



HOKKAIDO UNIVERSITY

Title	Elucidating the structural changes of cellulose molecules and dynamics of Na ions during the crystal transition from cellulose I to II in low temperature and low concentration NaOH solution
Author(s)	Kugo, Yuki; Nomura, Satoshi; Isono, Takuya et al.
Citation	Carbohydrate Polymers, 332, 121907 https://doi.org/10.1016/j.carbpol.2024.121907
Issue Date	2024-05-15
Doc URL	https://hdl.handle.net/2115/97182
Rights	© <2024>. This manuscript version is made available under the CC BY-NC-ND 4.0 license http://creativecommons.org/licenses/by-nc-nd/4.0/
Rights(URL)	https://creativecommons.org/licenses/by-nc-nd/4.0/
Type	journal article
File Information	Revised4_Unmarked_Document_Kugo 240129.pdf



**Elucidating the structural changes of cellulose molecules and dynamics
of Na ions during the crystal transition from cellulose I to II in low
temperature and low concentration NaOH solution**

Yuki Kugo¹, Satoshi Nomura¹, Takuya Isono², Shinichiro Sato², Masashi Fujiwara²,

Toshifumi Satoh^{2,3}, Hirofumi Tani², Tomoki Erata², and Kenji Tajima^{2*}

¹Graduate School of Chemical Sciences and Engineering, Hokkaido University, N13W8, Kita-ku,
Sapporo 060-8628, Japan

²Faculty of engineering, Hokkaido University, N13W8, Kita-ku, Sapporo 060-8628, Japan

³ICReDD List-PF, Hokkaido University, N21W10, Kita-ku, Sapporo 001-0021, Japan

*Corresponding author Email: ktajima@eng.hokudai.ac.jp

Emails: Yuki Kugo: kugo2318@eis.hokudai.ac.jp; Satoshi Nomura: satoshi054@gmail.com;

Takuya Isono: isono.t@eng.hokudai.ac.jp; Shinichiro Sato: s-sato@eng.hokudai.ac.jp;

Masashi Fujiwara: fujiwara@icredd.hokudai.ac.jp; Toshifumi Satoh:

satoh@eng.hokudai.ac.jp; Hirofumi Tani: tani@eng.hokudai.ac.jp; Tomoki Erata:

erata@eng.hokudai.ac.jp.

ORCID Takuya Isono: 0000-0003-3746-2084; Toshifumi Satoh: 0000-0001-5449-9642; Hirofumi

Tani: 0000-0002-5935-0756; Tomoki Erata: 0000-0003-3159-908X; Kenji Tajima: 0000-0002-3238-

813X

KEYWORDS

Mercerization; low-temperature alkali treatment; cellulose structure; alkali cellulose; intrachain hydrogen bonding; Na-ion dynamics

Author contributions

Yuki Kugo: Conceptualization, Methodology, Investigation of all experiments, Writing – Original Draft preparation. Satoshi Nomura: Conceptualization, Methodology, Takuya Isono: Investigation of WAXS measurements. Shinichiro Sato: Resources of the computing calculation. Masashi Fujiwara: Supervision. Toshifumi Satoh: Supervision. Hirofumi Tani: Supervision. Tomoki Erata: Methodology, Supervision, Writing – Review and Editing. Kenji Tajima: Supervision, Writing – Review and Editing

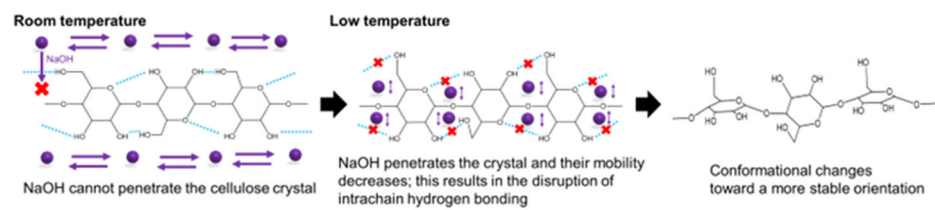
Declaration of competing interest

We declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that support the findings of this study are available from corresponding author, K.T., upon reasonable request.

35 **Graphical abstract**



36

37

Abstract

Low-concentration alkali treatments at low temperatures facilitate the crystal transition of cellulose I to II. However, the transition mechanism remains unclear. Hence, in this study, we traced the transition using in situ solid-state ^{13}C CP/MAS NMR, WAXS, and ^{23}Na NMR relaxation measurements. In situ solid-state ^{13}C CP/MAS NMR and WAXS measurements revealed that soaking cellulose in NaOH at low temperatures disrupts the intramolecular hydrogen bonds and lowers the crystallinity of cellulose. The dynamics of Na ions (NaOH) play a crucial role in causing these phenomena. ^{23}Na NMR relaxation measurements indicated that the Na-ion correlation time becomes longer during the crystal transition. This transition requires the penetration of Na ions (NaOH) into the cellulose crystal and a reduction in Na-ion mobility, which occurs at low temperatures or high NaOH concentrations. The interactions between cellulose and NaOH disrupt intramolecular hydrogen bonds, inducing a conformational change in the cellulose molecules into a more stable arrangement. This weakens the hydrophobic interactions of cellulose, and facilitates the penetration of NaOH and water into the crystal, leading to the formation of alkali cellulose. Our findings suggest that a strategy to control NaOH dynamics could lead to the discovery of a novel preparation method for cellulose II.

1. Introduction

Cellulose, which consists of glucose residues linked via β -1,4-glycosidic bonds, is the most abundant biopolymer. Natural cellulose found in plants, algae, and bacteria contains cellulose I crystals, which are soluble in a limited number of organic solvents (Bikova & Treimanis, 2002; Sayyed et al., 2019) and ionic liquids (Cao et al., 2009; Swatloski et al., 2002). However, cellulose I crystals are converted into cellulose II crystals upon treatment with a concentrated alkaline solution such as NaOH. This process is called mercerization and does not occur at low NaOH concentrations (8–10 wt%) at room temperature (Dinand et al., 2002; Wang et al., 2022). Nevertheless, cellulose is dissolved in a low-concentration NaOH aqueous solution at low temperatures (Isogai & Atalla, 1998; Kamide et al., 1984), and the crystal transition of cellulose I to II also proceeds in the insoluble portions (Isogai & Atalla, 1998). In the mercerization process, several intermediates known as alkali cellulose are generated during crystalline transitions (Okano & Sarko, 1984). When cellulose I is soaked in a 3–5 N NaOH solution, it converts into Na-cellulose I. Furthermore, when Na-cellulose I is soaked in a NaOH solution with a higher concentration, Na-cellulose II is obtained. Na-cellulose III is obtained by vacuum drying Na-cellulose I. In addition, Na-cellulose I transformed into Na-cellulose IV in low-temperature washing, whereas Na-cellulose I is reconverted to cellulose I in high-temperature washing (Kim et al., 1991). Na-cellulose IV can also be derived from Na-cellulose II, or III by washing with water, thereby eliminating NaOH. This structure is converted to cellulose II by drying (Nishimura &

Sarko, 1991). The region below 0 °C and at approximately 8 wt% NaOH concentration is referred to as the alkali cellulose Na–Cell Q, with Q representing Quellung (“swelling” in German) (Sobue et al., 1939). In other words, a detailed investigation of these alkali celluloses is crucial for discussing their crystalline transitions. Cellulose molecular chains are packed parallelly in cellulose I and anti-parallelly in cellulose II. Okano et al. proposed that swelling of cellulose fibrils with cellulose molecular chains oriented in opposite directions due to NaOH leads to formation of anti-parallel Na-cellulose I during the first step of mercerization (Okano & Sarko, 1985). Recently, Sawada et al. suggested that the molecular chains are packed antiparallely by folding back onto themselves (Sawada et al., 2022). However, the changes in the structure of the cellulose molecules and the driving force for the crystal transition remain unclear. Elucidating this crystal transition mechanism is crucial for understanding the crystal structure of cellulose and for developing its industrial use. This study aims to clarify the conformational changes in the molecular chain of cellulose during the crystal transition, especially the changes at the hydroxymethyl group at the C6 position.

The disruption of intrachain hydrogen bonds (Kamide et al., 1985) and hydrophobic interactions (Glasser et al., 2012; Lindman et al., 2010) are important in the crystal transition of cellulose I to II. The crystals of cellulose had different hydrophilic and hydrophobic planes (Medronho & Lindman, 2014). In the solid state, the hydrogen atoms in the axial planes of the glucose residues form hydrophobic planes between the molecular chain sheets. Similarly, the O6 hydroxy groups in the

equatorial planes of the glucose residues form intrachain hydrogen bonds with neighboring residues, and interchain hydrogen bonds with neighboring cellulose molecular chains, thus forming hydrophilic planes and flat sheets. Cellulose I has intrachain O2-O6 and O3-O5 hydrogen bonds (Nishiyama et al., 2002, 2003, 2008). On the other hand, cellulose II has intrachain O3-O6 and O3-O5 hydrogen bonds (Langan et al., 1999). Furthermore, Cellulose III_I, which is prepared by the treatments on cellulose I with liquid ammonia or organic amines, also has intrachain O3- O6 and O3-O5 hydrogen bonds (Ford et al., 2005). Hence, since the mode of intrachain hydrogen bonds in cellulose I to II, the crystal transition likely involves the disruption of intrachain hydrogen bonds and the formation of new intrachain hydrogen bonds. The differences in these intrachain hydrogen bonds are related to the conformation of the C6 hydroxymethyl group. Nuclear magnetic resonance (NMR) spectroscopy can clearly distinguish the conformation of the C6 hydroxymethyl group (Horii et al., 1983). The conformations of the C6 hydroxymethyl group in cellulose I, cellulose II and III_I, as well as in surface regions and dissolved cellulose, are *tg* (trans-gauche) (Nishiyama et al., 2002, 2003), *gt* (gauche-trans) (Langan et al., 1999; Wada et al., 2004), and *gg* (gauche-gauche) (Cai & Zhang, 2005; Newman & Davidson, 2004; Phyto et al., 2018), respectively. Thus, the C6 conformation is one of the guidelines for observing the crystal structures and the intrachain hydrogen bonds. Furthermore, our previously reported calculations show that the *tg* structure does not revert to its original structure after experiencing a change but changes to the *gt* structure (Nomura et al., 2020), which could contribute to

the crystal transition of cellulose I to cellulose II. Since the disruption of intrachain hydrogen bonds by the selective coordination of Na ions to the hydroxy group (Kamide, 1985) and the formation of the cellulose-O-Na-type structures by the partial dissociation of the hydroxy group of cellulose (Isogai, 1997) are considered to occur during the mercerization process, we hypothesized that dynamics of NaOH are deeply involved in the conformational change at the C6 position of cellulose and the disruption of intrachain hydrogen bonds. Therefore, we tracked the conformational changes in cellulose molecules and the behaviors of Na ions (NaOH) during the crystal transition to verify this hypothesis.

In this study, we analyzed cellulose soaked in low-concentration NaOH solutions via in situ solid-state ^{13}C NMR and wide-angle X-ray scattering (WAXS) at room temperature and low temperatures. The structure of the cellulose molecular chain was predicted via structural optimization calculations for cello-oligomers without hydrogen bonds. ^{23}Na NMR relaxation time measurements were acquired to elucidate the interactions between NaOH and cellulose at low temperatures.

2. Materials and Methods

2.1. Materials

Filter paper powder (100–200 mesh, cellulose > 99%) derived from cotton linter with DP (degrees of polymerization) 250, and kraft pulp paper (bleached hardwood kraft pulp, cellulose ca. 80% and hemicellulose ca. 20%) with DP 650–840 purchased from Advantech Co., Ltd (Tokyo, Japan)

and Nippon Paper Industries (Tokyo, Japan), respectively, were used as the cellulose materials. Cellobiose was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan) and used as received. NaOH, with a minimum purity of 97.0%, and H₂SO₄, with a minimum purity of 95.0 %, were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). All reagents were used as purchased. Deionized water (Elix®Essential3, Merck KGaA, Darmstadt, Germany) was used to prepare aqueous solutions of NaOH and H₂SO₄ and wash the cellulose samples after alkaline treatments and subsequent neutralization.

2.2. NMR measurements

All NMR measurements, including the NMR relaxation time measurements, were performed using a Bruker Avance 500 spectrometer (Bruker, Germany) at the Instrumental Analysis Support Office, Frontier Chemistry Center, Faculty of Engineering, Hokkaido University. Solid-state ¹³C CP/MAS NMR spectra were recorded at 125 MHz. The samples were packed in a 3.2-mm rotor and spun at a frequency of 10 kHz. All the CP/MAS high-resolution spectra were recorded with a ¹H 90° pulse length of 4.0 μs, a contact time of 1.5 ms, and a repetition time of 4 s. The ramp and tppm-15 sequences were used for the contact pulse and decoupling, respectively. The chemical shift was calibrated using the carbonyl carbon of glycine (176.46 ppm) as the reference.

²³Na NMR relaxation time measurements were performed at 133 MHz. The samples were packed into the MAS rotor; however, no MAS conditions were applied during the temperature control

and relaxation time measurements. Spin lattice relaxation time (T_1) measurements were performed using the inversion recovery method with a 90° pulse length of 3.1 μ s. The T_1 values were obtained using the fitting program of the TOPSPIN NMR software developed by Bruker Inc.

2.3. Content ratios of cellulose I and II after low-temperature NaOH treatments obtained using solid-state ^{13}C CP/MAS NMR

Cellulose powder was added to an 8 wt% NaOH solution at room temperature (24 $^\circ\text{C}$) and centrifuged at $1,000 \times g$ for 5 min. The supernatant was removed, and the precipitate was used for NaOH treatment. The reason for using the precipitation portion is to use as a sample only those regions involved in the crystal transition. The quenching involved chilling a 2 mL microtube, which contained 8 wt% NaOH-soaked cellulose in liquid nitrogen for up to 90 s. Similarly, for higher-temperature treatments, the centrifuge tubes with 8 wt% NaOH-soaked cellulose were chilled at -30°C , -25°C , -20°C , -18°C , -17°C , -16°C , -15°C , -10°C , and -5°C in a cryostat (EYELA PFR-1000, TOKYO RIKAKIKAI Co Ltd, Japan, Tokyo) for up to 90 min. The temperature was controlled by cooling ethanol in the instrument. The samples were then thawed to room temperature. A 10 vol% H_2SO_4 solution was added to the samples until the pH dropped below 7.0. The samples were washed with deionized water, air-dried, and analyzed via solid-state ^{13}C CP/MAS NMR. The C6 carbon signal in the spectrum was line-fitted to calculate the content rate of cellulose II. The C6 lines were deconvoluted using the DMfit2017 line-fitting program (Massiot et al., 2002). A Lorentzian shape was

used for each line. The C6 lines were deconvoluted based on the chemical shifts for the *tg* (64–66 ppm), *gt* (62–63 ppm), and *gg* (60–62 ppm) conformations (Horii et al., 1983) (Fig. 1) (Idström et al., 2016; Phyo et al., 2018). Additionally, the *gg* lines were divided into two categories: surfaces accessible to water and surfaces inaccessible to solvents but accessible to solute ions (Zuckerstätter et al., 2013). The content ratio of cellulose II crystals was calculated using the following equation:

$$\text{Content ratio of cellulose II} = \frac{A_{gt}}{A_{tg} + A_{gt}} \times 100$$

where A_{tg} is the integrated area of *tg* lines at 66.0, 65.5 and 65.2 ppm, and A_{gt} is the integrated area of *gt* lines at 63.3 and 62.6 ppm.

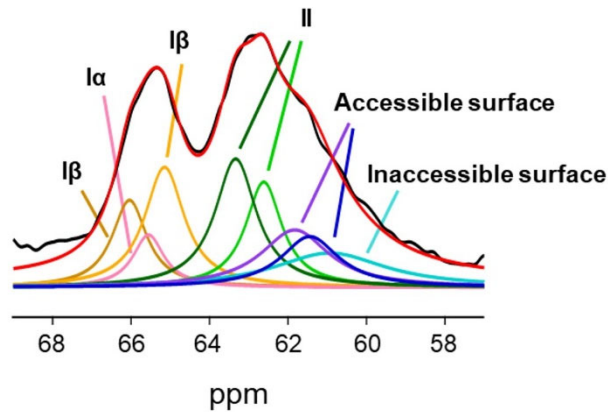


Fig. 1. C6 line fitting model in the solid-state ^{13}C CP/MAS NMR spectrum.

2.4. In situ solid-state ^{13}C CP/MAS NMR measurements at different low temperatures

Cellulose powder was added to a 10 wt% NaOH solution at 24 °C and centrifuged at 1,000 × g for 5 min. The supernatant was removed, and the precipitate was analyzed via in situ solid-state ^{13}C CP/MAS NMR. As a complete crystal transition could not be observed with 8 wt% NaOH-soaked

cellulose powder, we used a 10 wt% NaOH solution, which does not induce crystal transition at 24 °C. The sample was packed in a 3.2 mm rotor, and the rotor was set in the probe inside the NMR instrument. Solid-state ^{13}C CP/MAS NMR measurements were performed as the temperature was lowered from 24 °C to –25 °C and increased from –25 °C to 24 °C. A variable-temperature instrument system was used in the experiments.

First, the measurements were obtained at 24 °C, and MAS was stopped after the measurement. The temperature in the probe was lowered from 24 °C to 20 °C while the rotor remained stationary in the probe. The temperature was changed without MAS to minimize the discrepancy between the displayed and actual temperatures. MAS was restarted 30 min after the temperature reached 20 °C and the measurements were obtained, after which MAS was stopped. The same operation was performed while decreasing the temperature down to –25 °C at intervals of 5 °C and while increasing the temperature to room temperature in increments of 5 °C.

2.5. ^{23}Na NMR relaxation time measurements

Cellulose powder was added to 1, 5, 8, and 10 wt% NaOH solutions at 24 °C, and these mixtures were centrifuged at $1,000 \times g$ for 5 min. The supernatant was removed, and the precipitate was used for ^{23}Na NMR relaxation time measurements. These samples were packed in a 3.2-mm rotor and set in an NMR instrument. Furthermore, the relaxation times of ^{23}Na nuclei in 1 and 10 wt% NaOH solutions without cellulose powder were measured to evaluate the influence of cellulose.

First, the spin-lattice relaxation time (T_1) at 24 °C was measured. Then, the temperature inside the probe was decreased to 20 °C, and T_1 was measured after 10 min of temperature stabilization. The same operation was performed at 15 °C, 10 °C, 5 °C, 0 °C, –5 °C, –10 °C, –15 °C, –20 °C, and –25 °C. Subsequently, the temperature inside the probe was increased to –20 °C, –15 °C, –10 °C, –5 °C, 0 °C, 5 °C, 10 °C, 15 °C, 20 °C, and 24 °C, and the relaxation times were acquired. Then, solid-state ^{13}C CP/MAS NMR measurements were acquired to confirm the alkali concentration at which the crystal transition proceeded.

2.6. NMR measurements of NaOH-soaked cellulose using a high-concentration NaOH solution

In situ CP/MAS NMR and ^{23}Na NMR relaxation time measurements of cellulose soaked in a 25 wt% NaOH solution were performed at 24 °C to investigate mercerization in a concentrated NaOH solution. The samples were prepared as described in Sections 2.4 and 2.5, albeit with a 25 wt% NaOH solution. ^{23}Na NMR relaxation time measurements of a 25 wt% NaOH solution without cellulose powder were also performed at 24 °C for comparison.

2.7. In situ WAXS measurements at different temperatures

In situ WAXS measurements of the 10 wt% NaOH-soaked cellulose powder were performed at the BL-6A beamline, and measurements of the 10 wt% NaOH-soaked pulp paper were performed at the BL-10C beamline of the Photon Factory of the High-Energy Accelerator Research Organization (KEK, Tsukuba, Japan). The range of Q differed between these measurements as the beamlines were

different. The X-ray wavelength and exposure time were 1.50 Å (8.27 keV) and 60 s, respectively. PILATUS 100k detectors (Dectris Ltd., Switzerland) were used for the WAXS data acquisition. The sample-to-detector distance was calibrated using the scattering pattern of silver behenate (Nagara Science Co. Ltd., Japan). One-dimensional (1D) profiles were obtained as plots of scattering intensity vs. scattering vector (q), where $q = (4\pi/\lambda) \sin(\theta/2)$ (λ , wavelength; θ , scattering angle).

Variable-temperature measurements of 10 wt% NaOH-soaked cellulose samples were performed from room temperature (24 °C) to low temperatures as well as from low temperature to room temperature (24 °C). The samples were prepared using the procedures described in Sections 2.4. The sample was packed into the center hole of a 1.5-mm-thick flat washer and closed on both sides with a 1.2-mm-thick Kapton sheet. A flat washer with the samples was fixed on a stage (Linkam Scientific Instruments, UK), and the temperature was controlled by blowing air that was cooled with liquid nitrogen. In situ WAXS measurements were performed at 25 °C, 20 °C, 10 °C, 0 °C, –10 °C, –20 °C, –30 °C, and –35 °C, followed by increasing the temperature to –30 °C, –20 °C, –10 °C, 0 °C, 10 °C, and 20 °C.

The same 10 wt% NaOH-soaked cellulose samples used for in situ WAXS measurements were analyzed via solid-state ^{13}C CP/MAS NMR before neutralization. Then, the samples were neutralized with a 10 vol% H_2SO_4 solution, washed with water, and air-dried. The samples were again analyzed via solid-state ^{13}C CP/MAS NMR. These measurements were acquired to confirm the crystal

transition during the in situ WAXS measurements and after neutralization.

2.8. Calculations to identify stable structures of the cello-oligomers

Structure optimization calculations of cellobiose, cellotriose, and cellotetraose, which have no hydrogen bonds between glucose residues, were performed using the Gaussian G16 program with the B3LYP/6-31+G (d,p) basis set. Vibrational frequency calculations were performed on the optimized structure, and the absence of negative vibrational frequencies confirmed the adequacy of the results of the structure optimization calculation. The calculations were performed using a supercomputer system at the Information Initiative Center of Hokkaido University, Sapporo, Japan. Each initial structure was constructed using GaussView 6.0 software and then cleaned for creation.

3. Results and discussion

3.1. Crystal transition induced by low-concentration NaOH treatment at low temperatures

Fig. 2 shows the solid-state ^{13}C CP/MAS NMR spectra of 8 wt% NaOH-soaked cellulose quenched with liquid nitrogen at different time intervals. As the treatment time increased, the peak intensity at 65 ppm corresponding to the C6 carbon of the *tg* conformation decreased while the peak intensity at 62 ppm corresponding to the C6 carbon of the *gt* conformation II increased. The samples froze when cooled in liquid nitrogen for ~30 s, and the samples soaked for a longer duration completely transitioned to cellulose II after neutralization treatment. This suggests that cellulose II increased with treatment time, and the crystal transition was completed in an extremely short time at an extremely

low temperature. Hence, the liquid-nitrogen temperature is not suitable for tracking the crystal transition, and we decided to study the transition at $-20\text{ }^{\circ}\text{C}$, as reported in the literature.

Fig. 3 shows the treatment time dependence of content rates of cellulose II in the 8 wt% NaOH-soaked cellulose powder at various temperatures. The crystal transition was not completed at temperatures above $-16\text{ }^{\circ}\text{C}$ but only below $-17\text{ }^{\circ}\text{C}$. The lower the processing temperature, the shorter the time required to complete the crystal transition. As only a small amount of NaOH solution was used to soak the cellulose powder, it was not fully dissolved. This behavior indicates that the crystal transition from cellulose I to cellulose II was a non-Arrhenius-type, as it occurred only at low temperatures. This result suggests the importance of temperature conditions. In addition, Na ions (NaOH) in NaOH solutions with concentrations where the crystal transition progressed may exhibit specific dynamic behaviors compared to those in solutions where the crystal transition did not progress. Therefore, Na ions (NaOH) dynamics may affect crystal transition. Hence, Na-ion dynamics must be evaluated along with the conformational changes in the cellulose molecular chains to elucidate the crystal transition. Consequently, temperature-variable in situ solid-state ^{13}C CP/MAS NMR and WAXS measurements, as well as ^{23}Na NMR relaxation time measurements, were acquired using NaOH-soaked cellulose.

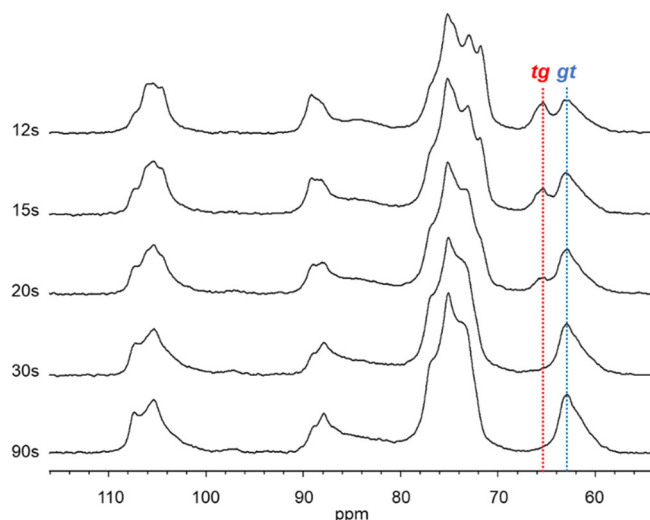


Fig. 2. Solid-state ^{13}C cross-polarization magic angle spinning (CP/MAS) NMR spectra of dried samples containing 8 wt% NaOH-soaked cellulose obtained by quenching with liquid nitrogen, recorded over a duration of up to 90 s. These spectra were recorded at recorded at 24 °C. After quenching, the samples were neutralized with a 10 vol% H_2SO_4 solution, washed with deionized water, and air-dried at 24 °C.

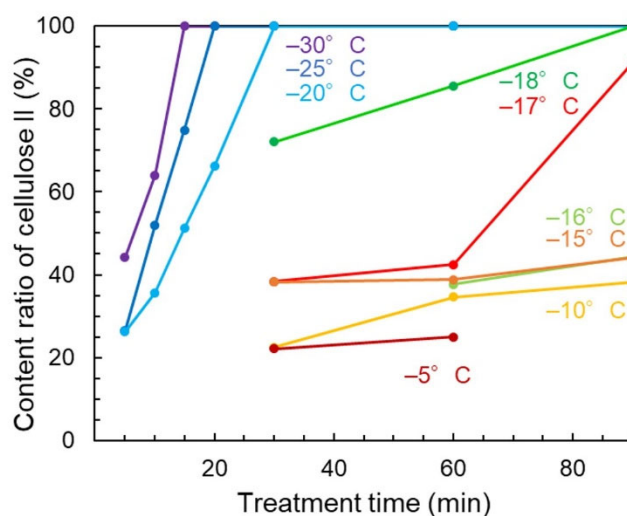


Fig. 3. Time-course changes for the content rates of cellulose II in a low-concentration (8 wt%) NaOH-soaked cellulose powder at various temperatures.

3.2. In situ solid-state ^{13}C CP/MAS NMR measurements at low temperatures

Fig. 4 shows the in situ solid-state ^{13}C CP/MAS NMR spectra of 10 wt% NaOH-soaked cellulose powder as the temperature was gradually decreased. The C6 peak shifted from 65 to 61 ppm,

274 indicating a change in the O6 conformation from *tg* to *gg*. Similar upfield shifts were observed for the
275 C1 and C4 peaks, indicating corresponding environmental changes. The large upfield shift of the C4
276 peak from 89 to ~80 ppm suggests that the conformational changes at C1 and C4 occurred after the
277 changes at C6. Fig. 5 shows the solid-state ^{13}C CP/MAS NMR spectra as the temperature was
278 increased from $-25\text{ }^{\circ}\text{C}$ to $24\text{ }^{\circ}\text{C}$. The C1 and C4 peaks shifted downfield, whereas the C6 peak was
279 maintained at 61 ppm, indicating that the *gg* conformation of O6 was maintained. The C4 peak shifted
280 to ~86 ppm, which is attributed to the surface/amorphous region (Park et al., 2009). The C4 upfield
281 shift observed at low temperatures and at $24\text{ }^{\circ}\text{C}$ after warming is consistent with that for alkaline
282 cellulose reported previously (Porro et al., 2007). In summary, the in situ solid-state ^{13}C CP/MAS
283 NMR spectra indicate that crystallinity reduction and conformational changes were observed when
284 the temperature was decreased, and these changes were maintained when the temperature was
285 increased.

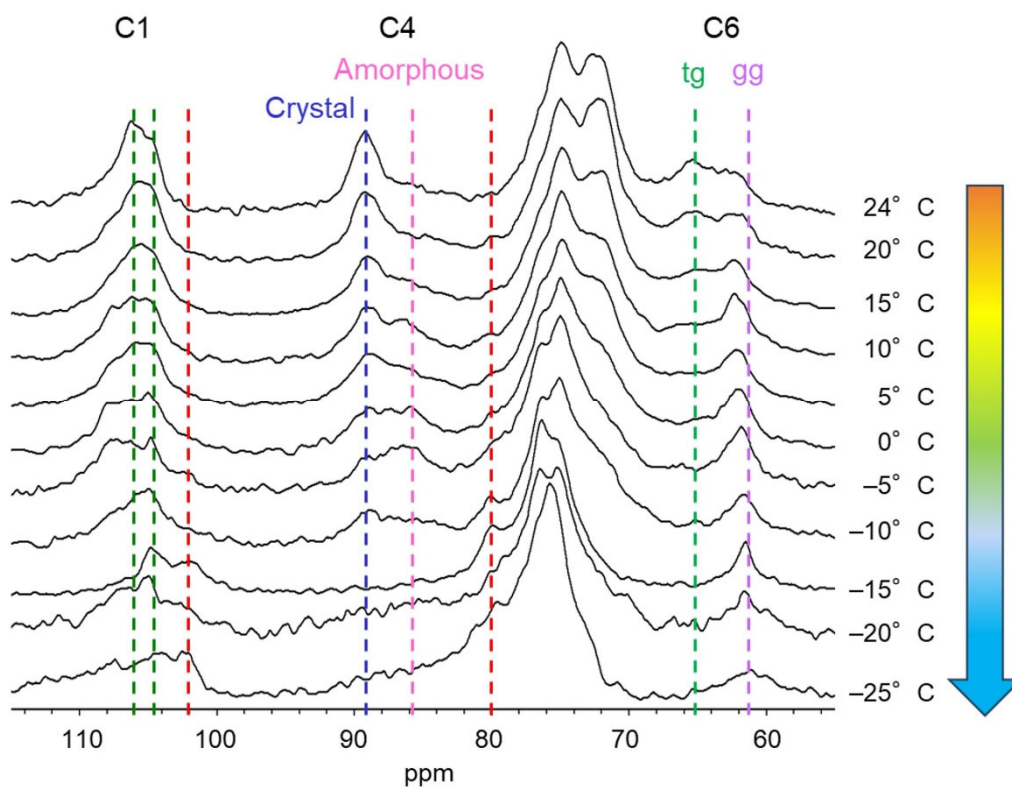


Fig. 4. Solid-state ^{13}C CP/MAS NMR spectra of 10 wt% NaOH-soaked cellulose powder as the temperature was lowered from 24 °C to 20 °C, 15 °C, 10 °C, 5 °C, 0 °C, -5 °C, -10 °C, -15 °C, -20 °C, and -25 °C.

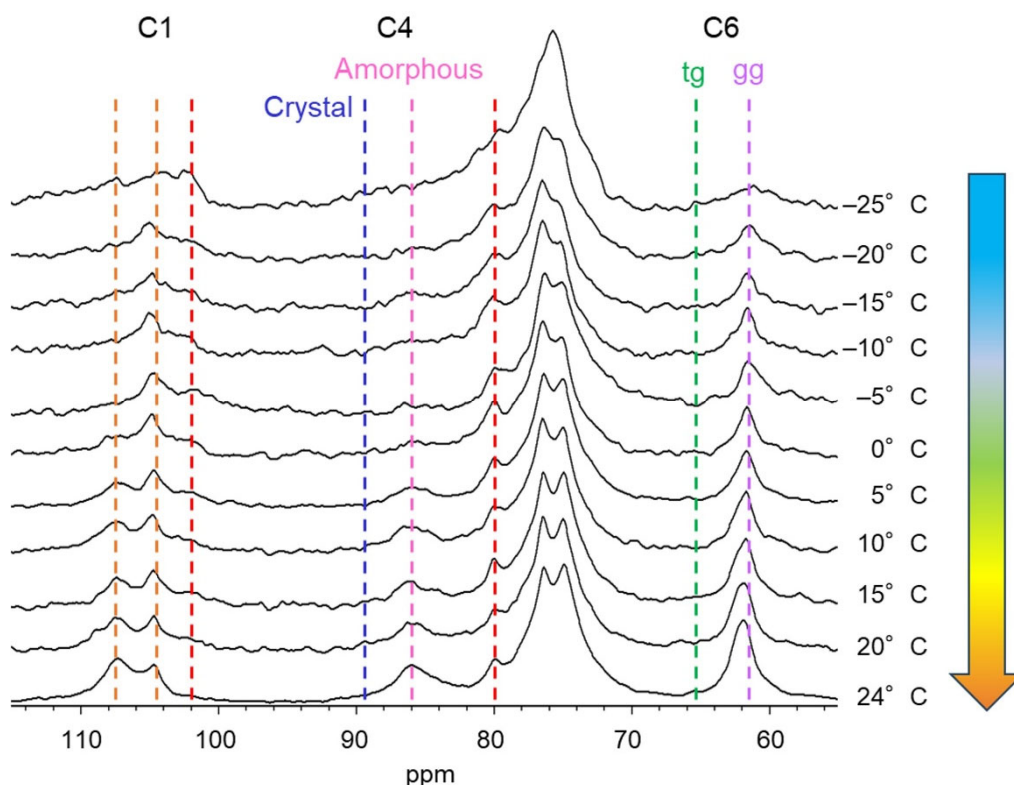


Fig. 5. Solid-state ^{13}C CP/MAS NMR spectra of 10 wt% NaOH-soaked cellulose powder as the temperature was increased from $-25\text{ }^{\circ}\text{C}$ to $-20\text{ }^{\circ}\text{C}$, $-15\text{ }^{\circ}\text{C}$, $-10\text{ }^{\circ}\text{C}$, $-5\text{ }^{\circ}\text{C}$, $0\text{ }^{\circ}\text{C}$, $5\text{ }^{\circ}\text{C}$, $10\text{ }^{\circ}\text{C}$, $15\text{ }^{\circ}\text{C}$, $20\text{ }^{\circ}\text{C}$, and $24\text{ }^{\circ}\text{C}$. The in situ ^{13}C NMR spectra shown in Figs. 4 and 5 were conducted consecutively. Fig. 4 also shows the spectrum recorded at $-25\text{ }^{\circ}\text{C}$, and in situ NMR spectra of this sample were recorded from room temperature ($24\text{ }^{\circ}\text{C}$) to low temperatures before obtaining this spectrum.

3.3. In situ wide-angle X-ray scattering (WAXS) measurements at different temperatures

Fig. 6 shows the in situ WAXS profiles of 10 wt% NaOH-soaked cellulose powder at different temperatures. As the temperature decreased, the intensity of the cellulose I crystal peaks decreased, and the width at half maximum increased, indicating a reduction in cellulose crystallinity. These WAXS profiles are consistent with those observed for cellulose powder dissolved in a LiOH/urea solution, which indicate a decrease in crystallinity (Isobe et al., 2013). Moreover, the crystal peaks of cellulose I almost disappeared at $-35\text{ }^{\circ}\text{C}$. This decrease in crystallinity confirms that

Na ions have penetrated the crystal region. However, no change in the crystal structure was observed as the temperature increased. The three sharp peaks at ~ 15 , 17, and 18 nm⁻¹ disappeared with increasing temperature, corresponding to ice.

The in situ WAXS results are consistent with the C4 peak observed in the in situ solid-state ¹³C CP/MAS NMR spectra, whose intensity decreased in the crystalline region and increased in the surface/amorphous region as the temperature was lowered. In summary, during the crystal transition induced by NaOH treatment at low temperatures, cellulose I crystals disappeared, alkali cellulose was formed as the temperature was decreased, and this amorphous structure remained unchanged as the temperature increased. Similar results were obtained in the experiment using pulp paper (Fig. S1).

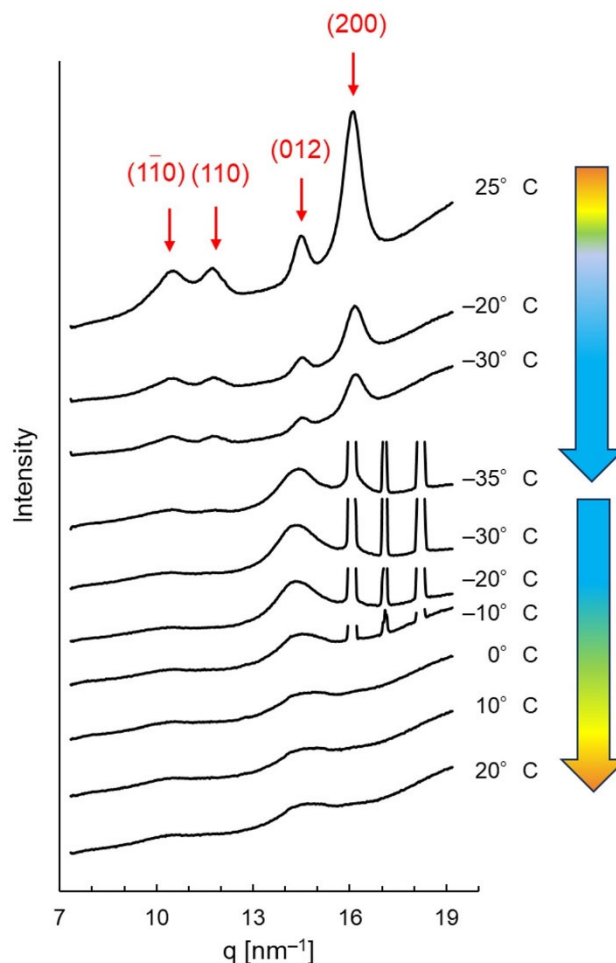


Fig. 6. Temperature-variable wide-angle X-ray scattering (WAXS) profiles of 10 wt% NaOH-soaked cellulose powder (25 °C > -35 °C > 20 °C).

Fig. 7 shows the solid-state ^{13}C CP/MAS NMR spectra of the 10 wt% NaOH-soaked cellulose powder samples used in the in situ WAXS measurements before and after neutralization. The sample before neutralization was determined to be alkali cellulose, whereas the sample after neutralization, washing, and drying was cellulose II. This confirms that cellulose I crystals disappeared, alkali cellulose was formed as the temperature decreased, and this amorphous structure remained unchanged as the temperature increased during the crystal transition induced by NaOH treatment at

low temperatures. Moreover, the transition from alkali cellulose to cellulose II occurred during neutralization, washing, and drying. Fig. S1 shows the in situ WAXS profiles of the 10 wt% NaOH-soaked pulp paper, and Fig. S2 shows the solid-state ^{13}C CP/MAS NMR spectra of the pulp paper samples used in the in situ WAXS measurements before and after neutralization. The 10 wt% NaOH-soaked pulp paper samples behaved identically to the 10 wt% NaOH-soaked cellulose powder samples (Figs 6 and 7). This indicates that regardless of the cellulose material, cellulose I crystals disappear, and alkali cellulose is formed as the temperature is lowered. Furthermore, this amorphous structure remains unchanged as the temperature increases, transitioning from alkali cellulose to cellulose II after the neutralization treatment.

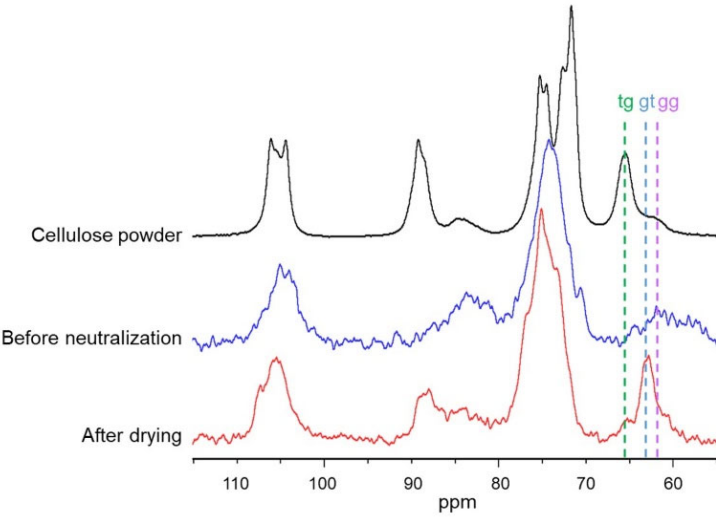


Fig. 7. Solid-state ^{13}C CP/MAS NMR spectra before and after neutralization of samples used in the temperature-variable WAXS measurements. *Color code*: black line, cellulose powder (cellulose I_β rich); blue line, before neutralization; red line, after washing and drying.

3.4. Structure of cellulose during the crystal transition determined via in situ NMR measurements and calculations of the stable structures of the cello-oligomers

Fig. 8 shows the ^{13}C CP/MAS NMR spectra of cellulose powder (cellulose I_β rich), the 10 wt% NaOH-soaked cellulose powder at -25°C and 25°C , and as-received cellobiose. The C6 peak shift indicates that the O6 conformation changed from *tg* to *gg*, where the hydroxy groups are perpendicular to the glucose residues and consequently do not form hydrogen bonds with neighboring glucose residues. Therefore, this C6 peak shift suggests disruption of the intrachain O6-O2 hydrogen bonds. Kamide et al. reported that the disruption of the O3-O5 hydrogen bonds has a de-shielding effect on C4 and C5, which explains the large C4 peak shift (Kamide et al., 1984). A similar upfield shift from 89 ppm to 80 ppm was observed for the C4 peak in this study, which suggests the disruption of the intrachain O5-O3 hydrogen bonds.

To confirm the conformation of the cellulose molecules after the intrachain hydrogen bonds were broken, we obtained computational simulations of the structures of cellobiose, cellotriose, and cellotetraose devoid of hydrogen bonds. The in situ ^{13}C NMR spectra show that the intrachain hydrogen bonds were broken during the crystal transition. The solid-state ^{13}C CP/MAS NMR spectrum of as-received cellobiose (Fig. 8) revealed that the C4 peak shifted upfield to the crystalline peak, suggesting that as-received cellobiose has a similar structure to that of cellulose during crystal transition. We hypothesized that the optimized structures of cellobiose, cellotriose, and cellotetraose,

and a portion of the cellulose molecular chain could be used to predict the structure of cellulose during the crystalline transition. Fig. 9 shows the optimized structures of cellobiose, cellotriose, and cellotetraose obtained by DFT calculations. No negative frequencies were observed in the optimized structures in the vibrational frequency calculations. The neighboring glucose residues are oriented in different directions in all optimized structures, suggesting the absence of a plane sheet structure. This suggests that the disruption of intrachain hydrogen bonds during the crystal transition results in a more stable structure. The absence of a plane sheet structure indicates weakened hydrophobic interactions between cellulose molecules, which would allow more water molecules to penetrate the crystal region and facilitate the presence of a higher number of Na ions (NaOH) in the vicinity of the cellulose molecule.

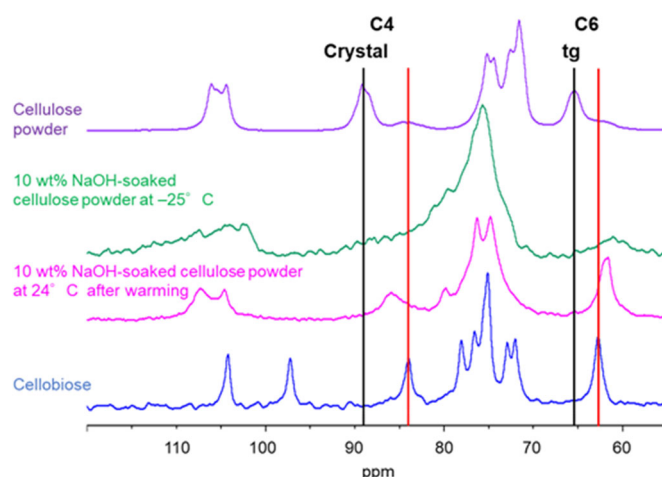
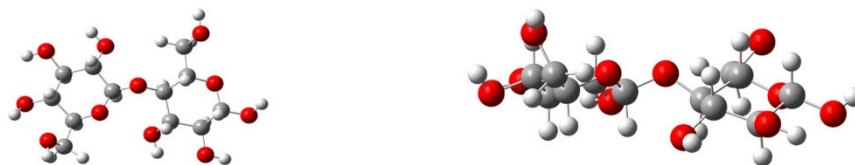


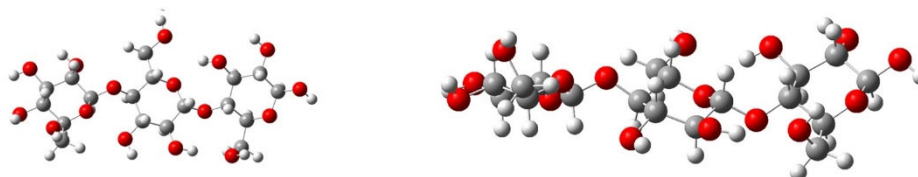
Fig. 8. Solid-state ^{13}C NMR CP/MAS NMR spectra. Color code: purple line, cellulose powder (cellulose I_β rich); green line, 10 wt% NaOH-soaked cellulose powder (the spectrum was recorded at -25°C after cooling the sample from 24°C to -25°C); pink line, 10 wt% NaOH-soaked cellulose powder (the spectrum was recorded at 24°C after cooling the sample from 24°C to -25°C , followed

368 by warming to 24 °C); blue line, as-received cellobiose.

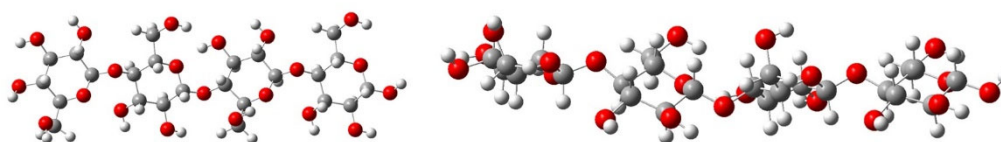
(a) Cellobiose



(b) Cellotriose



(c) Cellotetraose



369

370 Fig. 9. Three-dimensional (3D) optimized structures of (a) cellobiose, (b) cellotriose, and (c)
371 cellotetraose.

372 3.5. ^{23}Na NMR relaxation time measurements

373 Fig. S3 shows the solid-state ^{13}C CP/MAS NMR spectra of the 1 wt% to 10 wt% NaOH-

374 soaked cellulose powder samples used in the ^{23}Na NMR relaxation time measurements. These ^{13}C

375 NMR measurements were conducted to confirm the concentration of the NaOH solution at which the

376 crystal transition proceeded. The spectra of the samples soaked in 1 wt% and 5 wt% NaOH were

377 almost identical to those of the samples soaked in 10 wt% NaOH before measurement (uncooled).

378 However, C4 peaks were observed in both the amorphous and crystalline regions in the spectrum of

379 the 8 wt% NaOH-soaked sample. The spectrum of the sample soaked in 10 wt% NaOH showed that

380 the C4 peak completely disappeared in the crystal region. Moreover, the C6 peak shifted upfield,

confirming the conformational change from *tg* to *gg*. These results indicate that the crystal transition did not progress in the cellulose samples soaked in <5 wt% NaOH, partially progressed in 8 wt% NaOH, and was completed in 10 wt% NaOH. Thus, the degree of crystal transition depends on the NaOH concentration. Dinand et al. (2002) previously reported that the crystal transition did not progress at <5 wt% NaOH concentration, which is consistent with our results.

The spin-lattice relaxation times (T_1) of the Na nuclei in 1 wt% to 10 wt% NaOH-soaked cellulose powder samples and 1 wt% and 10 wt% NaOH solutions without cellulose powder were measured in the temperature range of 24 °C to −25 °C. Table 1 shows the T_1 of the Na nuclei in the 10 wt% NaOH-soaked cellulose powder while cooling. T_1 decreased with decreasing temperature, indicating that the Na-ion dynamics follow the Arrhenius law. Fig. S4 shows the ^{23}Na NMR spectra of the 10 wt% NaOH-soaked cellulose powder recorded at 24 °C, 0 °C, and −15 °C. The width of the peak increased as the temperature decreased, indicating that T_1 decreased as the temperature decreased. Because the spin number of the Na nucleus is 3/2, it will have quadrupolar moments, and consequently, the main component of the spin-lattice relaxation mechanism is the interaction between the quadrupolar moment and the electric field gradient. Figs. 10(a) and 10(b) show semi-logarithmic Arrhenius plots during cooling and heating, respectively, with the reciprocal of the absolute temperature ($1/T$) on the x-axis and the reciprocal of T_1 ($1/T_1$) on the y-axis. Although the observed temperature range is not sufficient, the changes in $1/T_1$ exhibit a roughly linear trend in this

semilogarithmic plot, except around the lowest temperature end where the solution begins to freeze.

The term $1/T_1$ is proportional to the term $\langle e^2qQ/h \rangle^2 \times J(\omega)$, where eq and eQ are the electric field gradient and the quadrupolar moment of the ^{23}Na nuclei, respectively. Moreover, $J(\omega)$ is the spectral density, and ω is the resonance frequency of the nucleus. The term, $\langle e^2qQ/h \rangle^2$ and ω were considered constants as only the ^{23}Na nuclei were experimentally measured. Therefore, $1/T_1$ becomes proportional to only $J(\omega)$, which is expressed as $\omega\tau_c/(1+\omega^2\tau_c^2)$, where τ_c is the correlation time. At extremely narrow conditions such as $\omega\tau_c \ll 1$, and assuming that $\omega^2\tau_c^2 \approx 0$, $J(\omega)$ can be approximated as $J(\omega) = \omega\tau_c$, and $1/T_1$ can directly represent the correlation time. Generally, the correlation time for the motion of ions should increase with decreasing temperature. In the present study, the $1/T_1$ of the Na nuclei increases as temperature decreases. This indicates that the extremely narrow condition can be adopted. Furthermore, $J(\omega)$ becomes proportional to τ_c , suggesting that $1/T_1$ is proportional to the correlation time. Notably, Na ions will have low mobility at long correlation times and high mobility at short correlation times. In addition, the slope of the semi-log plot is proportional to the activation energy for Na-ion mobility. However, this slope is not precise and provides only an approximate trend.

The term $1/T_1$ of the sample that underwent crystal transition increased with decreasing temperature (Fig. 10(a)), indicating that the mobility of Na ions decreases with decreasing temperature. The 10 wt% NaOH-soaked sample, in which the crystal transition occurred, showed lower Na-ion mobility than the 8 wt% NaOH-soaked sample. However, the mobilities of Na ions in the 1 wt%

NaOH-soaked sample and the 1 wt% and 10 wt% NaOH solutions without cellulose powder were comparable. The Na ions in the 1 wt% and 10 wt% NaOH solutions without cellulose powder were expected to move freely. Notably, the Na ions in the 1 wt% NaOH-soaked sample moved freely, with no significant increase in the interaction between Na ions and cellulose. In addition, the slope of the semi-log plot was larger for the concentration at which crystal transition progressed than for the concentration that did not induce crystal transition. This indicates that the activation energy for the motion of the Na ions was higher. Therefore, the interaction between Na ions and cellulose molecules is essential for the crystal transition.

The mobility of Na ions at NaOH concentrations that induced crystal transition and those that did not induce the crystal transition was different when the temperature was increased (Fig. 10(b)). The $1/T_1$ of 1 wt% NaOH-soaked samples exhibited no significant changes, which is attributed to the absence of interactions between the Na ions and cellulose chains as the temperature increased. However, $1/T_1$ of the 8 wt% and 10 wt% NaOH-soaked samples at 25 °C was larger than that before cooling. This is attributed to the penetration of Na ions (NaOH) into the crystalline region and subsequent interaction with the cellulose crystals, which facilitates the crystal transition to alkali cellulose.

In summary, the mobility of Na ions decreased significantly during the crystal transition. This suggests that the residence time of Na ions (NaOH) around the cellulose chain increased,

facilitating crucial interactions between the cellulose chain and Na ions (NaOH) for the crystal transition of cellulose I to alkali cellulose.

Table 1. Spin-lattice relaxation time (T_1) of Na ions in 10 wt% NaOH-soaked cellulose powder during cooling from 24 °C to −25 °C

Temperature (°C)	24	20	15	10	5	0	−5	−10	−15	−20	−25
T_1 (ms)	13.9	11.9	9.8	7.1	5.2	3.6	3.0	2.5	2.0	1.7	0.53

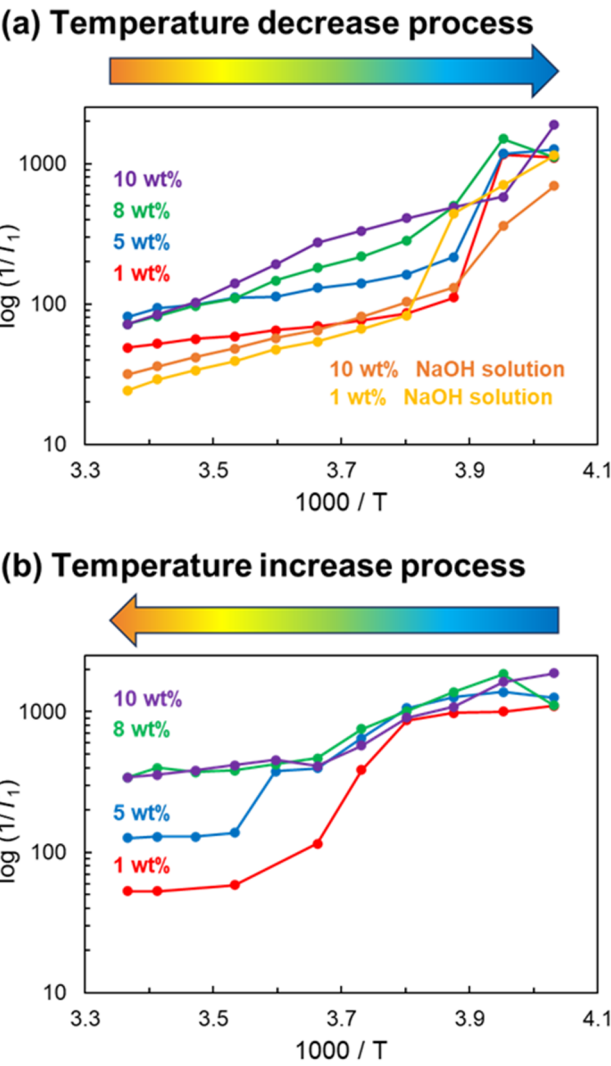


Fig. 10. Semi-logarithmic Arrhenius plots of the ^{23}Na -NMR relaxation time measurements of NaOH-soaked cellulose powder during (a) temperature-decrease and (b) temperature-increase processes.

Color code: purple line, 10 wt%; green line, 8 wt%; blue line, 5 wt%; red line, 1 wt%; orange line, a 10 wt% NaOH solution without cellulose; yellow line, a 1 wt% NaOH solution without cellulose.

3.6. Mercerization induced by a concentrated NaOH solution

Fig. 11 shows the in situ solid-state ^{13}C NMR spectra of 25 wt% NaOH-soaked cellulose powder at 24 °C. The C4 and C6 peaks were similar to those observed in the spectrum of 10 wt% NaOH-soaked cellulose recorded at 24 °C after the in situ NMR measurements (Fig. 5), suggesting that the conformational changes in cellulose were the same in the cases of both mercerization with a high-concentration NaOH solution at room temperature and low-concentration alkali treatment at low temperatures.

In addition, the T_1 of Na ions in the 25 wt% NaOH-soaked cellulose powder at 24 °C was 1.40 ms, which is comparable to the T_1 value of Na ions in 10 wt% NaOH-soaked cellulose at low temperatures (Table 1). This also suggests that the Na-ion dynamics, which facilitate the crystal transition, in both cases were comparable. That is, the interactions between Na ions and cellulose decrease the mobility of Na ions (NaOH) because they penetrate the crystal regions, resulting in a longer residence time. Table 2 shows the T_1 values of 1, 10, and 25 wt% NaOH solutions without cellulose powder measured at 24 °C. T_1 decreased with increasing NaOH concentration, which confirms a reduction in the mobility of Na ions (NaOH) and an increase in their correlation time. This was attributed to the high alkali concentration, which suppresses the mobility of Na ions (NaOH).

These results suggest that an elongated correlation time for Na ions (NaOH) is imperative

for crystal transition. At high alkali concentrations or low alkali concentrations combined with low temperatures, Na ions (NaOH) penetrate the crystal and interact with cellulose, disrupting intrachain hydrogen bonds. The NaOH is removed by neutralization and washing, which completes the crystal transition to cellulose II.

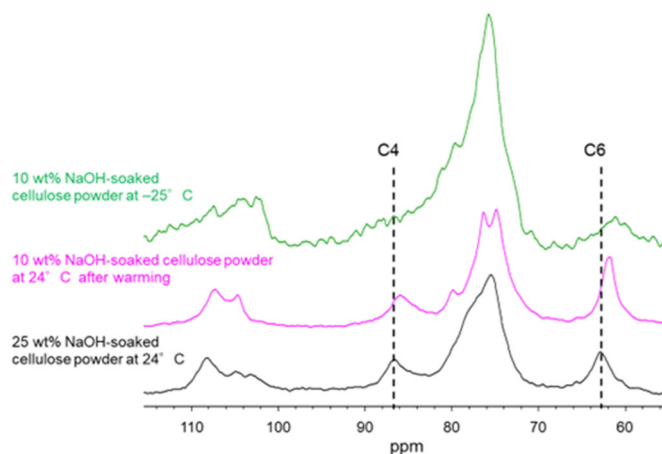


Fig. 11. In situ solid-state ^{13}C CP/MAS NMR spectra. *Color code:* green line, 10 wt% NaOH-soaked cellulose powder (the spectrum was recorded at -25°C after cooling the sample from 24°C to -25°C); pink line, 10 wt% NaOH-soaked cellulose powder (the spectrum was recorded at 24°C after cooling the sample from 24°C to -25°C , followed by warming to 24°C); and black line, 25 wt% NaOH-soaked cellulose powder at 24°C .

Table 2. T_1 values obtained from the ^{23}Na NMR relaxation time measurements of 1, 10, and 25 wt% NaOH solutions without cellulose powder at 133 MHz

NaOH concentration (wt%)	T_1 (ms)
1	41.3
10	31.7
25	11.7

3.7. Mechanism of crystal transition from cellulose I to alkali cellulose

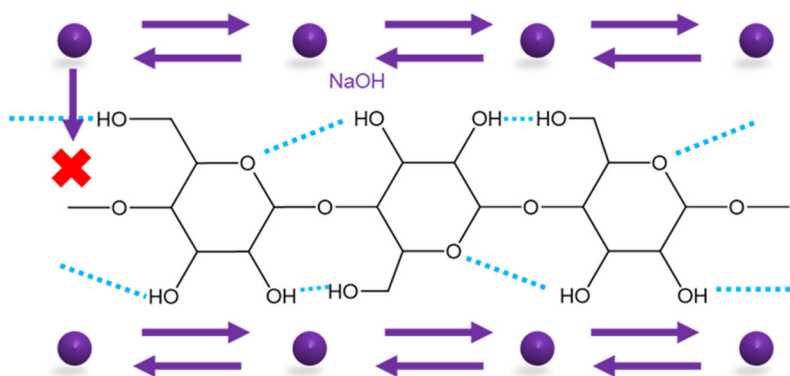
Fig. 12 shows the conformational changes of cellulose molecular during the crystal

transition from cellulose I to alkali cellulose during the temperature-decrease process. In this study, we observed that the crystal transition in a low-concentration alkali solution occurs only at low temperatures. This transition involves the disruption of intrachain hydrogen bonds and subsequent conformational changes in the cellulose molecular chains toward a more stable orientation. The disruption of hydrogen bonds is attributed to the interactions between cellulose and Na ions (NaOH). These interactions are associated with their penetration into the crystal structures of cellulose, resulting in the prolonged residence time of Na ions (NaOH).

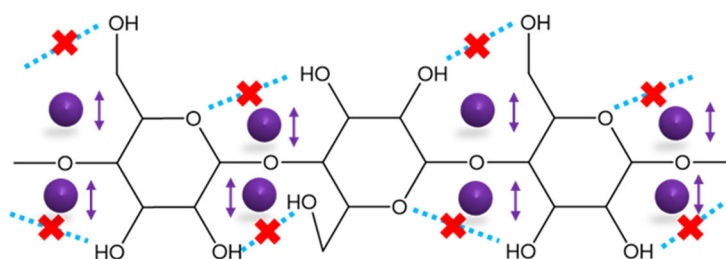
Na ions (NaOH) cannot penetrate the cellulose crystals at room temperature. However, Na ions adopt a solvated conformation in 7–10 wt% NaOH solutions at low temperatures (Yamashiki et al., 1988). Miyamoto et al. reported an increase in the density of water near cellulose at low temperatures based on computational simulations (Miyamoto et al., 2015), which explains the facile penetration of Na ions (NaOH) into the crystals. Consequently, their mobility decreases and residence time around the cellulose molecular chains increases, facilitating interactions, such as the bulkiness of Na ions (NaOH) and electrophilic interactions, between them. These interactions must be further clarified. When a 4–30% NaOD solution was used during mercerization, the dissociated form of C3-OH increased substantially with an increase in NaOD concentration, whereas that of C6-OH increased negligibly (Isogai, 1997). This suggests that the partial dissociation of the OH group at the C3 position of cellulose occurs only under the conditions in which mercerization proceeds. In other words, not

496 only are the intrachain hydrogen bonds of O2-O6 associated with the conformation at the C6 position
497 important, but the changes in the intrachain hydrogen bonds of O3-O5 also play a crucial role. The
498 partial dissociation of the OH groups of cellulose is expected to produce cellulose-ONa-type structures.
499 This is because NaOH solutions are commonly used to pretreat cellulose during the preparation of
500 cellulose derivatives, wherein OH ions withdraw protons to produce the cellulose-ONa-type structure.
501 Furthermore, hydroxide ions are presumed to penetrate the cellulose crystal and be present in the
502 vicinity of Na ions as counter ions. It is possible that hydroxide ions may influence the crystal
503 transition; however, their specific role could not be determined in this study. However, they may
504 disrupt intramolecular hydrogen bonds by partially dissociating the OH groups of cellulose molecules
505 in the vicinity of Na ions. These interactions disrupt the intrachain hydrogen bonds between glucose
506 residues, contributing to conformational changes in the cellulose molecular chains from a plane-sheet
507 structure toward a more stable orientation with a disrupted plane. This results in lower hydrophobic
508 interactions among the cellulose molecular chains. This further facilitates the penetration of molecules,
509 such as water, into the crystal, thereby increasing the mobility of the cellulose molecular chains.
510 Finally, during neutralization, washing, and drying, new O6-O3 intrachain hydrogen bonds associated
511 with the *gt* conformation are formed, completing the crystal transition to cellulose II.

Na ions (NaOH) cannot penetrate the crystal at room temperature



Na ions (NaOH) penetrate the crystal in the temperature-decrease process and their mobility decreases. This results in the partial dissociations of OH group of cellulose and the disruption of the intrachain hydrogen bonds



Conformational changes toward a more stable orientation

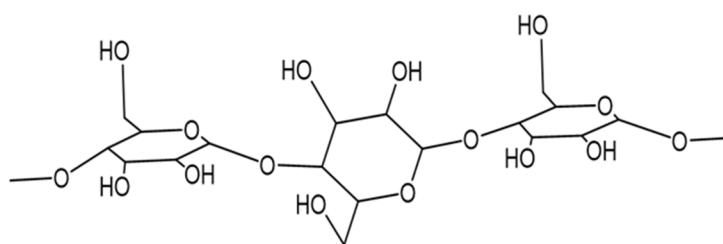


Fig. 12. Proposed mechanism for the defibrillation of the cellulose I molecular chains induced by alkali treatment at low temperatures.

4. Conclusion

In this study, we traced the mechanism of the crystal transition from cellulose I to cellulose II during low-concentration alkali treatment at low temperatures, especially the behavior of the cellulose chain of cellulose I and the dynamics of Na ions (NaOH) during the crystal transition. In situ solid-state ^{13}C NMR spectra revealed a shift in the C6 peak, indicating a conformational change from trans-gauche (*tg*) to gauche-gauche (*gg*), and an upfield shift in the C4 peak at low temperatures. This indicates the disruption of the O6-O2 and O5-O3 intrachain hydrogen bonds, and the partial dissociation of the OH group of cellulose in the crystal transition process, and we confirmed the hypothesis that the dynamics of Na ions (NaOH), particularly the prolonged correlation times, have a significant impact on the disruption of these hydrogen bonds. ^{23}Na NMR relaxation measurements showed that the mobility of Na ions was significantly reduced at the alkali concentration at which the crystal transition progressed, suggesting that Na ions (NaOH) penetrate the crystal region and interact with cellulose, resulting in sufficient residence times and longer correlation times for Na ions (NaOH). These increased interactions could facilitate the disruption of the intrachain hydrogen bonds, thus contributing to the conformational changes in the cellulose molecular chains towards a more stable orientation. A similar increase in the correlation time of the Na ions was observed during mercerization with a high-concentration NaOH solution. We believe that a strategy to control the Na-ion (NaOH) dynamics could lead to a novel preparation method for cellulose II.

534 **Acknowledgements**

535 We would like to express our gratitude to Mr. Naoya Nakagawa, Instrumental Analysis
536 Support Office, the Frontier Chemistry Center, Faculty of Engineering, Hokkaido University, for
537 allowing us to conduct the NMR measurements. We also thank the High-Energy Accelerator Research
538 Organization for their help with the WAXS measurements at the Photon Factory, KEK, Tsukuba, Japan.
539 This study was partly performed using a supercomputer system at the Information Initiative Center,
540 Hokkaido University, Sapporo, Japan. We would like to thank Professor Chihiro Yamane of Kobe
541 Women's University for valuable discussions. We would like to thank Editage (www.editage.jp) for
542 English language editing.

References

- Bikova, T., & Treimanis, A. (2002). Problems of the MMD analysis of cellulose by SEC using DMA/LiCl: A review. *Carbohydrate Polymers*, 48(1), 23–28. [https://doi.org/10.1016/S0144-8617\(01\)00207-7](https://doi.org/10.1016/S0144-8617(01)00207-7)
- Cai, J., & Zhang, L. (2005). Rapid dissolution of cellulose in LiOH/urea and NaOH/urea aqueous solutions. *Macromolecular Bioscience*, 5(6), 539–548. <https://doi.org/10.1002/mabi.200400222>
- Cao, Y., Wu, J., Zhang, J., Li, H., Zhang, Y., & He, J. (2009). Room temperature ionic liquids (RTILs): A new and versatile platform for cellulose processing and derivatization. *Chemical Engineering Journal*, 147(1), 13–21. <https://doi.org/10.1016/j.cej.2008.11.011>
- Dinand, E., Vignon, M., Chanzy, H., & Heux, L. (2002). Mercerization of primary wall cellulose and its implication for the conversion of cellulose I → cellulose II. *Cellulose*, 9(1), 7–18. <https://doi.org/10.1023/A:1015877021688>
- Ford, Z. M., Stevens, E. D., Johnson, G. P., & French, A. D. (2005). Determining the crystal structure of cellulose III by modeling. *Carbohydrate Research*, 340(5), 827–833. <https://doi.org/10.1016/j.carres.2005.01.028>
- Glasser, W. G., Atalla, R. H., Blackwell, J., Brown Jr, M. M., Burchard, W., French, A. D., Klemm, D. O., & Nishiyama, Y. (2012). About the structure of cellulose: Debating the Lindman hypothesis. *Cellulose*, 19(3), 589–598. <https://doi.org/10.1007/s10570-012-9691-7>

561 Horii, F., Hirai, A., & Kitamaru, R. (1983). Solid-state ^{13}C -NMR study of conformations of
 562 oligosaccharides and cellulose: Conformation of CH_2OH group about the exo-cyclic C-C bond.
 563 *Polymer Bulletin*, 10(7–8), 357–361. <https://doi.org/10.1007/BF00281948>
 564 Idström, A., Schantz, S., Sundberg, J., Chmelka, B. F., Gatenholm, P., & Nordstierna, L. (2016). ^{13}C
 565 NMR assignments of regenerated cellulose from solid-state 2D NMR spectroscopy.
 566 *Carbohydrate Polymers*, 151, 480–487. <https://doi.org/10.1016/j.carbpol.2016.05.107>
 567 Isobe, N., Noguchi, K., Nishiyama, Y., Kimura, S., Wada, M., & Kuga, S. (2013). Role of urea in
 568 alkaline dissolution of cellulose. *Cellulose*, 20(1), 97–103. [https://doi.org/10.1007/s10570-012-](https://doi.org/10.1007/s10570-012-9800-7)
 569 9800-7
 570 Isogai, A. (1997). NMR analysis of cellulose dissolved in aqueous NaOH solutions. *Cellulose*, 4(2),
 571 99–107. <https://doi.org/10.1023/A:1018471419692>
 572 Isogai, A., & Atalla, R. H. (1998). Dissolution of cellulose in aqueous NaOH solutions. *Cellulose*, 5(4),
 573 309–319. <https://doi.org/10.1023/A:1009272632367>
 574 Kamide, K., Kowsaka, K., & Okajima, K. (1985). Determination of intramolecular hydrogen bonds
 575 and selective coordination of sodium cation in alkalicellulose by cp/mass ^{13}C nmr. *Polymer*
 576 *Journal*, 17(5), 707–711. <https://doi.org/10.1295/polymj.17.707>
 577 Kamide, K., Okajima, K., Matsui, T., & Kowsaka, K. (1984). Study on the solubility of cellulose in
 578 aqueous alkali solution by deuteration IR and ^{13}C NMR. *Polymer Journal*, 16(12), 857–866.

579 <https://doi.org/10.1295/polymj.16.857>

580 Kim, N. H., Sugiyama, J., & Okano, T. (1991). X-ray and electron diffraction study of Na-cellulose I:

581 The effect of washing temperature on the structure of Na-cellulose I. *Mokuzai Gakkaishi*, 37(7),

582 637–643.

583 Langan, P., Nishiyama, Y., & Chanzy, H. (1999). A revised structure and hydrogen-bonding system in

584 cellulose II from a neutron fiber diffraction analysis. *Journal of the American Chemical Society*,

585 121(43), 9940–9946. <https://doi.org/10.1021/ja9916254>

586 Lindman, B., Karlström, G., & Stigsson, L. (2010). On the mechanism of dissolution of cellulose.

587 *Journal of Molecular Liquids*, 156(1), 76–81. <https://doi.org/10.1016/j.molliq.2010.04.016>

588 Massiot, D., Fayon, F., Capron, M., King, I., Le Calvé, S., Alonso, B., Durand, J. O., Bujoli, B., Gan,

589 Z., & Hoatson, G. (2002). Modelling one- and two-dimensional solid-state NMR spectra.

590 *Magnetic Resonance in Chemistry*, 40(1), 70–76. <https://doi.org/10.1002/mrc.984>

591 Medronho, B., & Lindman, B. (2014). Competing forces during cellulose dissolution: From solvents

592 to mechanisms. *Current Opinion in Colloid and Interface Science*, 19(1), 32–40.

593 <https://doi.org/10.1016/j.cocis.2013.12.001>

594 Miyamoto, H., Schnupf, U., Ueda, K., & Yamane, C. (2015). Dissolution mechanism of cellulose in a

595 solution of aqueous sodium hydroxide revealed by molecular dynamics simulations. *Nordic Pulp*

596 *and Paper Research Journal*, 30(1), 67–77. <https://doi.org/10.3183/npprj-2015-30-01-p067-077>

597 Newman, R. H., & Davidson, T. C. (2004). Molecular conformations at the cellulose-water interface.
 598 *Cellulose*, 11(1), 23–32. <https://doi.org/10.1023/B:CELL.0000014778.49291.c6>
 599 Nishimura, H., & Sarko, A. (1991). Mercerization of Cellulose. 6. Crystal and Molecular Structure of
 600 Na-Cellulose IV. *Macromolecules*, 24, 771–778.
 601 Nishiyama, Y., Johnson, G. P., French, A. D., Forsyth, V. T., & Langan, P. (2008). Neutron
 602 crystallography, molecular dynamics, and quantum mechanics studies of the nature of hydrogen
 603 bonding in cellulose I β . *Biomacromolecules*, 9(11), 3133–3140.
 604 <https://doi.org/10.1021/bm800726v>
 605 Nishiyama, Y., Sugiyama, J., Chanzy, H., & Langan, P. (2002). Crystal Structure and Hydrogen
 606 Bonding System in Cellulose I β from Synchrotron X-ray and Neutron Fiber Diffraction. *Journal*
 607 *of the American Chemical Society*, 124(31), 9074–9082. <https://doi.org/10.1021/ja037055w>
 608 Nishiyama, Y., Sugiyama, J., Chanzy, H., & Langan, P. (2003). Crystal Structure and Hydrogen-
 609 Bonding System in Cellulose Ia from Synchrotron X-ray and Neutron Fiber Diffraction. *Journal*
 610 *of the American Chemical Society*, 125(47), 14300–14306.
 611 <https://doi.org/https://doi.org/10.1021/ja0257319>
 612 Nomura, S., Sato, S. ichiro, & Erata, T. (2020). DFT approach to the pathway of conformational
 613 changes of cellulose C6-hydroxymethyl group with simple cellotetraose model involving the
 614 mechanism of mercerization process. *Chemical Physics Letters*, 742(October 2019), 137154.

615 <https://doi.org/10.1016/j.cplett.2020.137154>

616 Okano, T., & Sarko, A. (1984). Mercerization of cellulose. I. X-ray diffraction evidence for
617 intermediate structures. *Journal of Applied Polymer Science*, 29(12), 4175–4182.
618 <https://doi.org/10.1002/app.1984.070291247>

619 Okano, T., & Sarko, A. (1985). Merceization of cellulose II. Alkali-cellulose intermediates and a
620 possible mercerization mechanism. *Journal of Applied Polymer Science*, 30(1), 325–332.
621 <https://doi.org/10.1002/app.1985.070300128>

622 Park, S., Johnson, D. K., Ishizawa, C. I., Parilla, P. A., & Davis, M. F. (2009). Measuring the
623 crystallinity index of cellulose by solid state ¹³C nuclear magnetic resonance. *Cellulose*, 16(4),
624 641–647. <https://doi.org/10.1007/s10570-009-9321-1>

625 Phyto, P., Wang, T., Yang, Y., O'Neill, H., & Hong, M. (2018). Direct determination of hydroxymethyl
626 conformations of plant cell wall cellulose using ¹H polarization transfer solid-state NMR.
627 *Biomacromolecules*, 19(5), 1485–1497. <https://doi.org/10.1021/acs.biomac.8b00039>

628 Porro, F., Bedue, O., Chanzy, H., & Heux, L. (2007). Solid-state ¹³C NMR study of Na⁺-cellulose
629 complexes. *Biomacromolecules*, 8(8), 2586–2593. <https://doi.org/10.1021/bm0702657>

630 Sawada, D., Nishiyama, Y., Shah, R., Forsyth, V. T., Mossou, E., O'Neill, H. M., Wada, M., & Langan,
631 P. (2022). Untangling the threads of cellulose mercerization. *Nature Communications*, 13(1), 1–
632 6. <https://doi.org/10.1038/s41467-022-33812-w>

633 Sayyed, A. J., Deshmukh, N. A., & Pinjari, D. V. (2019). A critical review of manufacturing processes
 634 used in regenerated cellulosic fibres: viscose, cellulose acetate, cuprammonium, LiCl/DMAc,
 635 ionic liquids, and NMMO based lyocell. *Cellulose*, 26(5), 2913–2940.
 636 <https://doi.org/10.1007/s10570-019-02318-y>

637 Sobue, H., Kiessig, H., & Hess, K. (1939). Das System Cellulose–Natriumhydroxyd–Wasser in
 638 Abhängigkeit von der Temperatur. *Zeitschrift für Physikalische Chemie*, 43B(1), 309–328.
 639 <https://doi.org/10.1515/zpch-1939-4324>

640 Swatloski, R. P., Spear, S. K., Holbrey, J. D., & Rogers, R. D. (2002). Dissolution of cellose with ionic
 641 liquids. *Journal of the American Chemical Society*, 124(18), 4974–4975.
 642 <https://doi.org/10.1021/ja025790m>

643 Wada, M., Chanzy, H., Nishiyama, Y., & Langan, P. (2004). Cellulose III I crystal structure and
 644 hydrogen bonding by synchrotron X-ray and neutron fiber diffraction. *Macromolecules*, 37(23),
 645 8548–8555. <https://doi.org/10.1021/ma0485585>

646 Wang, H., Kataoka, H., Tsuchikawa, S., & Inagaki, T. (2022). Terahertz time-domain spectroscopy as
 647 a novel tool for crystallographic analysis in cellulose: Cellulose I to cellulose II, tracing the
 648 structural changes under chemical treatment. *Cellulose*, 29(6), 3143–3151.
 649 <https://doi.org/10.1007/s10570-022-04493-x>

650 Yamashiki, T., Kamide, K., Okajima, K., Kowsaka, K., Matsui, T., & Fukase, H. (1988). Some

651 characteristic features of dilute aqueous alkali solutions of specific alkali concentration (2.5
652 mol l⁻¹) which possess maximum solubility power against cellulose. *Polymer Journal*, 20(6),
653 447–457. <https://doi.org/10.1295/polymj.20.447>

654 Zuckerstätter, G., Terinte, N., Sixta, H., & Schuster, K. C. (2013). Novel insight into cellulose
655 supramolecular structure through ¹³C CP-MAS NMR spectroscopy and paramagnetic relaxation
656 enhancement. *Carbohydrate Polymers*, 93(1), 122–128.
657 <https://doi.org/10.1016/j.carbpol.2012.05.019>

658