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Note

Determination of the cellulose Iα allomorph content in a tunicate cellulose by CP/MAS ^{13}C-NMR spectroscopy

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The ordered regions in native celluloses are composites of two crystalline phases, cellulose Iα and cellulose Iβ [1–3]. Cellulose Iα is believed to be the dominant form in bacterial and algal celluloses and cellulose Iβ the dominant form in higher plants such as cotton and ramie [1–6]. The existence of substantial amounts of cellulose Iα in different wood pulp samples was recently demonstrated [7] using a quantitative partial least-squares model established between estimates of amorphicity index, cellulose Iα-content, cellulose Iβ-content and the CP/MAS ^{13}C-NMR data of calibration samples. In the present work we have shown that a highly crystalline animal cellulose, tunicate cellulose isolated from *Halocynthia sp.*, contains about 10% of the cellulose Iα allomorph. This finding is in agreement with the coexistence of the two cellulose allomorphs generally found in native celluloses [1,2,6].

So far, to our knowledge, all native celluloses recently reported in the literature have contained the cellulose Iα allomorph, with the exception of the tunicate celluloses, which first were suggested to consist principally (*Salapa* tunicin) [8] and later entirely (*Salapa* and *Halocynthia* tunicin) [9] of the Iβ allomorph. This exception intrigued us since it indicates the existence of differences in the biosynthetic assembly of the cellulose fibrils. An understanding of this phenomenon could be important for the elucidation of the mechanisms governing the architecture of crystalline domains in native celluloses (ref. [9]). Since the tunicate cellulose is highly ordered and not contaminated with hemicelluloses and since the microfibrils are relatively large, the

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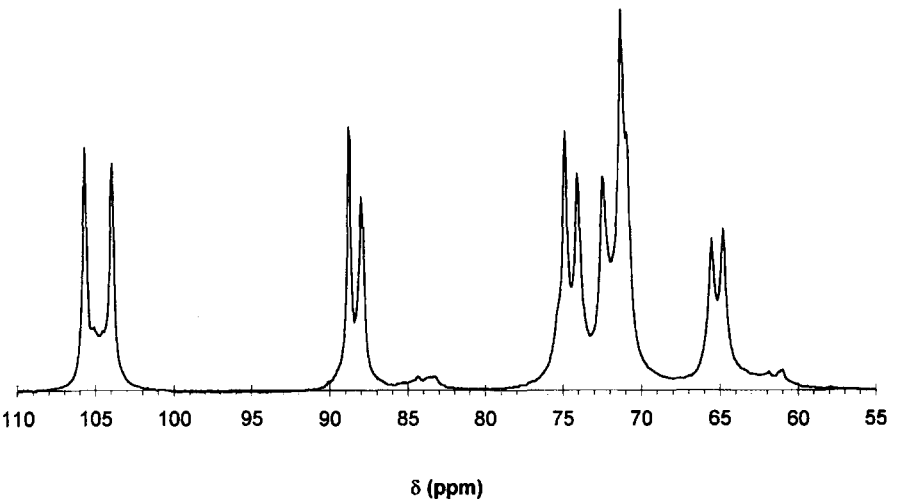

Fig. 1. The CP/MAS ^{13}C-NMR spectrum of tunicate cellulose.

allomorph composition of this cellulose is well suited for a detailed examination by NMR (Fig. 1). We thus decided to subject appropriate regions of the spectrum to non-linear least-squares regression in order investigate the presence of cellulose Iα, at levels not easily detectable by visual inspection.

1. Experimental

Sample preparation.—A cellulose sample (generously supplied by Professor Akira Isogai) was prepared from commercially available tunicate (*Halocynthia sp.*) by removing the interior organs manually. The outer skin, cut into small pieces, was boiled for 6 h in a 0.1 M NaOH solution, washed thoroughly with water, soaked with 0.05 M HCl overnight at ambient temperature and finally washed thoroughly with water. The whole procedure was repeated twice and followed by repeated bleaching with $NaClO_2$ under acidic conditions at 70°C. Completely white pieces were sampled and converted to fine particles using a cylindrical type of homogenizer, followed by freeze-drying [10].

NMR spectroscopy.—The CP/MAS ^{13}C-NMR spectrum was recorded on a Bruker AMX-300 instrument (at ambient temperature) operating at 75.47 MHz. A double air-bearing probe and zirconium oxide rotor was used. The MAS rate was 5000 Hz. Acquisition was performed with a standard CP pulse-sequence using a 3.5 μs proton 90° pulse, a 800 μs contact pulse and a 2.5 s delay between repetitions. For the spectrum, 24,000 transients were recorded, each consisting of 4096 data points, covering a spectral width of 368 ppm. Glycine was used for the Hartmann–Hahn matching procedure and as an external standard for the calibration of chemical shifts relative to tetramethylsilane ($(CH_3)_4Si$). The data point of maximum intensity in the glycine carbonyl line was assigned a chemical shift of 176.03 ppm.

2. Results and discussion

The non-linear least-squares regression was performed by assuming, ad hoc, that the observed spectrum ($S(\omega)$) could be modelled as a superposition of Lorentzian lines [6,11]:

$$S(\omega) = \sum_{i=1}^{n} \frac{a_i}{\pi} \frac{2\tau_i}{1 + 4(\omega - \omega_i)^2 \tau_i^2} \quad (1)$$

Using this model, a χ^2-fit to experimental data was achieved using a Levenberg–Marquardt algorithm [12]. In eq (1), a_i denotes the superposition weight of line i (i.e., its intensity), ω_i gives the centre of the line and τ_i is the inverse of the full width at half-height of the line. Each line is normalized to a_i. In this work all the intensities (a_i) are assumed to be proportional (with the same proportionality factor) to substance amount.

It is difficult to estimate the relative importance of the different factors responsible for the experimentally observed line-shape in CP/MAS ^{13}C-NMR spectra of cellulose. However, it is believed [13] that factors such as distribution of isotropic chemical shifts, inhomogeneous bulk susceptibility and incomplete proton decoupling affect the spectral features most. Some of these features may result in non-Lorentzian lines, making a Lorentzian model inadequate. In such a case, a signal from a nucleus at one specific molecular position may be decomposed into several Lorentzian lines. To prevent such a misinterpretation, it is important to limit the number of lines used (n in eq (1)) by prior knowledge of the sample or similar samples, rather than by the best possible fit in a χ^2-sense. In this work, the number of superimposed Lorentzian lines ($n = 4$ and $n = 5$ for the C-1 and C-4 regions, respectively) was the smallest number giving results in accordance with the composite crystal model [1,2,6].

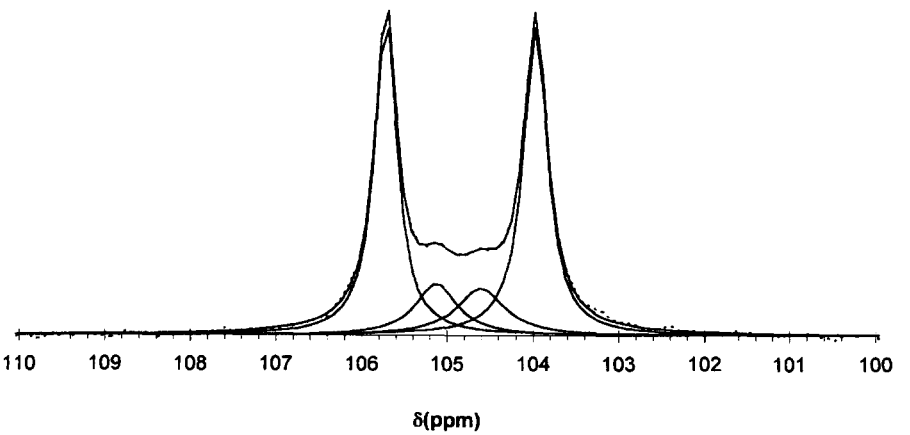

Fig. 2. Non-linear least-squares regression, using four Lorentzian lines, of the C-1 spectral region of tunicate cellulose. The experimental spectrum is shown as a dashed line, and the individual Lorentzian lines and the calculated spectrum are shown as solid lines.

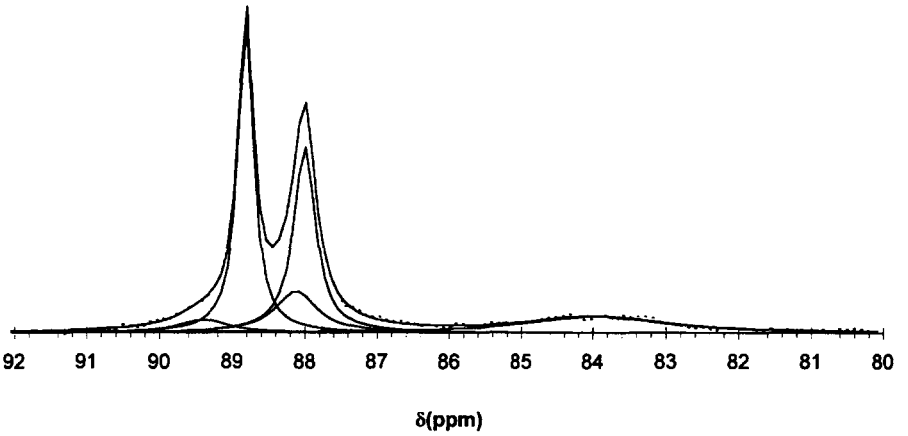

Fig. 3. Non-linear least-squares regression, using five Lorentzian lines, of the C-4 spectral region of tunicate cellulose. The experimental spectrum is shown as a dashed line, and the individual Lorentzian lines and the calculated spectrum are shown as solid lines.

The non-linear least-squares regression results, from the C-1 and C-4 spectral regions of tunicate cellulose, are shown in Figs. 2 and 3 and numerical results are given in Table 1. The estimates of relative amount in Table 1 show that the tunicate cellulose contains about 10% of the cellulose Iα allomorph. As a check of the internal consistency of these results, the relative amounts of crystalline cellulose and cellulose Iα allomorph were determined independently from the C-1 (crystalline = 88(4)%, Iα = 11(3)%) and C-4 (crystalline = 83(8)%, Iα = 9(2)%) spectral regions. The results agree within experimental error.

The unassigned C-4 line at $\delta = 88.1$ ppm has been observed in other highly crystalline celluloses and, although not completely accounted for, is considered to originate from crystalline C-4 [6]. The unambiguous assignment of this extra C-4 line

Table 1
The assignments, full width at half-height (FWHH) of the individual Lorentzian lines and relative amounts of the different allomorphs found in the C-1 and C-4 spectral regions of tunicate cellulose. The numbers in parentheses are one standard deviation

Atom	Allomorph	δ (ppm)	FWHH (ppm)	Relative amount (%)
C-1	Iβ	105.7	0.313 (0.002)	38 (2)
C-1	Iα	105.1	0.591 (0.071)	11 (3)
C-1	amorphous	104.6	0.670 (0.099)	12 (3)
C-1	Iβ	104.0	0.335 (0.002)	39 (2)
C-4	Iα	89.4	0.817 (0.151)	5 (1)
C-4	I$\alpha + \beta$	88.8	0.258 (0.001)	36 (3)
C-4	–	88.1	0.681 (0.123)	13 (3)
C-4	Iβ	88.0	0.357 (0.013)	30 (7)
C-4	amorphous	84.0	2.332 (0.435)	17 (2)

requires a thorough investigation involving a large set of samples. However, as a tentative explanation to its origin we either suggest the presence of partially distorted domains (paracrystalline regions) in the cellulose fibril, or the oversimplification of the spectra due to the use of Lorentzian line-shapes.

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