordered Cellulose Film Using Polarized FTIR Accompanied with a Vapor-phase Deuteration Method. *Cellulose* 2010, 17, 539–545.

(33) Nishino, T.; Takano, K.; Nakamae, K. Elastic Modulus of the Crystalline Regions of Cellulose Polymorphs. *J. Polym. Sci., Part B: Polym. Phys.* 1995, 33, 1647–1651.

(34) Togawa, E.; Kondo, T. The assignment of IR absorption bands due to free hydroxyl groups in cellulose. *Cellulose* 1997, 4, 281–292.