The Iα → Iβ Transformation of Highly Crystalline Cellulose by Annealing in Various Mediums

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ABSTRACT: The Iα → Iβ conversion of cellulose was investigated by annealing *Valonia* and bacterial cellulose in a series of mediums ranging from helium gas to organic solvents of various polarities. The annealing treatment was followed by ^{13}C CP/MAS NMR and infrared spectroscopy together with electron diffraction analysis. These techniques revealed that when annealing conditions ranging from 260 °C and 30 min to 280 °C and 60 min were selected, around 80% of the Iα phase could be converted into the Iβ, whereas a residual 20% remained unmodified. With polar solvents such as glycol, glycerol, etc., the transformation occurred at temperatures lower than with nonpolar mediums such as dibenzyl ether, heptane, helium, etc. Also, samples of lower crystallinity, e.g. bacterial cellulose, were converted more easily than specimens of high crystallinity such as *Valonia*.

Introduction

The interest for the structure of cellulose has been revived recently by the discovery of a crystalline dimorphism which existed in most celluloses and in particular with some samples of high crystallinity. In 1984, Atalla and VanderHart[1] were the first to establish by ^{13}C CP/MAS NMR spectroscopy that native cellulose or cellulose I was a composite of two distinct crystalline phases, namely cellulose Iα and cellulose Iβ. Their observation was based on the detailed analysis of the multiplicity occurring at the signal of each carbon atom in a variety of celluloses. Typically, there were two families of cellulose specimens: samples such as bacterial or *Valonia* cellulose were rich in Iα, whereas ramie, cotton, or wood were dominated by Iβ.[2] More recently, the analysis of the highly crystalline cellulose from tunicin showed that it consisted entirely of the phase Iβ.[3,4] Therefore tunicin could be selected as the crystalline standard for the Iβ phase. As of today, no cellulose specimen of the pure Iα phase has been discovered or produced.

One of the advantages of the Iα/Iβ dimorphism concept is that it explains several singularities which have been reported when celluloses were examined by infrared[5-7] or Raman [8,9] spectroscopy. In diffraction analysis unusual data were also found as it was observed that the diffraction diagrams of cellulose from *Valonia*[10,11] or other algae contained more diffraction spots than those of the cellulose of higher plants such as ramie.[12,13] Quite recently, the same phenomenon was also observed when the diffraction diagram of *Valonia* was compared to that of tunicin.[14] Despite the fact that this animal cellulose has a crystallinity as high as that of *Valonia*, its diffraction diagram corresponds only to a subset of that of *Valonia*. This is well accounted for in the biphasic hypothesis where tunicin cellulose, being a pure Iβ phase, should match only a fraction of *Valonia* cellulose which contains both celluloses Iα and Iβ. In a recent study, the coexistence of the two phases was even demonstrated when we succeeded in recording electron diffractograms of each phase during an investigation of the cellulose from the green alga *Microdictyon*. The analysis of these diagrams showed that the phase Iα was a $P1$ triclinic allomorph with one cellulose chain per unit cell whereas the Iβ component was attributed to a monoclinic $P2_1$ structure with two chains per unit cell.[15]

The thermal stability of the two phases Iα and Iβ was investigated by Horii et al.[16] In a study performed with *Valonia* cellulose these authors concluded that the Iα phase was metastable and could be converted almost entirely into the Iβ by annealing the samples with saturated steam at a temperature higher than 260 °C. When trying to reproduce this experiment, we found that this transformation led to a drastic destruction of the microfibrillar structure of *Valonia* which became converted into small microcrystalline elements. More recently, Yamamoto et al. have shown that the Iα → Iβ transformation could also be obtained by an hydrothermal treatment at 260 °C in the presence of 0.1 N NaOH.[4] In that case, the ultrastructural integrity of the sample was maintained as the treated and untreated samples were undistinguishable by electron microscopy in image mode.[17] The conversion had nevertheless taken place as the triclinic Iα cellulose could no longer be observed in the annealed specimens by electron diffraction analysis.[17]

In the Iα → Iβ conversion of cellulose, the temperature seems to be the determinant parameter which governs the transformation. The role played by water and alkali to achieve the conversion is not obvious as, in particular, NaOH can act either as a stabilizing additive[4] to the medium or as a cellulose swelling agent. In order to clarify the transformation mechanism, conditions where the conversion could be obtained in a variety of mediums should be interesting to follow. With this goal in mind, we have attempted in this work, to achieve the Iα → Iβ transformation not only in inert gases but also in a series of solvents of various polarities. Both protic and aprotic solvents were tested under annealing conditions ranging from 260 to 280 °C. The effect of these treatments on *Valonia* and bacterial cellulose was followed by ^{13}C CP/MAS NMR spectroscopy, Fourier transform infrared spectroscopy (FT-IR), and electron diffraction analysis.

Experimental Section

Materials. Cellulose Samples. *Valonia ventricosa* vesicles were harvested from the sea bed in the Lower Keys, FL. The fresh cells were slit open and emptied and their walls allowed to

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dry by pressing between two sheets of tissue paper. The dried walls were scraped with tweezers to remove most of the noncellulosic components. The cell walls were then boiled 3 times for 3 h in distilled water and thereafter 3 times for 2 h in 0.1 N NaOH under nitrogen. The purified cell walls were then rinsed in distilled water until neutrality, before being dried between two sheets of filter paper.

Bacterial cellulose from *Acetobacter xylinum* was cultivated for 18 days at 30 °C in Roux culture bottles. The resulting cellulose pads were washed under tap water for two days, extracted for 6 days with 0.5% NaOH, and washed with distilled water until neutrality was reached. The purified pads were then freeze dried and stored until usage.

Chemicals. Reagent-grade glycol, glycerol, heptane, ethanol, and acetone were purchased from Prolabo, whereas 1,2-dimethoxyethane (monoglyme), polyethylene glycol 300, and dibenzyl ether were obtained from Fluka. These solvents were used without further purification.

Diethylene glycol dimethyl ether (diglyme) from Fluka was dried with NaOH pellets first at room temperature and then by refluxing under reduced nitrogen pressure. It was then distilled at 10 Torr.

Helium gas of purity U was obtained from Air Liquide.

Annealing Treatment. The cellulose specimens were inserted under nitrogen in a small glass ampule filled with the given solvent. The ampule was sealed and positioned inside a 10-cm^3 vapor pressure bomb to which a small amount of the solvent used was added to build a counter pressure during the experiment. The bomb was hermetically sealed and heated in an oil bath to the given temperature (between 260 and 280 °C) for a variable time ranging from 30 to 240 min. The bomb was then cooled by quenching under tap water. The annealed sample was removed and washed thoroughly with distilled water until it reached neutrality.

For the samples annealed in helium gas, the cellulose specimens were inserted inside the pressure bomb which was flushed for 15 min with helium. The bomb was then sealed hermetically and annealed for 30 or 60 min in an oil bath before being cooled by quenching under tap water.

Electron Microscopy. All observations and diffraction experiments were achieved with a Philips EM 400 T electron microscope operated at 120 kV. Electron diffraction diagrams were recorded with electron doses kept to a minimum and the diagrams were recorded on Mitsubishi (MEM) emulsion.

Infrared Spectroscopy. Thin layers of *Valonia* cell wall having thicknesses between 5 and 20 μm were prepared by delamination in water. These specimens were mounted with tweezers on micro disk sample holders perforated by a 500-μm hole.

A Fourier transform infrared (FT-IR) spectrophotometer (Perkin Elmer 1720 X), equipped with a microfocus accessory, was used. All the spectra were recorded in transmission mode with an accumulation of 20 scans and a resolution of 2 cm^{-1}, in the range of 4000 cm^{-1} to 400 cm^{-1}.

^{13}C CP/MAS NMR Spectroscopy. The spectra were recorded with a Bruker MSL 300 at 75.47 MHz under a static field of 7.04 T. The samples were inserted in a zirconia rotor and the spinning frequency was set between 3.0 and 4.0 kHz. All spectra were obtained with a contact time of 0.5 ms and a repetition time of 8 s. Resolution enhanced spectra were obtained by multiplication of the free-induction decay using a function of the form: $\exp\{-at - bt^2\}$ where $a = \pi \times LB$ and $b = -a/(2 \times GB \times AQ)$ with the line broadening LB = − 50 Hz, the Gaussian broadening factor GB = 0.5, and the acquisition time AQ = 40 ms.

In a given sample, the quantitative measurement of the Iα and Iβ phases was obtained by deconvoluting the triplet corresponding to the C1 carbon. This was achieved with the initial spectra before resolution enhancement. A line-shape simulation program was selected: by generating several Lorentzian lines, it takes into account the chemical shifts, the intensities and line widths of the corresponding signals. The content in Iα phase was taken as proportional to the ratio of the surface of the deconvoluted peak at 105.6 ppm over the sum of the surfaces of the three deconvoluted peaks at 104.5, 105.6, and 106.2 ppm. The percentage of conversion was obtained from

$$\%\ I\alpha \rightarrow I\beta = \{1 - (\%\ I\alpha \text{ after annealing} / \%\ I\alpha \text{ initial})\}100$$

Results

Fourier Transform Infrared Analysis. In the infrared spectra of cellulose, it was shown[14] that an OH stretching band, present as a secondary maximum near 3240 cm^{-1}, could be attributed to cellulose Iα: it occurred in the spectra of initial *Valonia* and bacterial cellulose but was not found in those of pure Iβ cellulose such as tunicin.[6] Also, this band disappeared when the conversion Iα → Iβ took place during the annealing treatment of *Valonia* or bacterial cellulose.[14] Similarly, a band near 750 cm^{-1} which behaved as that near 3240 cm^{-1}, could also be attributed to cellulose Iα. This band was better resolved than the band near 3240 cm^{-1}: its presence, decrease, or disappearance was thus a good way to estimate the extent of the transformation Iα → Iβ in a given annealing experiment. The recording of infrared spectra by FT-IR analysis is quite rapid and can furthermore be applied to minute amounts of sample. It was used here to quickly monitor a series of annealing experiments where the annealing mediums, the temperatures, and the treatment times were varied.

Typical FT-IR spectra in the region 800–400 cm^{-1} corresponding to annealing experiments of *Valonia* cellulose in glycol and diglyme are shown in Figures 1 and 2. In Figure 1 the annealing in glycol is compared with that in diluted NaOH following the Yamamoto et al. procedure.[4] In this figure, one verifies that the band near 750 cm^{-1} which was clearly observed in the initial sample (Figure 1a) has almost disappeared when *Valonia* is annealed in dilute NaOH at 260 °C for 30 min (Figure 1b). When glycol is used instead of dilute NaOH, there is only a small decrease of this band after an annealing at 260 °C for 30 min (Figure 1c). In order to obtain a spectrum identical to that in Figure 1b, one needs to raise the temperature to 270 °C and to wait also for 30 min at this temperature (Figure 1d). In both, Figure 1, parts b and d, one notices also that a band near 710 cm^{-1} which was attributed to Iβ cellulose[14] is markedly increased.

When diglyme was used as the annealing medium, significant conversion could not be obtained at 270 °C after 30 min of annealing. In Figure 2, the FT-IR spectra of *Valonia* samples treated in diglyme are compared with those annealed in glycol. As in Figure 1, one sees that the band near 750 cm^{-1} was sensitive to the annealing treatment. Whereas it had almost disappeared in the sample treated in glycol for 30 minutes at 270 °C (Figure 2b), it was unaffected in the spectrum of the sample treated in diglyme at 270 °C for 30 min: this spectrum (Figure 2c) was identical to that of the initial material (Figure 2a). When however, the annealing in diglyme was extended to 60 min (Figure 2d), a noticeable decrease in the intensity of the band near 750 cm^{-1} can be observed, whereas that of the band near 710 cm^{-1} is increased. It can be estimated that under such conditions, a conversion of nearly 75% has been achieved. If one wants to achieve a nearly total conversion with diglyme, an annealing temperature of 280 °C and a treatment time of 30 min have to be applied.

Table I is a survey of all the experiments which were done in this study. This table indicates that at a given temperature and for a given annealing time, the polarity of the solvent is the determinant factor which controls the ease of the transformation Iα → Iβ. Dilute NaOH is the most polar solvent which was used. In such medium, the Iα → Iβ transformation is quite rapid as it occurs within 30 min at a temperature as low as 260 °C. Glycol, glycerol,

Figure 1. Details of the FT-IR spectra in the region 800–400 cm^{-1} of cellulose from *Valonia ventriocosa*: a, initial sample; b, as in a but after annealing in 0.1 N NaOH at 260 °C for 30 min; c, as in a but after annealing in glycol at 260 °C for 30 min; d, as in a but after annealing in glycol at 270 °C for 30 min. The band near 750 cm^{-1} (identified by *) is specific of the Iα phase. The band near 710 cm^{-1} (identified by **) is specific of the Iβ phase.

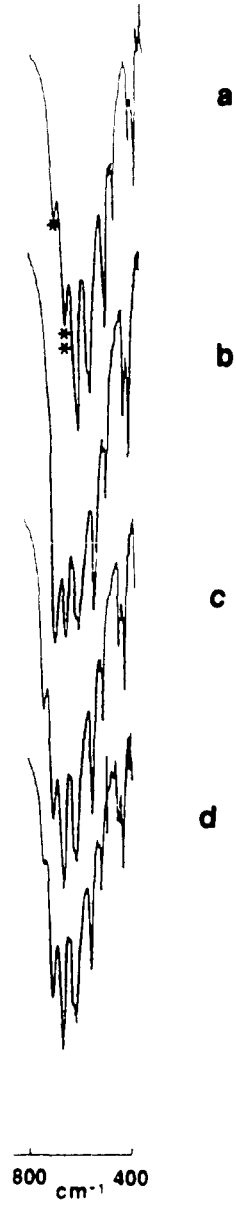

Figure 2. Details of the FT-IR spectra in the region 800–400 cm^{-1} of cellulose from *Valonia ventricosa*: a, initial sample; b, as in a but after annealing in glycol at 270 °C for 30 min; c, as in a but after annealing in diglyme at 270 °C for 30 min; d, as in a but after annealing in diglyme at 270 °C for 60 min. The band near 750 cm^{-1} (identified by *) is specific of the Iα phase. The band near 710 cm^{-1} (identified by **) is specific of the Iβ phase.

ethanol, and polyethylene glycol are less polar solvents and with these, the temperature has to be raised to 270 °C for at least 60 min to obtain maximal conversion. In an aprotic solvent such as diglyme the conversion is also observed but only at 280 °C and after 30 min of annealing treatment.

In Table I, one sees that for inert or nonpolar mediums, the conversion requires a temperature of 280 °C and an annealing time of 60 min to take place: this is the case in particular when the experiments were achieved in dibenzyl ether, heptane, or even helium gas. Thus these conditions appear to be the upper limit beyond which most of the Iα phase existing in *Valonia* has been converted to Iβ cellulose.

^{13}C CP/MAS NMR Spectroscopy. ^{13}C CP/MAS NMR spectroscopy is another tool for monitoring the Iα → Iβ transformation. As it requires substantial amount of sample, it was limited only to two series of experiments, namely those achieved in glycol and diglyme.

Figure 3 reproduces some of the spectra recorded during the annealing of *Valonia* in glycol for different times and at different temperatures. Figure 3a shows the initial *Valonia* spectrum which matches closely those reported by Atalla and VanderHart.[1,2] In this spectrum, the resonance centered at 105.6 ppm for the C1 carbon, together with that at 90.4 for C4 are typical of the Iα component which is dominant in *Valonia* cellulose. During the annealing in glycol, one notices a sharp decrease of these two resonance signals. If the sample is treated at 260 °C for 60 min, these peaks lose more than half of their intensity (Figure 3b). If higher temperatures are selected, the extent of the conversion goes further as these peaks have almost disappeared after an annealing period of 1 h at 270 °C (Figure 3d). If still more severe conditions (e.g. higher temperatures or longer annealing period) are selected, the

Table I
Extent of the Iα → Iβ Conversion of *Valonia* Cellulose after Annealing in a Series of Mediums (Estimated from the Decrease of the Infrared Absorption Band near 750 cm^{-1})

	annealing conditions, %					
medium	260 °C for 30 min	260 °C for 60 min	270 °C for 30 min	270 °C for 60 min	280 °C for 30 min	280 °C for 60 min
aqueous NaOH 0.1 N	maximum	maximum	maximum	maximum	maximum	maximum
glycol	25	50	maximum	maximum	maximum	maximum
glycerol	25		50		75	maximum
PEG 300	25		50		maximum	maximum
ethanol	25		75		maximum	maximum
acetone	25		50		maximum	maximum
diglyme	no	no	no	50	75	maximum
monoglyme	no		50		maximum	maximum
dibenzyl ether	no	no	no	no	25	maximum
heptane	no	no	no	25	50	maximum
helium gas	no	no	25	25	75	maximum

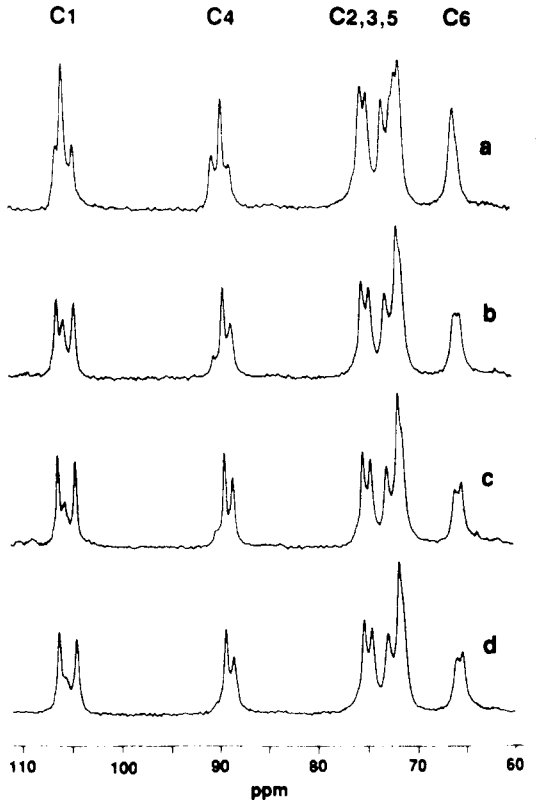

Figure 3. ^{13}C CP/MAS NMR spectra at 75.47 MHz of *Valonia ventricosa*: a, initial sample; b, as in a but after annealing in glycol at 260 °C for 60 min; c, as in a but after annealing in glycol at 270 °C for 30 min; d, as in a but after annealing in glycol at 270 °C for 60 min. The resonances attributed to the carbons C1, C4, and C6 and the group C2, C3, and C5 have been identified at the top of the figure.

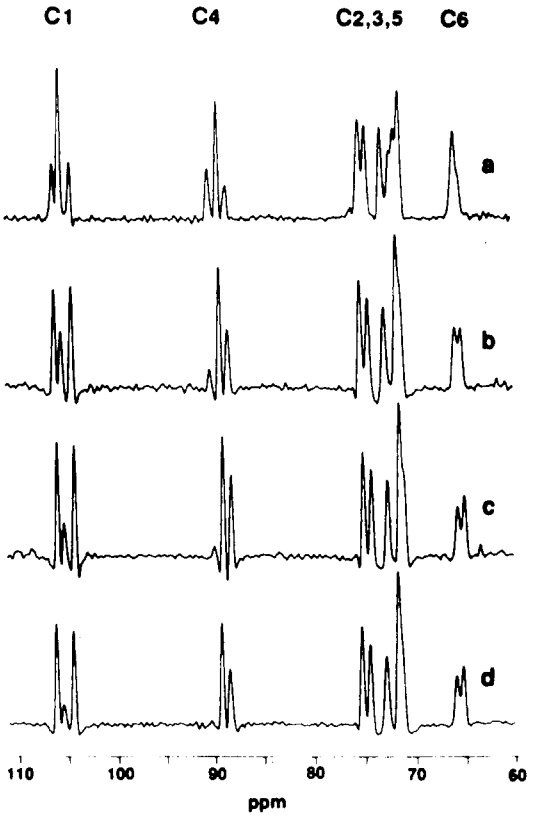

Figure 4. CP/MAS ^{13}C NMR spectra as in Figure 3 but after resolution enhancement, applying the parameters LB = −50 Hz and GB = 0.5.

spectra remain as in Figure 3d which seems to be the ultimate that can be obtained, at least in this study.

In Figure 4, the spectra shown in Figure 3 have been resolution enhanced. This allows the visualization of the relative intensity changes of the peaks corresponding to the Iα and Iβ phases. At this resolution, the C1 and C4 carbons signals occur as triplets. For quantitative purpose, their deconvolution was performed on the original spectra, assuming three Lorentzian curves. This is illustrated in Figure 5 which shows the deconvolution of the C1 carbon peak of a *Valonia* sample annealed in glycol at 260 °C for 60 min. In Figure 5, as well as in all the other spectra, a good agreement between experimental and simulated spectra was always obtained.

The results of the deconvolution treatment applied to the spectra shown in Figure 3 are listed in Table II which gives the percentage of Iα as well as the extent of conversion Iα → Iβ for each sample. This treatment gives a content of 58% of phase Iα for the initial *Valonia* sample, 24% in the spectrum shown in Figure 4b, 17% in that in Figure 4c, and 13% in the final spectrum in Figure 4d. The corresponding percentage of Iα → Iβ transformation is 0, 59, 70, and 77%.

In Figures 6 (initial) and 7 (resolution enhanced) are shown some of the ^{13}C CP/MAS NMR spectra corresponding to the annealing experiments in diglyme medium. Both *Valonia* and bacterial cellulose are considered and their initial spectra represented (Figure 6, part a for bacterial and part b for *Valonia* cellulose). One sees that in diglyme, the transformation is nearly complete at 270 °C and after 60 min of annealing in the case of bacterial cellulose (Figure 6d). On the other hand for *Valonia* (Figure 6e), 270 °C for 60 min is not enough since less than half of the initial Iα phase has been converted to Iβ under such conditions. One has to go to a temperature of 280

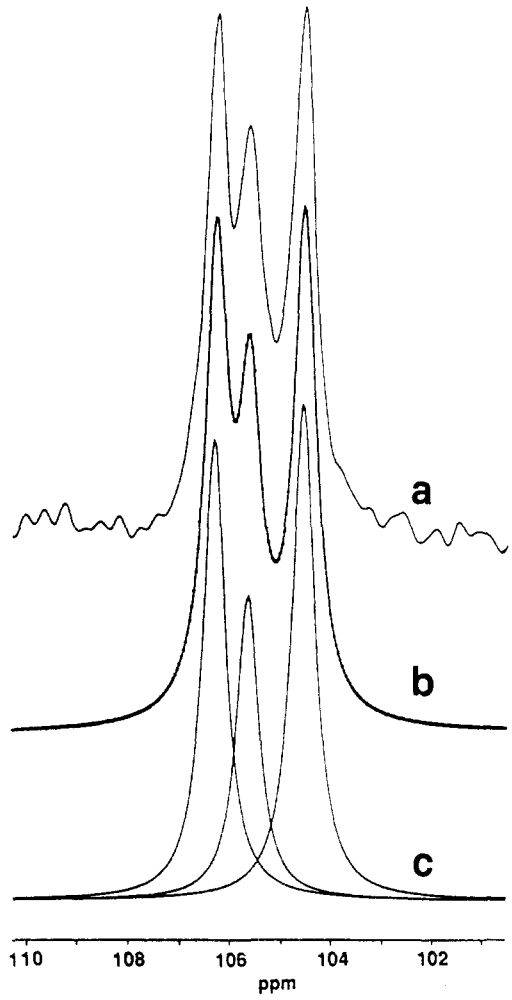

Figure 5. Deconvolution of the C1 signal in Figure 3, part b: a, initial signal; b, sum of the three Lorentzian curves shown in c; c, the three Lorentzian curves with the middle one corresponding to cellulose Iα and the two external to cellulose Iβ. Parts a and b are quasi identical.

Table II
Percentage of the Iα Phase and of the Iα → Iβ Conversion of Cellulose Samples Annealed in Glycol and Diglyme

sample	solvent	temperature, °C	time, min	% of Iα phase[b]	% of Iα → Iβ conversion[c]
Valonia[a]				58	
bacterial[a]				60	
Valonia	glycol	260	60	24	59
Valonia	glycol	270	30	17	70
Valonia	glycol	270	60	13	77
Valonia	diglyme	270	60	38	34
bacterial	diglyme	270	30	32	47
bacterial	diglyme	270	60	21	65

[a] Initial cellulose sample. [b] Calculated by measuring the relative intensity of the C1 central component at 105.6 ppm of deconvoluted ^{13}C CP/MAS NMR C1 signals. [c] Calculated as described in the Experimental Section.

°C for 60 min to obtain a spectrum such as in Figures 6d or 3d.

The percentages of Iα remaining after the annealing treatment as well as the extent of conversion are listed in Table II. One sees in particular that the *Valonia* cellulose sample annealed at 270 °C for 60 min in diglyme still contained 38% of Iα phase (only 34% of conversion in Iβ form). It is remarkable that under the same conditions, only 21% of the Iα phase of bacterial cellulose has been left after the annealing treatment. With bacterial cellulose which initially contained roughly as much Iα cellulose as

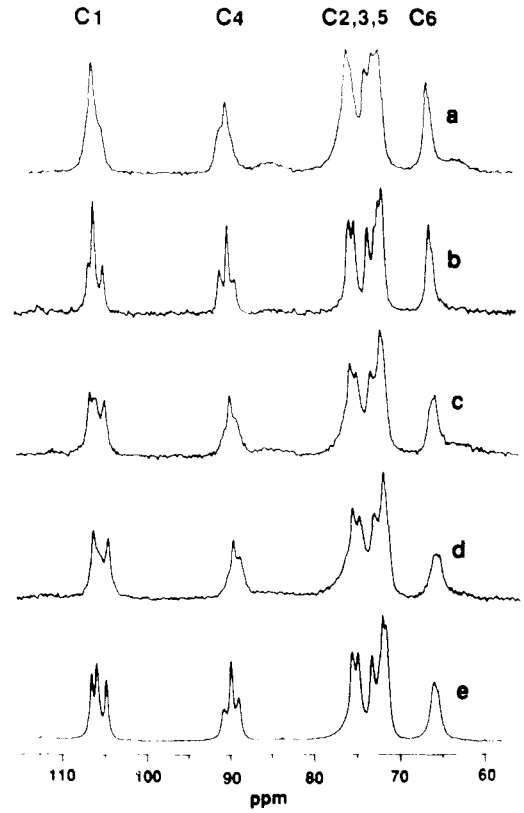

Figure 6. ^{13}C CP/MAS NMR spectra at 75.47 MHz of cellulose samples: a, initial bacterial cellulose; b, initial *Valonia ventricosa* cellulose; c, as in a but after annealing at 270 °C in diglyme for 30 min; d, as in a but after annealing at 270 °C in diglyme for 60 min; e, as in b but after annealing at 270 °C in diglyme for 60 min. The resonance attributed to the carbons C1, C4, and C6 and the group C2, C3, and C5 have been identified at the top of the figure.

Valonia, this represents 65% conversion in the Iβ form.

Electron Diffraction Analysis. The data resulting from FT-IR and ^{13}C CP/MAS NMR spectroscopy can be confirmed by electron diffraction analysis. This is exemplified in Figure 8 which is representative of the annealing experiments of *Valonia* that were achieved in this study. Figure 8a shows a diagram of initial *Valonia*. Following the work of Sugiyama et al.,[15,17] this pattern corresponds to the superposition of a triclinic (Iα) fiber diagram to that of the monoclinic (Iβ). In the pattern of the specimen annealed for 30 min in diglyme at 280 °C (Figure 8b) all the information of the triclinic phase has disappeared and only that of the monoclinic has remained. This diagram has become identical to those of tunicin[14] or *Valonia* cellulose after annealing in dilute NaOH.[17]

Discussion

In the present study, we have combined the data from three techniques, namely FT-IR, ^{13}C CP/MAS NMR, and electron diffraction, to obtain information on the conditions under which the metastable cellulose Iα could be converted into the more stable cellulose Iβ.

Among the parameters which seem to influence the Iα → Iβ transformation, the temperature is the most important. A high temperature is indeed absolutely necessary to achieve the conversion which takes place at 260 °C in the most favorable case (in dilute NaOH), whereas a temperature of 280 °C is required in nonpolar medium or in inert gases. In between these limits, the ease of transformation seems to be directly related to the polarity of the annealing medium, no specific difference being found between protic and aprotic solvents. Among the solvents

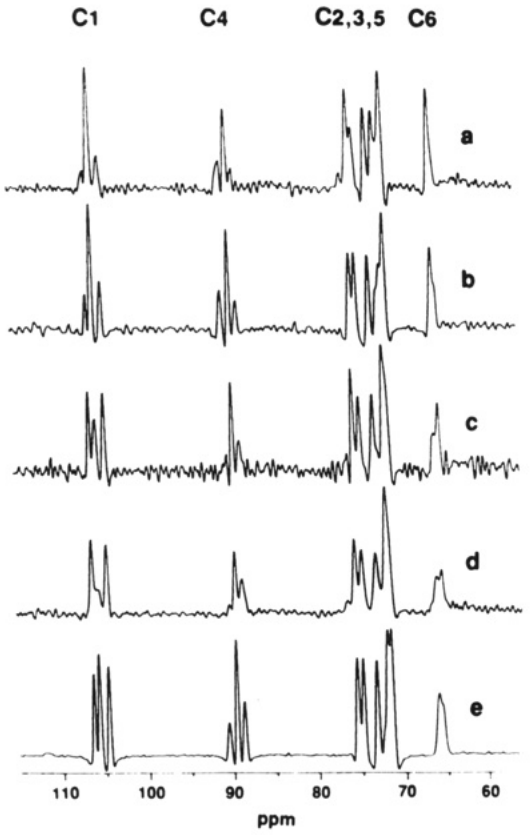

Figure 7. ^{13}C CP/MAS NMR spectra as in Figure 6 but after resolution enhancement, applying the parameters LB = −50 Hz and GB = 0.5.

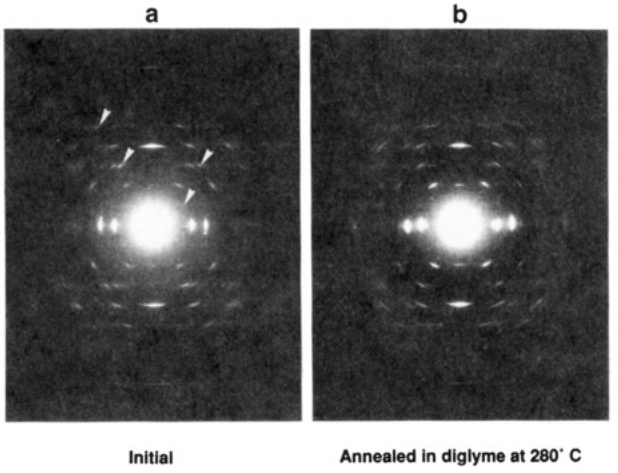

Figure 8. Fiber electron diffraction diagrams taken from a bundle of cellulose microfibrils from *Valonia ventricosa*: a, initial specimen; b, as in a, but after annealing at 280 °C in diglyme for 30 min. The arrows in part a correspond to reflections that can be assigned unambiguously to the triclinic Iα phase.

which were tested, glycol was found to be the best medium to achieve the conversion: with this solvent, nearly total transformation was obtained in 30 min at 270 °C. This temperature is nevertheless 10 °C higher than that required to achieve the annealing in dilute NaOH, which is the most efficient medium reported so far. Other solvents such as glycerol, ethanol, polyethyleneglycol, acetone, monoglyme, and diglyme require an additional 10 °C, i.e. a temperature of 280 °C to give the same results (only 75% conversion in the case of diglyme). As for the nonpolar mediums such as heptane, dibenzyl ether, and inert gases, they require an additional 30 min at 280 °C to yield the maximum conversion.

Another parameter of importance in the Iα → Iβ conversion of cellulose during annealing is the crystallinity and the crystalline width of the cellulose sample that were investigated: specimens of lower crystallinity are converted faster and more easily than those of higher crystallinity. This is exemplified when the results obtained with bacterial cellulose are compared with those of *Valonia*, two samples that are particularly rich in the Iα phase.[2] *Valonia* cellulose has a crystallinity which approaches the 100% value.[18] When studied by X-ray line-broadening measurements, *Valonia* is shown to consist of crystals having average lateral width of the order of 100–120 Å.[19,20] As shown in Table II and Figures 6 and 7, its resistance toward the Iα → Iβ transformation is substantially higher than that of bacterial cellulose which has a crystallinity of only 76% of that of *Valonia*,[8] whereas the average crystalline width of its crystals is only 70% of those of *Valonia* cellulose.[19,20] The dependence of the transformation on the crystallinity and crystalline width of the specimens must reflect a scheme where the progression of the conversion occurs slowly through the Iα crystalline domains of cellulose. Such slow process is corroborated by dependence of the conversion on the annealing time which is also a parameter which has to be accounted for to obtain a successful conversion.

A parallel may be drawn between the heat annealing of cellulose III_I to obtain cellulose IV_I[5,21-23] and the present heat treatment which converts cellulose Iα into cellulose Iβ. The preparation of cellulose IV_I takes place at temperatures that have to be at least 250 °C.[21] In the preparation of cellulose IV_I, as in the above annealing treatment, parameters such as the polarity of the annealing medium or the crystallinity of the specimens influence the temperature at which the conversion is obtained.[21] By infrared analysis, the conversion of cellulose III_I into cellulose IV_I has been explained as resulting from a weakening of the intermolecular hydrogen bonds that hold the cellulose III_I crystal together.[24] A similar reasoning must apply also to the Iα → Iβ conversion. The preliminary data on the two crystal structures of cellulose Iα and Iβ indicate that the density of the Iα crystals is lower than that of the Iβ.[15] Thus, the packing energy of the Iα phase is higher than that of the Iβ. It is therefore logical to find temperatures where the Iα phase will be unstable whereas the Iβ phase remains unaffected. According to this scheme, 280 °C appears to be the ultimate temperature beyond which the intermolecular hydrogen bonds of the phase Iα are no longer stable, no matter what the annealing medium is. In polar mediums, this temperature is depressed by a factor of 10–20 degrees, depending upon the affinity of cellulose for the annealing medium. It would be interesting to test temperatures higher than 280 °C to probe the stability of the Iβ phase and also to see whether cellulose IV_I could result from an annealing of *Valonia* or bacterial cellulose. This however is not possible as the thermal degradation of cellulose becomes quite severe when *Valonia* cellulose is heated beyond 280 °C or when annealing times at this temperature, greater than 60 min are selected. The occcurrence of this degradation is denoted by the presence of new peaks in the 1600–1650 cm^{-1} area of the FT-IR spectra, together with extensive blackening of the samples.

In this study, three independent techniques have been used to prove the Iα → Iβ transformation of cellulose. FT-IR and electron diffraction give essentially the same qualitative results. In particular, they seem to indicate that total or nearly total conversion can be achieved in the temperature range 260–280 °C. The resolution of ^{13}C CP/

MAS NMR data is far better and as a consequence is more suitable to evaluate the extent of conversion.

In the above NMR spectra, as well as those reported by Yamamoto et al.,[4] one sees that the total disappearance of the Iα phase is never achieved. This is illustrated by the fact that starting with *Valonia* or bacterial cellulose, pure Iβ spectra, similar to those reported for tunicin,[3,4] could not be obtained. With either *Valonia* or bacterial cellulose, residual signals of the crystalline Iα phase were always found—in particular at the C1 carbon atom—even in the experiments where the most severe annealing conditions were used. Such residual signals could tentatively be attributed to disordered domains rather than to residual Iα phase. However, when considering the signals at C4, the occurrence of a disordered structure should correspond to the presence of a shoulder at around 85 ppm. Such a shoulder is not observed, at least with the experiments dealing with *Valonia*. Therefore, it is likely that the residual signals denotes the presence of Iα domains that are particularly resistant toward the annealing treatment. The occurrence of these domains may be explained by considering that the Iα → Iβ transformation is achieved in the solid state. During the conversion process some of the cellulose chains have either to slide or rotate past one another when going from the Iα lattice to the 1β.[15] Depending on how the crystalline domains of Iα and Iβ are intermingled, these chain slidings or rotations may or may not take place easily. Previously,[15] it was also shown in *Microdictyon tenuius*—a seaweed whose cell wall resembles closely that of *Valonia*—that the Iα and Iβ crystalline domains alternated at short distances along a given microfibril. Therefore in *Microdictyon* as well as in *Valonia*, there are cellulose chains which go through a succession of Iα and Iβ crystals. As during the annealing treatment, the Iβ domains remain unperturbed, the cellulose chains which cross these domains will resist any molecular movement which is required by the Iα → Iβ transformation in adjacent areas. Thus it is likely that a percentage of Iα domains will be maintained as such, even though a temperature where the Iα phase is no longer stable has been reached. In order to achieve a total conversion, one would look for conditions where some mobility could be imparted to the cellulose chains. This could be obtained by either reducing the crystallinity of the sample or shortening the length of cellulose chains by selective hydrolysis.

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References and Notes

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Registry No. PEG, 25322-68-3; He, 7440-59-7; NaOH, 1310-73-2; glycol, 107-21-1; glycerol, 56-81-5; ethanol, 64-17-5; acetone, 67-64-1; diglyme, 111-96-6; monoglyme, 110-71-4; ether, 103-50-4; heptane, 142-82-5; cellulose, 9004-34-6.