Temperature-Dependent Changes in Hydrogen Bonds in Cellulose Iα Studied by Infrared Spectroscopy in Combination with Perturbation-Correlation Moving-Window Two-Dimensional Correlation Spectroscopy: Comparison with Cellulose I*β*

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Our recent IR study demonstrated that hydrogen-bond structure in cellulose Iβ drastically changes around 220 °C (Watanabe et al. *Biomacromolecules* **2006**, *7*, 3164). In the present study, temperature-dependent IR spectra of cellulose Iα from 30 to 260 °C were analyzed by use of perturbation-correlation moving-window two-dimensional correlation spectroscopy. It was observed that as in the case of cellulose Iβ abrupt changes in the hydrogen-bond structure occur around 220 °C in cellulose Iα. It was also revealed that although weakly hydrogen-bonded OH groups in Iβ are stable below 230 °C thermal oxidation of those in Iα is accelerated around 220 °C. In this way, the present study has clarified a difference between the thermal behavior of Iα and that of Iβ at the functional group level. Our result suggests that the drastic change in the hydrogen-bond structure around 220 °C makes cellulose Iα much more unstable than Iβ.

Introduction

Cellulose, a linear 1,4-β-glucan (Figure 1), is one of the most abundant biopolymers on the earth.[1−3] It is biosynthesized not only in higher plants but also in other living systems such as bacteria and algae.[1−3] According to the literature,[1−10] native cellulose, so-called cellulose I, has two different intrachain hydrogen bonds (a O3−H3···O5 intrachain hydrogen bond and a O2−H2···O6 intrachain hydrogen bond) and possesses an interchain hydrogen bond (O6−H6···O3′ interchain hydrogen bond). It has been considered that these intrachain hydrogen bonds are concerned with the single-chain conformation and the stiffness of cellulose, while the interchain hydrogen bond is responsible for the sheetlike nature of cellulose.[1−4]

Recently, Atalla and Van der Hart[11,12] have shown from the results obtained by the solid-state ^{13}C cross-polarization with magic-angle spinning NMR spectroscopy that native cellulose exists in two crystalline forms, Iα and Iβ. The ratio of the two crystalline phases depends on the origin.[3,11−13] Celluloses produced by primitive organisms (bacteria, algae, etc.) are predominantly of the Iα phase, whereas celluloses of higher plants (wood, cotton, ramie, etc.) consist mainly of the Iβ phase. On the basis of an electron diffraction study, Sugiyama et al. have revealed that cellulose Iα has a triclinic unit cell containing one chain, while cellulose Iβ has a monoclinic unit cell containing two parallel chains.[14] One of the most notable phenomena of the two crystalline phase system of native cellulose is that cellulose Iα is transformed into Iβ by a hydrothermal treatment at 260 °C in an alkaline solution.[15,16] Although some of the Iα phase remains intact, the transition also takes place in an inert gas atmosphere above 260 °C.[17] On the basis of an X-ray diffraction study,[18] Wada has shown that a phase transition from cellulose Iβ to a high-temperature phase occurs around 220−230 °C. After the study, Wada et al.[19] have revealed that the increase in *d*-spacings of *Cladophora* cellulose (Iα-rich type) with temperature is accelerated above 200 °C and that converting the ratio from Iα to Iβ increases above 250 °C. From these results, they have proposed that the high-temperature structure is an intermediate for the Iα → Iβ transition.[19] However, the mechanism of this phenomenon has not sufficiently been elucidated.

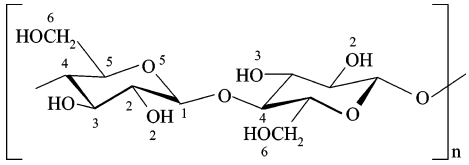

Figure 1. Structure of cellulose.

Infrared spectroscopy has widely been employed to study the structure of cellulose.[4,10,20−29] One of the advantages of IR spectroscopy in cellulose research is that an IR spectrum in the O−H stretching vibration region can directly give information about the hydrogen-bond structure. Our research group has shown that temperature-dependent IR spectroscopy is a useful tool for investigating thermal behavior of the hydrogen bonds in native celluloses.[26,27,29] In one of these studies, the study on the thermal behavior of cellulose Iβ,[29] we have revealed that drastic changes in the hydrogen-bond structure of cellulose Iβ take place around 220 °C and that they induce the phase transition of cellulose Iβ proposed by Wada.[18] In the present study, we explore the thermal behavior of the hydrogen bonds in cellulose Iα by temperature-dependent IR spectroscopy. The cellulose was prepared from cell walls of *Glaucocystis nostochinearum*, which is considered to be essentially cellulose Iα.[30] Since the thermal behavior of cellulose Iα has not been clarified sufficiently at the functional group level, information about

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temperature-dependent changes in the hydrogen bonds in cellulose Iα may be a clue for understanding the mechanism of the Iα → Iβ transition.

Bands due to the O−H stretching vibration modes, which are located in the 3700−3000 cm^{-1} region, are considered as the most useful bands for exploring the hydrogen-bond structure of cellulose.[4,10,20,22−24,26−29] However, it is not straightforward to monitor spectral variations in this region from a large number of IR spectra of cellulose collected directly under a particular external perturbation (here, temperature) because many bands arising from OH groups with various strength of hydrogen bonds heavily overlap with each other, and the overlapping bands often shift with the perturbation. We have solved the above problems by using perturbation-correlation moving-window two-dimensional (PCMW2D) correlation spectroscopy.[27,29,31] This method is derived from generalized two-dimensional correlation spectroscopy (2DCOS)[32−34] and moving-window two-dimensional (MW2D) correlation spectroscopy.[35] The PCMW2D correlation analysis is expressed as a pair of synchronous and asynchronous 2D correlation spectra plotted on a plane between a spectral variable (here, wavenumber) axis and a perturbation variable (here, temperature) axis. According to the study by Morita et al.,[31] the synchronous Π_Φ and asynchronous Π_Ψ PCMW2D correlation spectra are proportional to the first perturbation derivative and the opposite sign of the perturbation second derivative, respectively

$$\Pi_\Phi(\upsilon,T) \sim \left(\frac{\partial y(\upsilon,T)}{\partial T}\right)_\upsilon \quad (1)$$

$$\Pi_\Psi(\upsilon,T) \sim -\left(\frac{\partial^2 y(\upsilon,T)}{\partial T^2}\right)_\upsilon \quad (2)$$

where y is the spectral intensity as function of wavenumber υ and temperature T. These features of the PCMW2D correlation analysis described above make it possible to extract both a specific perturbation (e.g., melting temperature) and a characteristic spectral variable (e.g., corresponding band position) from complicated spectral variations induced by a perturbation[27,29,31]

The purpose of the present study is to investigate the thermal behavior of the hydrogen bonds in cellulose Iα by temperature-dependent IR spectroscopy. To unravel the complicated spectral variations in the O−H stretching region, the PCMW2D correlation analysis is utilized. Furthermore, in the present article, we have compared the results obtained from the temperature-dependent IR spectra of cellulose Iα with those of cellulose Iβ to investigate a difference in the stability of the structure between them especially in the temperature range of 210−230 °C. The reason for the existence of two kinds of crystalline phases in nature is a central issue.[3,13] It has been considered that the ratio of cellulose Iα to Iβ in organisms is correlated with evolution.[3,13,36] An elucidation of the difference in the thermal behavior of the hydrogen bonds between cellulose Iα and Iβ may contribute to clarification of the process of the evolution of various living systems.

Experimental Section

Preparation of a Cellulose Film.[18,19,22,25] *Glaucocystis nostochinearum* was obtained from the Institute of Applied Microbiology Culture Collection, The University of Tokyo, and it was cultivated in Murashige and Skoog medium (MS medium)[37] for 7 months. Its cellulose was purified by immersion in a 5% (w/v) KOH aqueous solution for 12 h at room temperature and then treated with a 0.3% (w/v) $NaClO_2$ aqueous solution buffered at pH 4.9 with an acetate buffer at 70 °C for 3 h. After the sample had become completely white by the above procedure, it was hydrolyzed by 50% (w/w) sulfuric acid at 50 °C for 8 h to make a suspension of cellulose microcrystals. The solid content of the sample after the hydrolysis was collected by centrifugation at 600*g*, and then it was diluted with deionized water. These procedures were repeated until the supernatant in the tube became turbid. This turbid supernatant contained microcrystals of cellulose Iα. The turbid supernatant was collected and then neutralized by a 1% (w/v) NaOH aqueous solution (cellulose suspension). The suspension was dialyzed against deionized water. A few drops of the cellulose suspension was placed onto a ZnSe plate and then dried at 40 °C. A film of cellulose Iα prepared on the ZnSe plate was carefully washed by deionized water and then dried under the purge of dried nitrogen gas at room temperature for 12 h. An IR spectrum of the purified sample in the 3600−3100 and 800−500 cm^{-1} regions almost corresponded to that in the literature.[22,25,30]

Methods. The cellulose film was prepared on a CaF_2 plate by the same procedure described above, and then it was placed into a homemade cell equipped with a temperature controller for transmittance IR measurements. The temperature-dependent IR spectra were measured under the purge of dried nitrogen gas. All of the IR spectra were obtained at a resolution of 2 cm^{-1} with a NEXUS 870 FT-IR spectrometer (Thermo Nicolet) equipped with a deuterated tryglycine sulfate (DTGS) detector, and 128 scans were coadded for each spectrum. A series of transmission IR spectra was collected from 30 to 260 °C at 1 °C increments at a rate of ca. 0.2 °C/min. That is, 231 spectra were obtained. All of the IR spectra are given in an absorbance unit defined as $-\log(I/I_0)$, where I and I_0 are the intensities of signals from the sample on the CaF_2 plate at a given temperature and the CaF_2 plate without the sample at 30 °C, respectively. The second derivative spectra were calculated by the Savitzky-Golay method[38] using homemade software after the spectra were subjected to Kawata-Minami smoothing.[39] This smoothing was useful for the calculation of the second derivative spectra, especially in the high-temperature range where the signal-to-noise ratio becomes low. Before the calculation of the PCMW2D correlation spectra, all of the spectra were subjected to smoothing and baseline correction in the wavenumber region of 3900−2000 cm^{-1}. In the present study, the PCMW2D correlation spectra were calculated by homemade software named "2Dshige" (http://sci-tech.ksc.kwansei.ac.jp/~ozaki/).[31] The mathematical procedure and numerical computation of the PCMW2D correlation spectra are described in detail elsewhere.[31] The basic concepts of the analytical method and rules of the interpretation for the PCMW2D correlation spectra are explained also in our previous report.[29]

Results and Discussion

O−H Stretching Region of Cellulose Iα. Figure 2a shows temperature-dependent IR spectra in the 3600−3100 cm^{-1} region of cellulose Iα. Only 17 spectra from 30 to 260 °C are shown here. Bands observed in this region are assigned to the stretching modes of OH groups of cellulose.[10,20,22−24,26−29] Two pronounced peaks are observed at 3349 and 3240 cm^{-1} in the spectrum at 30 °C. The intensities of these peaks decrease, and the peaks shift to a higher wavenumber with temperature. These observations reflect temperature-dependent variations in the hydrogen-bond structure of cellulose Iα.

Second derivative spectra of the temperature-dependent IR spectra (3600−3100 cm^{-1}) are shown in Figure 2b. The wavenumbers in the figure represent peak positions in the spectrum at 30 °C. At least seven peaks are identified in the second derivative spectra. By using polarized IR spectra, Liang and Marchessault assigned a band at 3350 cm^{-1} observed in an IR spectrum of bacterial cellulose,[20] which has been considered to be rich in cellulose Iα,[1−3] to the O3−H3···O5 intrachain

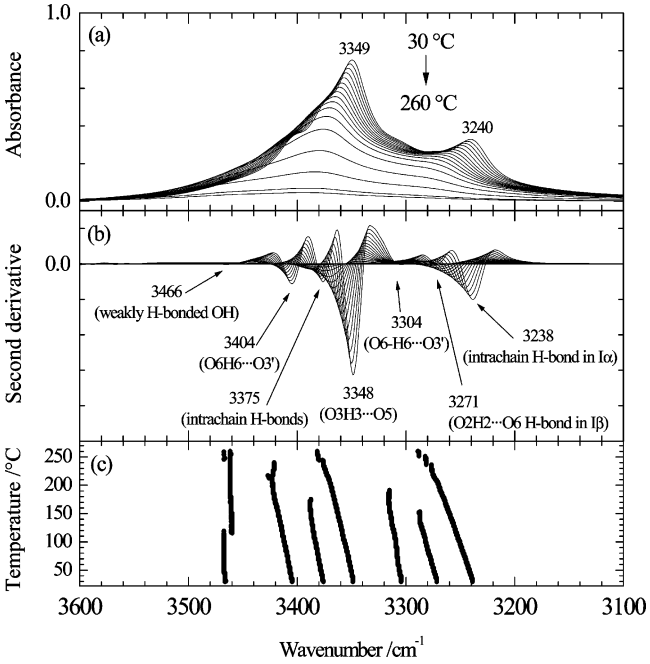

Figure 2. (a) Temperature-dependent IR spectra in the 3600−3100 cm^{-1} region of cellulose Iα (30−260 °C). Only representative spectra (17 spectra) are shown in the figure. (b) Second derivative spectra of the spectra shown in panel a. (c) Peak positions as a function of temperature determined by the local minimum of the second derivative spectra.

hydrogen bonds. In the same study, they correlated bands at 3404 and 3304 cm^{-1} to the interchain hydrogen bonds. Other research groups also assigned a band around 3350−3340 cm^{-1} to the O3−H3···O5 intrachain hydrogen bonds.[10,26] In the IR study by Sugiyama et al.,[22] the transition dipole moments of the bands at 3404 and 3304 cm^{-1} observed in IR spectra of Iα-rich celluloses such as bacterial and algae celluloses are perpendicular to the axis of the cellulose chain. Their results also suggested that these bands are due to the OH groups forming the interchain hydrogen bonds because the interchain hydrogen bonds are formed almost perpendicularly to the cellulose chain.[1−10] Although it was a study on cellulose Iβ, Maréchal and Chanzy proposed that bands at 3410 and 3305 cm^{-1} are due to the O6−H6···O3′ interchain hydrogen bonds.[10] Therefore, it is very likely that the features at 3404 and 3304 cm^{-1} in the second derivative spectra (Figure 2b) are correlated with the O6−H6···O3′ interchain hydrogen bonds. A peak at 3238 cm^{-1} was assigned to the hydrogen bonds constructed only in the cellulose Iα phase by Sugiyama et al.[22] This peak is not observed in an IR spectrum of cellulose Iβ.[10,22,29] According to the IR studies not only by Sugiyama et al.[22] but also by Liang and Marchessault,[20] transition dipole moments of the bands at 3375 and 3240 cm^{-1} are parallel to the axis of the cellulose chain. Since the intrachain hydrogen bonds are constructed almost parallel to the cellulose chain,[1−10] the bands at 3375 and 3240 cm^{-1} are probably due to the intrachain hydrogen bonds. Especially, the band at 3240 cm^{-1} is attributed to the intrachain hydrogen bonds existing only in cellulose Iα.[22] A weak peak at 3466 cm^{-1} is assigned to the OH groups with weak hydrogen bonds. (This hydrogen-bond structure has also not been revealed.[10]) A feature at 3271 cm^{-1} is correlated with the O2−H2···O6 intrachain hydrogen bonds, which exist only in cellulose Iβ.[22] Thus, cellulose Iβ is present slightly in the cell walls of *Glaucocystis nostochinearum*.

Figure 2c plots the peak positions as a function of temperature determined from local minima of the second derivative spectra.

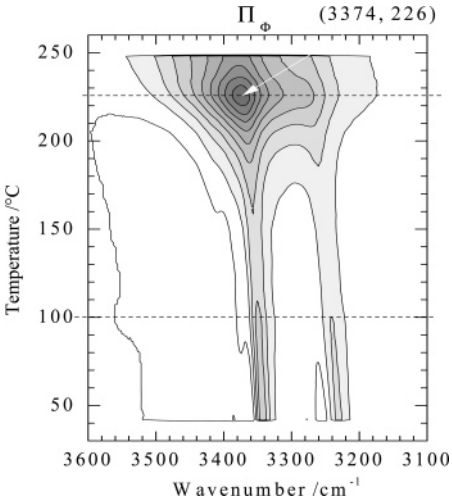

Figure 3. Synchronous PCMW2D correlation spectrum in the 3600−3100 cm^{-1} region calculated from the temperature-dependent IR spectra of cellulose Iα measured over a temperature range of 30−260 °C.

The peaks except for the peak at 3466 cm^{-1} show a shift to a higher wavenumber with temperature. These shifts reflect the fact that the inter- and intrachain hydrogen bonds become weak with temperature. The shifts of the peaks at 3348 and 3238 cm^{-1}, which are assigned to the intrachain hydrogen bonds, seem to become larger above 220 °C. The change in the peak position at 3466 cm^{-1} above 240 °C is not continuous. This is because the raw spectra around 3470 cm^{-1} become broad above the temperature. Although second derivative analysis provides information about changes in the strength of the hydrogen bonds, it cannot always elucidate intensity changes of observed bands. It is expected that the raw spectra in the O−H stretching vibration region (Figure 2a) contain much information about temperature-dependent changes in the hydrogen-bond structure of cellulose Iα. However, the spectra become broad in the high-temperature range, and many OH bands due to the OH groups with different strength of hydrogen bonds are heavily overlapping. Therefore, it is difficult to extract detailed information about structural changes from the spectra directly. As in the case of our previous study on the thermal behavior of the hydrogen bonds in cellulose Iβ,[29] PCMW2D correlation spectroscopy was utilized for analysis of the temperature-dependent IR spectra of cellulose Iα.

PCMW2D Correlation Analysis. Figure 3 shows a synchronous PCMW2D correlation spectrum constructed from the temperature-dependent IR spectra in the region of 3600−3100 cm^{-1} of cellulose Iα. A window size of 25 °C was applied. Positive and negative correlation areas are represented by white and gray, respectively. In the temperature range of 30−200 °C, two negative correlation valleys are observed in the 3390−3320 and 3270−3200 cm^{-1} regions. A negative PCMW2D correlation intensity corresponds to a decrease in the observed spectral intensity along temperature (eq 1). Therefore, these negative correlation valleys elucidate that the intensities of the bands in the 3390−3320 and 3270−3200 cm^{-1} regions decrease in the temperature range of 30−200 °C. The bands presented in the 3390−3320 and 3270−3200 cm^{-1} regions are assigned to the O3−H3···O5 intrachain hydrogen bonds and the intrachain hydrogen bonds in the Iα phase, respectively (Figure 2b). This result reveals that structural changes in these intrachain hydrogen bonds are significant in this temperature range. These two negative correlation valleys shift to a high wavenumber with temperature, reflecting that the intrachain hydrogen bonds are

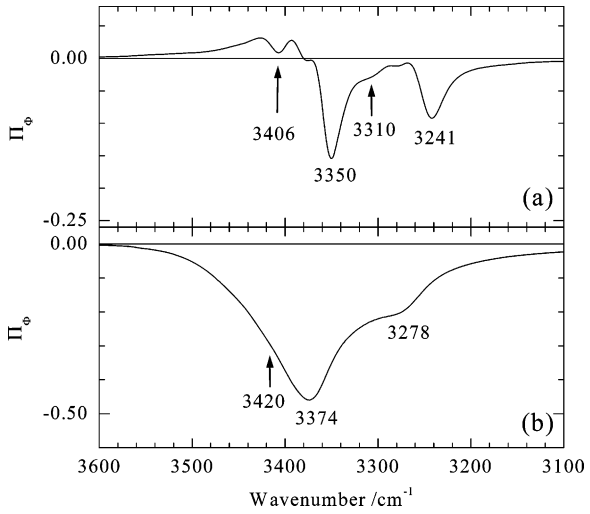

Figure 4. Slice spectrum extracted from the synchronous PCMW2D correlation spectrum in Figure 3 at (a) 100 and (b) 226 °C.

gradually weakened with temperature. Figure 4a shows a slice spectrum at 100 °C. The slice point is given in Figure 3 by a dashed line. A dip at 3406 cm^{-1} and a negative peak at 3310 cm^{-1} are correlated with the O6−H6···O3′ interchain hydrogen bonds. Thus, the structural changes in the interchain hydrogen bonds also occur with temperature. An intensity of the PCMW2D correlation does not respond directly to an intensity of the corresponding IR absorption (eq 1). The intensities of the bands at 3304 and 3240 cm^{-1} are in the same level in the IR spectrum at 30 °C (Figure 2a), whereas the negative PCMW2D correlation intensity of the band at 3241 cm^{-1} is much larger than that of the band at 3310 cm^{-1}.

An intense negative correlation peak at Π_Φ (3374 cm^{-1}, 226 °C) is identified in the synchronous PCMW2D correlation spectrum. Since a synchronous PCMW2D correlation spectrum is proportional to a spectral gradient along temperature, the negative correlation peak indicates that the slope of the decrease in the intensity of the band at 3374 cm^{-1} becomes the maximum at 226 °C. The band at 3374 cm^{-1} at 226 °C is assigned to the O3−H3···O5 intrachain hydrogen bonds (Figure 2b). Therefore, this negative correlation peak elucidates that the slope of the structural change in the intrachain hydrogen bonds becomes the maximum at the temperature. Figure 4b depicts a slice spectrum at 226 °C extracted from the synchronous PCMW2D correlation spectrum in Figure 3. In the slice spectrum, shoulders at 3420 and 3278 cm^{-1}are observed. Although it is difficult to recognize the former shoulder, this peak can be detected by the second derivative of this slice spectrum. (The spectrum is not shown.) The positions of these peaks correspond to those of second derivative peaks attributed to the intrachain hydrogen bonds in the Iα phase and the O6−H6···O3′ interchain hydrogen bonds at 226 °C, respectively (Figure 2b). Therefore, not only the structural change in the O3−H3···O5 intrachain hydrogen bonds but also that in the intrachain hydrogen bonds in the Iα phase and the O6−H6···O3′ interchain hydrogen bonds are important at 226 °C. In the synchronous PCMW2D correlation spectrum of cellulose Iβ, a strong negative peak is identified at Π_Φ (3361 cm^{-1}, 221 °C).[29] These observations suggest that the response of the O3−H3···O5 intrachain hydrogen bonds in cellulose Iα to temperature is very similar to that of cellulose Iβ. In the slice spectrum at 221 °C extracted from the synchronous PCMW2D correlation spectrum of cellulose Iβ, the synchronous PCMW2D correlation intensity of the band due to the interchain hydrogen bonds is around zero.[29] It has been suggested that the structural changes in the interchain hydrogen bonds in cellulose Iβ are competed below 200 °C.[29] The temperature-dependent change in the interchain hydrogen bonds in cellulose Iα may be different from that of cellulose Iβ. It can be seen from Figure 3 that the intensity change in the band at 3240 cm^{-1}, attributed to the intrachain hydrogen bonds constructed only in the Iα phase,[22] shows synchronicity with that in the band due to the O3−H3···O5 intrachain hydrogen bonds. In the previous IR study on cellulose Iβ,[29] it has been revealed that the intensity of the band at 3270 cm^{-1}, assigned to the O2−H2···O6 intrachain hydrogen bonds formed only in the Iβ phase[22] decreases with the decrease in the intensity of the band of the O3−H3···O5 intrachain hydrogen bonds. These observations imply that the band at 3240 cm^{-1} is correlated with the O2−H2···O6 intrachain hydrogen bonds in cellulose Iα.

In the 3500−3400 cm^{-1} region, a broad, positive PCMW2D correlation intensity is observed. This result indicates that the intensities of the bands around this region increase with temperature. Since the intensities of the bands due to the inter- and intrachain hydrogen bonds decrease with temperature, the increase in the intensity in the region of 3500−3400 cm^{-1} suggests that a weak hydrogen-bond structure is constructed by the structural changes in the inter- and intrachain hydrogen bonds with temperature. In our previous study,[29] a strong positive peak at Π_Φ (3470 cm^{-1}, 221 °C) is observed in the synchronous PCMW2D correlation spectrum of cellulose Iβ. However, it can be seen from Figure 3 that the synchronous correlation intensities around 3470 cm^{-1} show negative correlation above 210 °C and that there is no positive correlation peak. The band around 3470 cm^{-1} is assigned to the OH groups with weak hydrogen bonds (Figure 2b). This result indicates that the response of the OH groups in cellulose Iα to temperature is different from that of cellulose Iβ. It should be noted that the PCMW2D correlation analysis can easily provide a difference between temperature-dependent changes in the weakly hydrogen-bonded OH groups in cellulose Iα and those in Iβ from spectral variation around 3470 cm^{-1} where the peak poison cannot be identified in the raw spectra. In later subsections, the difference between the thermal behavior of cellulose Iα and that of cellulose Iβ will be discussed in detail.

Comparison of the Thermal Behavior of Hydrogen Bonds in Cellulose Iα with That in Iβ. In the previous subsection, it has been shown from the synchronous PCMW2D correlation spectrum that the decrease in the intensity of the band due to the O3−H3···O5 intrachain hydrogen bonds represents a decrease in the hydrogen-bond structure with temperature (Figure 3). On the contrary, the increase in the intensity of the band around 3500−3400 cm^{-1} provides information about the OH groups with hydrogen bonds formed by heating. It has been revealed that the intensity changes in the band attributed to the intrachain hydrogen bonds and that around 3470 cm^{-1} are important indices for exploring structural changes in the crystal of cellulose Iβ.[29] To discuss differences in temperature-dependent structural changes between cellulose Iα and cellulose Iβ, we compared the intensity changes in these bands of cellulose Iα with those of cellulose Iβ.

Figure 5a plots absorbance (y_1) of the band observed around the 3400−3340 cm^{-1} region due to the O3−H3···O5 intrachain hydrogen bonds as a function of temperature. Open circles and closed circles represent the absorbance of the band of cellulose Iα and that of Iβ, respectively. The intensities of these OH bands of cellulose Iβ are those obtained in our previous study.[29] Since the OH band due to the O3−H3···O5 intrachain hydrogen bonds (in both Iα and Iβ) shifts to a higher wavenumber with temperature, the band positions for the plots correspond to the

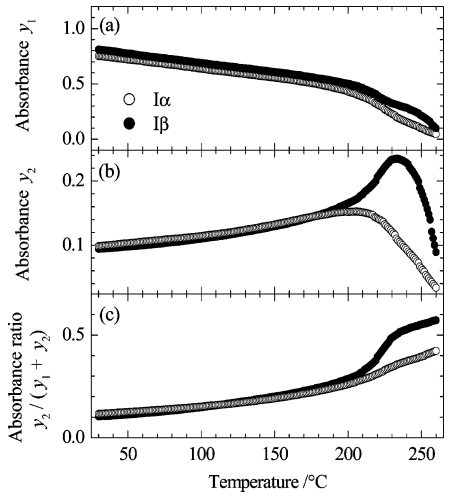

Figure 5. Temperature-dependent variations in absorbance of the typical OH bands due to (a) the intrachain O3−H3···O5 hydrogen bonds (the band positions for the plots correspond to the peaks in the raw spectra at each temperature) and (b) the OH groups with weak hydrogen bonds (fixed wavenumbers of 3466 and 3470 cm^{-1} are selected for the plots of cellulose Iα and Iβ, respectively) for cellulose Iα (○) and Iβ (●) as a function of temperature. (c) Ratio of absorbances of the latter bands of cellulose Iα (○) and Iβ (●) as a function of temperature. The ratio is given as $y_2/(y_1 + y_2)$, where y_1 and y_2 are absorbances of the bands due to the intrachain O3−H3···O5 hydrogen bonds and the OH groups with weak hydrogen bonds, respectively.

peak in the observed spectra at each temperature. It can be seen from Figure 5a that the absorbance of both bands of Iα and Iβ linearly decreases in the temperature range of 30−200 °C, whereas the slopes of absorbance decreases become significantly larger above 220 °C. The maximum slopes of the bands of Iα and Iβ have been seen at 226 (Figure 3) and 221 °C,[29] respectively, from the PCMW2D correlation analysis. There is no important difference between the intensity change in the band due to the intrachain hydrogen bonds of cellulose Iα and that of Iβ, indicating that the response of the intrachain hydrogen bonds of cellulose Iα to temperature is almost the same as that of Iβ. On the basis of the calculation of the strain energy distribution of native cellulose by Tashiro et al.,[40] the intrachain hydrogen bonds suppress torsional deformation of the flexible β-1,4-glycosidic bonds. We have suggested that the increase in the flexibility of β-1,4-glycosidic bonds induces the drastic rupture of the intrachain hydrogen bonds in cellulose Iβ.[29] It is likely that the β-1,4-glycosidic bonds and the intrachain hydrogen bonds in cellulose Iα as well as Iβ affect each other in the heating process.

Figure 5b plots absorbance (y_2) of the band at 3466 cm^{-1} (Iα) and that of the band at 3470 cm^{-1} (Iβ) as a function of temperature. Both bands are assigned to the OH groups with weak hydrogen bonds. The wavenumbers for the plots are fixed because the second derivative peaks of these bands do not show a shift. The intensities of the bands around 3470 cm^{-1} of cellulose Iα and Iβ gradually increase in the temperature range of 30−200 °C, whereas their intensity changes are significantly different above 200 °C. In cellulose Iβ, the intensity of the band, reflecting the formation of weak hydrogen bonds, drastically increases in the temperature range of 210−230 °C and decreases above 234 °C. However, in cellulose Iα, the decrease in the intensity of the band starts above 207 °C. This result indicates that the thermal behavior of the OH groups with weak hydrogen bonds formed with temperature in cellulose Iα is very much different from that of cellulose Iβ above 200 °C.

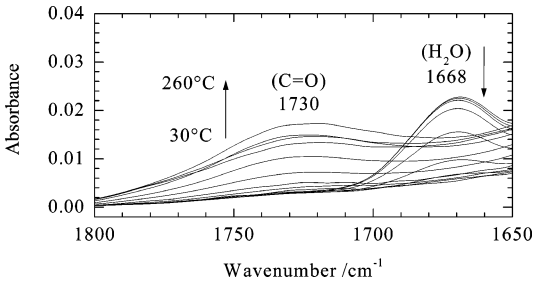

Figure 6. Temperature-dependent IR spectra in the 1800−1650 cm^{-1} region of cellulose Iα (30−260 °C). Only representative spectra (17 spectra) are shown in the figure.

Temperature-dependent variations in the absorbance ratio of the band due to the OH groups with weak hydrogen bonds are plotted for both cellulose Iα and Iβ in Figure 5c. The ratio is calculated as $y_2/(y_1 + y_2)$. This ratio is considered to be a rough index for the ratio of weak hydrogen-bond structure in the respective cellulose crystals. Below 200 °C, the ratio of both cellulose Iα and Iβ gradually increases, and it is almost the same for them. In the temperature range of 210−230 °C, the ratio of cellulose Iβ rapidly increases, whereas the increase in the ratio of cellulose Iα is not so pronounced. Above 230 °C, the slopes of the increase in the ratio are similar. It should be noted that the remarkable difference in the slope of the increasing ratio between cellulose Iα and Iβ is observed only in the temperature range of 210−230 °C. This result suggests that the response of cellulose Iα to temperature and that of cellulose Iβ are significantly different in this temperature range where drastic changes in the hydrogen-bond structure take place.

On the basis of an X-ray diffraction study, Wada[18] has shown that a phase transition of cellulose Iβ prepared from the mantle of tunicate occurs above 220−230 °C. The drastic intensity changes in the selected OH bands in the temperature range of 210−230 °C of cellulose Iβ elucidate the existence of the transition (Figure 5a).[29] It is suggested that the intense negative correlation peak at Π_F (3374 cm^{-1}, 226 °C) in Figure 3 also indicates the phase transition of cellulose Iα. The difference between the intensity change in cellulose Iα and that in Iβ described above suggests that the stability of weakly hydrogen-bonded OH groups in cellulose Iα is different from that of cellulose Iβ in the heating process.

Comparison of the Thermal Oxidation of Cellulose Iα with That of Iβ. Figure 6 shows temperature-dependent IR spectra in the 1800−1650 cm^{-1} region of cellulose Iα. Only 17 spectra from 30 to 260 °C are shown here. A peak observed around the 1750−1700 cm^{-1} region is assigned to the C═O stretching mode. According to the literature,[41] a spectral variation in the region of 1750−1700 cm^{-1} is a good index for the investigation of the thermal oxidation of cellulose. The intensity of a band at 1730 cm^{-1} increases with temperature, indicating that the thermal oxidation of cellulose Iα takes place with temperature. Figure 7 plots absorbance of the C═O stretching band as a function of temperature. The open circles and closed circles represent the absorbance of the band of cellulose Iα and that of Iβ, respectively. The wavenumber positions at 1730 and 1733 cm^{-1}, respectively, of cellulose Iα and Iβ are used for the plots. The C═O stretching band increases above 180 °C in cellulose Iα, whereas the intensity increase starts above 230 °C in cellulose Iβ. These results indicate that the initiating temperature of thermal oxidation is much lower for cellulose Iα than for cellulose Iβ. Both in cellulose Iα and Iβ, the ratio of absorbance of the bands due to the OH groups with weak hydrogen bonds increases in all of the temperature ranges investigated (Figure 5c). This result indicates that the

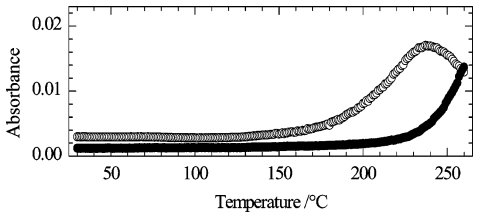

Figure 7. Absorbance of the C═O bands of cellulose Iα (○) and Iβ (●) as a function of temperature. The wavenumber positions at 1730 and 1733 cm^{-1}, respectively, of cellulose Iα and Iβ are used for the plots.

structural changes from the strong hydrogen bonds to the weak hydrogen bonds in both celluloses continue until 260 °C. Therefore, the decrease in the intensity of the band around 3470 cm^{-1}, assigned to the OH groups with weak hydrogen bonds (Figure 5b), suggests that the rate of the structural change from the OH groups with weak hydrogen bonds to the C═O group becomes faster than that of the change from the strong hydrogen bonds to the weak hydrogen bonds. It is obvious from Figure 5b that the temperature where the intensity of the band of the weakly hydrogen-bonded OH groups decreases is lower in cellulose Iα than in Iβ. From these observations, it is suggested that the weak hydrogen-bond structures in cellulose Iα are much more unstable than those in Iβ. The intensity increase in the C═O band in cellulose Iα is accelerated above 200 °C (Figure 7). The PCMW2D correlation analysis in this region reveals that the slope of the increase in the C═O band shows a maximum at 218 °C. (The result is not shown.) However, the intensity increase in the C═O band of cellulose Iβ is not observed around 220 °C. The slope of the absorbance ratio of the weakly hydrogen-bonded OH group in cellulose Iα (Figure 5c) shows a significant difference from that in cellulose Iβ only in the temperature range of 210−230 °C. These results lead us to conclude that the hydrogen-bond structure of cellulose Iα become less stable than that of cellulose Iβ, especially in the temperature range of 210−230 °C. This may be because the unstable hydrogen bonds are rapidly constructed in cellulose Iα by drastic changes in the hydrogen-bond structure in this temperature range.

According to the literature,[13,41] the formation of ketones at C2 and C3 and the change from a hydroxymethyl group to an aldehyde or carboxyl are considered as possible processes of cellulose oxidation; cleavage at the C2−C3 glycol may partially occur with the oxidation. It is likely that rapid structural change in the intrachain hydrogen bonds makes the C2−C3 glycol structure in cellulose Iα unstable, and as a result the formation of ketones at the C2 and C3 positions is accelerated. It is suggested that the intrachain hydrogen bonds in cellulose Iα contribute to depress the oxidation of C2−O2−H2 and C3−O3−H3 more than those of cellulose Iβ. As described above, β-1,4-glycosidic bonds become flexible with temperature; our results indicate that the increase in the flexibility of β-1,4-glycosidic bonds, structural changes in the intrachain hydrogen bonds, and the oxidation of C2−O2−H2 and C3−O3−H3 are closely linked in the heating process of cellulose Iα. It is also probable that the interchain hydrogen bonds in cellulose Iα also prevent oxidation of the hydroxymethyl group.

In Figure 6, a water deformation band is observed at 1668 cm^{-1}, indicating that a very slight amount of water exists in cellulose Iα.[41] The band disappears around 180 °C. This water cannot be removed by low-temperature drying. In the IR spectrum of cellulose Iβ, this water band is not identified. In our previous IR study on the drying process of microcrystalline cellulose,[27] it has been suggested that evaporation of adsorbed water disturbs the hydrogen-bond structure. The decrease in the intensities of the intrachain hydrogen bonds in cellulose Iα are accelerated above 200 °C. In Figure 5a, the acceleration is slightly faster than that in cellulose Iβ. These observations imply that a loss of water is responsible for the acceleration of the structural changes in the intrachain hydrogen bonds in cellulose Iα.

It is well-known that cellulose Iα transforms into Iβ by hydrothermal treatment in an alkaline solution at 260 °C.[15,16] Although the transition also takes place in an inert gas atmosphere above 260 °C, some Iα phase remains intact in this condition.[17] It has been considered that annealing in a NaOH solution is the most efficient treatment for the transition.[17] It has been confirmed from the raw spectra after cooling that the Iα → Iβ transformations do not arise in the present heating conditions. It has been reported that the NaOH solution plays a key role for preventing the thermal degradation of native cellulose.[42] The hydrothermal treatment with alkaline solution may be a key factor for stabilization of the weak hydrogen-bond structure of cellulose Iα in the temperature range of 210−230 °C. In the cooling process, a reversible formation of strong hydrogen bonds is observed below 200 °C. However, it is difficult to interpret spectral variations in the temperature range of 260−200 °C because a change from weak hydrogen bonds to strong hydrogen bonds and thermal oxidation arise at the same time. (The result is not shown.)

Conclusion

The present study has aimed to explore the thermal behavior of the hydrogen bonds in cellulose Iα by using temperature-dependent IR spectra. To extract useful information about the response of the hydrogen bonds to temperature from complicated spectral variations in the OH stretching region, PCMW2D correlation spectroscopy has been applied for analysis of the spectra. The PCMW2D correlation analysis has revealed that drastic changes in the hydrogen-bond structure of cellulose Iα as well as cellulose Iβ take place around 220 °C. It also clarified that the intensity changes in the bands due to the O3−H3···O5 intrachain hydrogen bonds and OH groups with weak hydrogen bonds are good indices for comparing the thermal behavior of cellulose Iα with that of cellulose Iβ. On the basis of the comparison of the intensity changes in these characteristic OH bands and the C═O band of cellulose Iα with those of Iβ, it was revealed that in cellulose Iα thermal oxidation of the OH groups with weak hydrogen bonds is accelerated in the temperature range of 210−230 °C; however, in cellulose Iβ, the oxidation does not occur below 230 °C. From these observations, it may be concluded that the hydrogen-bond structure of cellulose Iα becomes much more unstable than that of cellulose Iβ in the temperature range of 210−230 °C where the hydrogen-bond structure drastically changes. It is suggested from the present study that the inter- and intrachain hydrogen bonds in cellulose Iα contribute to the stabilization of the C2−C3 glycol and hydroxymethyl groups more significantly than those in cellulose Iβ. It should be noted that a difference between the thermal behavior of cellulose Iα and that of cellulose Iβ has been elucidated at the functional group level from the present study.

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