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Second-derivative FTIR spectra of native celluloses from *Valonia* and tunicin

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ABSTRACT

The greater resolution afforded by the second-derivative mode has enabled differences previously observed in bands near 3240, 750, and 710 cm^{-1} in the FTIR spectra of *Valonia* and tunicin celluloses to be confirmed, and revealed new differences near 2900 and 650 cm^{-1}. These bands are assigned largely to vibrations of groups involved in the system of hydrogen bonds. The remaining bands in the spectra correspond well, which indicates strong similarities in the structures at the conformational level. Differences between the spectra obtained for films and potassium bromide discs are attributed largely to the different orientations of the samples.

INTRODUCTION

Marrinan and Mann[1] found, and Liang and Marchessault[2] confirmed, that the IR spectra of native celluloses could be categorised as (*a*) bacterial and algal celluloses and (*b*) all other types. Moreover, significant differences in the unit-cell parameters were found by X-ray diffraction[3]. However, these observations were not rationalised until the discovery of the crystalline dimorphism of native celluloses by Atalla and VanderHart[4,5]. These workers showed, by using ^{13}C CP/MAS NMR spectroscopy, that native cellulose (cellulose I) was composed of two crystalline phases, namely, celluloses Iα and Iβ, and found that cellulose Iα preponderated in celluloses produced by primitive organisms, whereas cellulose Iβ preponderated in those produced by higher plants. Wiley and Atalla[6] proposed, on the basis of Raman spectral data, that the two phases differed in their hydrogen bonding rather than in their conformations. The highly crystalline cellulose from tunicin consists entirely of the Iβ phase[7], and the cellulose from *Valonia* consists of 60% of the Iα phase and 40% of the Iβ phase[8].

Sugiyama et al.[9] showed that the Iα phase could be converted into the Iβ phase by annealing the cellulose and used FTIR spectroscopy to follow the conversion. Bands near 3240 and 750 cm^{-1} were assigned to the Iα phase and bands near 3270 and 710 cm^{-1} to the Iβ phase. Michell[10] showed that the IR spectra of polysaccharides with much improved resolution could be obtained from the sec-

ond-derivative mode, and used this technique to investigate native celluloses and to confirm the classification of Marrinan and Mann[11]. The second-derivative FTIR spectra of the celluloses from *Valonia* and tunicin have now been examined further.

EXPERIMENTAL

Valonia ventricosa and tunicin celluloses were gifts from Dr. H. Chanzy (see ref. 9). They were purified further by boiling under reflux for 1 h in water then for 8 h in aq 1% NaOH, collected, washed with water, left overnight in 0.05 M HCl, collected, boiled under reflux for 3 h in 2.5 M HCl, collected, washed with water then EtOH, and stored under EtOH.

Valonia filaments, which had separated during the treatment, were sucked with EtOH into a pipette, spread on a rock-salt plate, allowed to dry, lifted from the plate, and placed against the orifice in a microdisc holder. The tunicin sample required shearing under EtOH for delamination but, otherwise, it was prepared as for the *Valonia* sample.

The spectra of the celluloses in KBr discs, obtained for purposes of comparison, were recorded as described[11]. A further spectrum of the *Valonia* cellulose was obtained with the film rotated through ~90°.

RESULTS

The normal and second-derivative spectra of *Valonia* and tunicin celluloses are shown in Figs. 1–5. Peaks in the second-derivative spectra are oriented downwards. Rotation of the *Valonia* cellulose film though ~90° did not change the spectrum significantly.

In the OH stretching region (Fig. 1), the normal spectra of films of the *Valonia* and tunicin celluloses have their strongest bands and centres near 3345 cm^{-1}, but the overall envelope of bands in the *Valonia* spectrum appears to be broader. This view is supported by the second-derivative spectra where that of the *Valonia* cellulose shows several features of roughly equal strength, whereas that of tunicin has only two strong features near 3345 and 3270 cm^{-1}. In addition, the strong band near 3240 cm^{-1} in the spectrum of the *Valonia* cellulose is absent from that of the tunicin cellulose, as noted previously[1,2,9]. However, there is good correspondence of the frequencies between bands in this region of the spectra of the two celluloses.

The spectrum of each cellulose film lacks the strong feature near 3445 cm^{-1} found[10,11] in the second-derivative spectra for the potassium bromide disc of each type of native cellulose and confirmed here for the *Valonia* and tunicin celluloses. The differences observed are probably related to the different orientations of the cellulose molecules in the films and potassium bromide discs relative to the IR radiation. The feature at 3240 cm^{-1} is present in the second-derivative spectrum

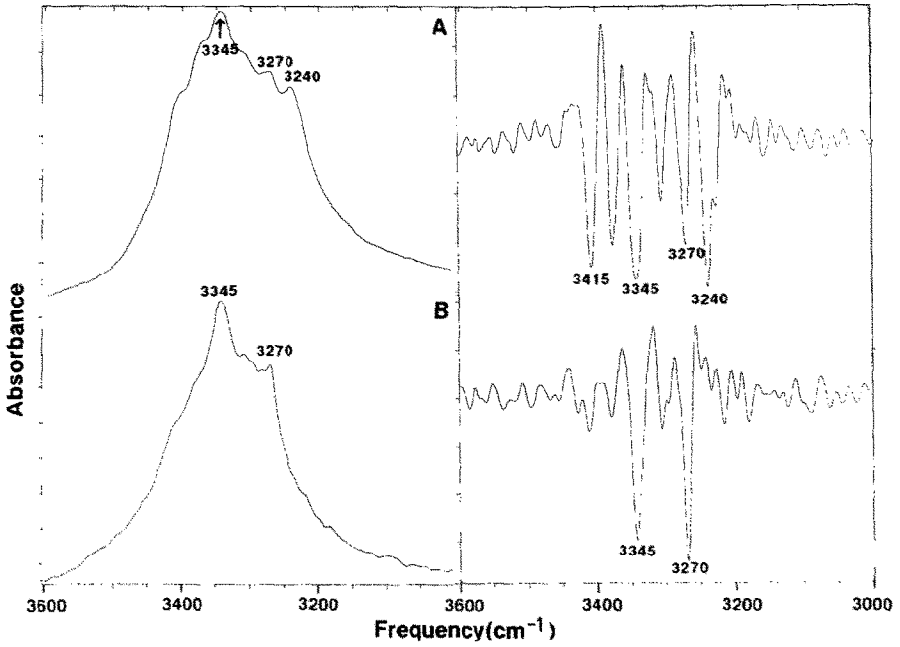

Fig. 1. FTIR spectra (3600–3000 cm^{-1}) of films of native celluloses: A, *Valonia*; B, tunicin. The left-hand spectra are normal, the right-hand spectra are second derivative.

of the disc containing *Valonia* cellulose and absent from that of the disc containing tunicin cellulose.

In the CH stretching regions shown in Fig. 2, there is good correspondence of bands in the normal spectra except for small differences near 2900 cm^{-1}. The difference can be seen more readily in the second-derivative spectrum of the tunicin cellulose, which shows strong features near 2910 and 2890 cm^{-1} that are not matched in the spectrum of *Valonia* cellulose. The same differences are evident in the second-derivative spectra of potassium bromide discs of the two celluloses. However, the film and the disc spectra differ in the absence from the latter of strong features near 2850 cm^{-1} probably due to differences in orientation of the molecules between the films and the discs.

The sub-spectra for tunicin cellulose recorded for a film and a potassium bromide disc in the regions 1500–1200 and 1200–800 cm^{-1}, respectively, and shown in Figs. 3 and 4, were almost identical with the corresponding spectra of *Valonia* cellulose. There are small differences of intensity between the spectra of tunicin cellulose in the film and potassium bromide disc. In Fig. 3, bands near 1361 and 1338 cm^{-1} are weaker in the normal spectrum of the disc, and the feature near 1361 cm^{-1} in the second-derivative spectrum is also weaker. On the other hand, the features near 1486 and 1457 cm^{-1} are more pronounced in the second-derivative spectrum. One noteworthy difference in Fig. 4 concerns the

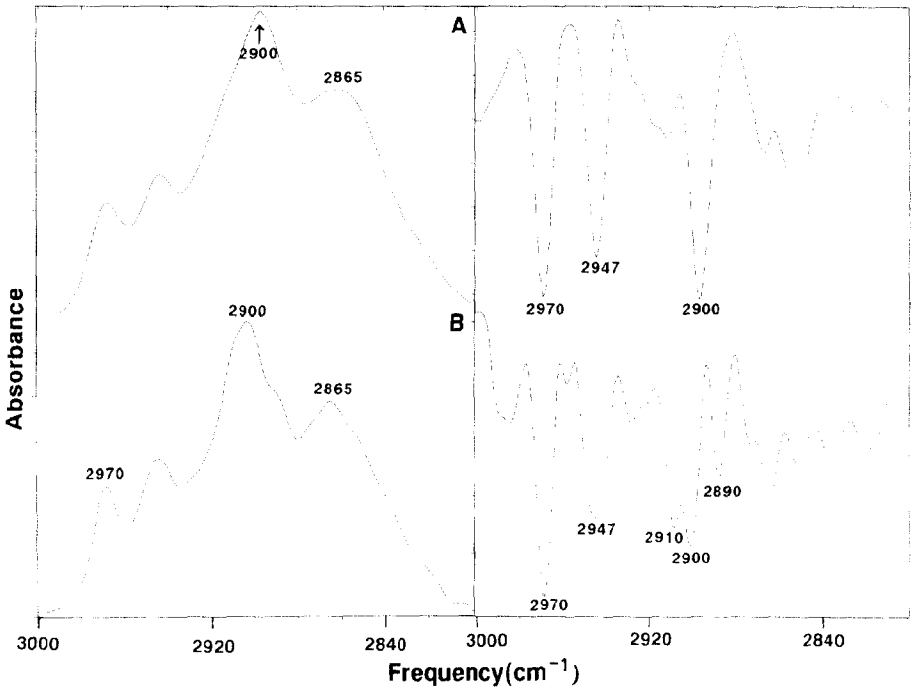

Fig. 2. FTIR spectra (3000–2800 cm^{-1}) of films of native celluloses: A, *Valonia*; B, tunicin; the left-hand spectra are normal, the right-hand spectra are second derivative.

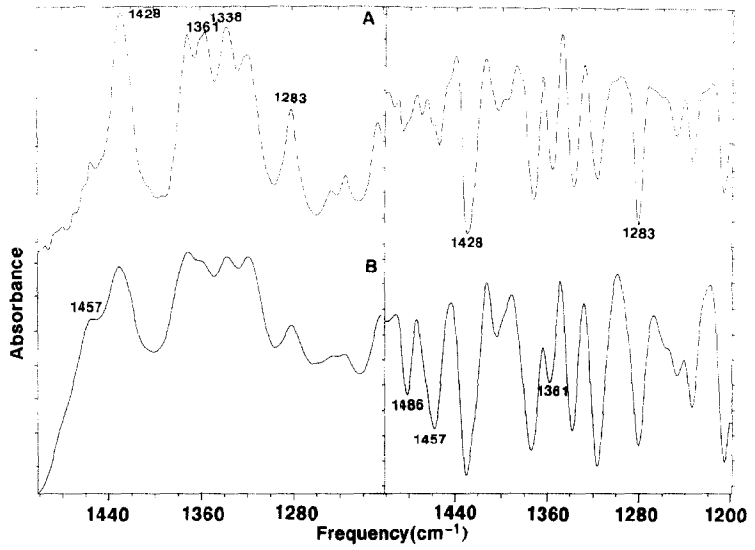

Fig. 3. FTIR spectra (1500–1200 cm^{-1}) of a film and disc of tunicin cellulose. The left-hand spectra are normal, the right-hand spectra are second derivative.

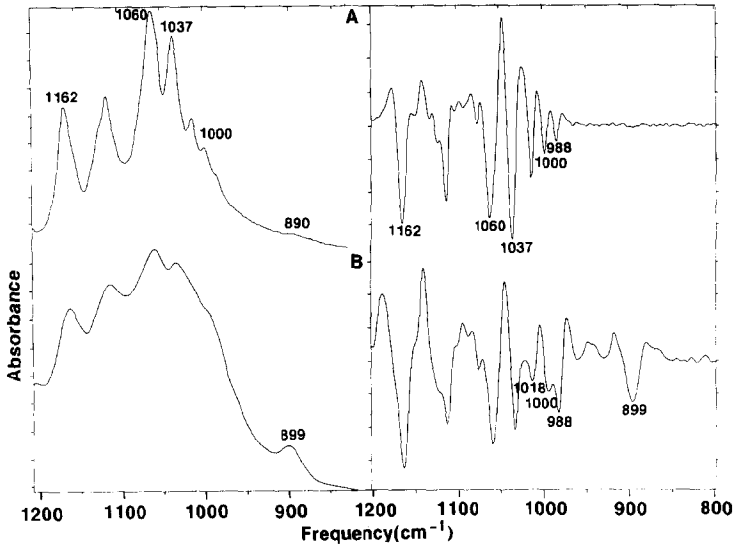

Fig. 4. FTIR spectra (1200–800 cm^{-1}) of a film and disc of tunicin cellulose. The left-hand spectra are normal, the right-hand spectra are second derivative.

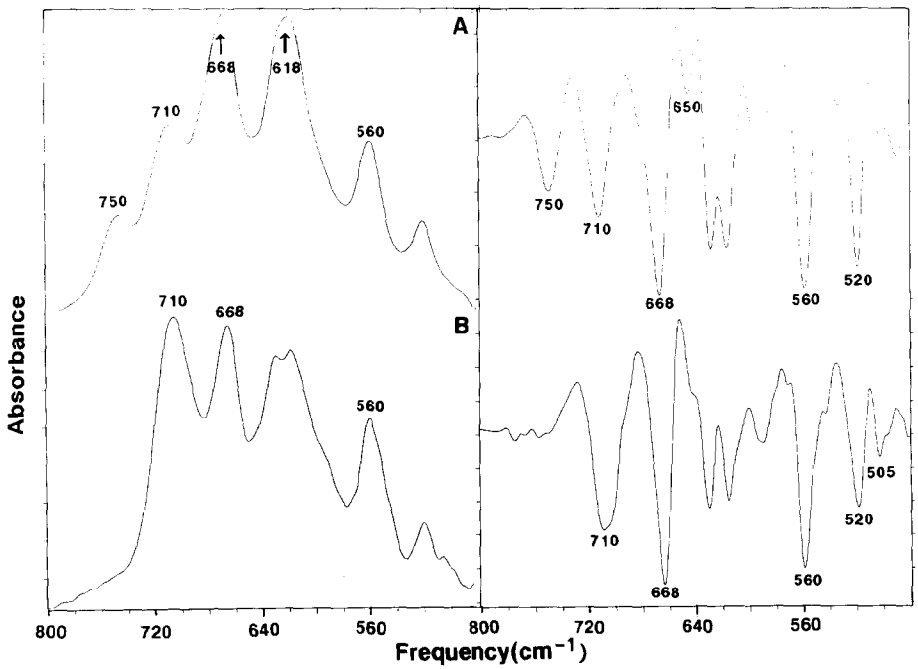

Fig. 5. FTIR spectra (800–480 cm^{-1}) of films of native celluloses: A, *Valonia*; B, tunicin. The left-hand spectra are normal, the right-hand spectra are second derivative.

band near 895 cm^{-1}, which is hardly observable in the spectra of the film but can be seen clearly in those of the disc.

The region 800–480 cm^{-1} of the spectra of the films of *Valonia* and tunicin celluloses is shown in Fig. 5. Comparison of the normal spectra shows the absence from the spectrum of the tunicin cellulose of the band at 750 cm^{-1} as noted by Sugiyama et al.[9]. This band was absent from the spectra of the disc and the film. The band at 710 cm^{-1} is more intense relative to that at 668 cm^{-1} in the spectrum of tunicin cellulose than in that of *Valonia* cellulose. Sugiyama et al.[9] suggested the bands at 668, 618, and 560 cm^{-1} to be composite. Use of the second-derivative mode has resolved several features from the peak near 618 cm^{-1}, but the peaks at 668 and 550 cm^{-1} appear to be single. A feature at 650 cm^{-1} is much better resolved in the spectrum of the *Valonia* cellulose. These differences are observable in the spectra for the discs and the films, otherwise, the bands in the spectra are well matched.

DISCUSSION

Sugiyama et al.[9] found that bands at 3240 and 750 cm^{-1} in the spectrum of *Valonia* cellulose were absent from that of tunicin cellulose. The presence of an additional feature near 650 cm^{-1} in the second-derivative spectrum of *Valonia* cellulose has now been found, which is absent from that of tunicin cellulose, and a single feature near 2900 cm^{-1} in the spectrum of *Valonia* cellulose instead of two features near 2910 and 2890 cm^{-1} in that of tunicin cellulose.

Bands in the region 3600–3000 cm^{-1} arise from hydrogen-bonded hydroxyl groups. Differences of intensity without differences in frequency, as observed here for several bands, may be explained by differences in the number of hydrogen bonds or their orientation but not of hydrogen-bond strength since such differences usually produce changes in frequency and intensity of the bands. It is more likely that the differences observed arise from differences in orientation relative to the surface and thus to the IR radiation. This view is supported by the greater similarity in this region between the spectra for potassium bromide discs, in which the crystals are randomised, than between the spectra of the cellulose films. It is also possible that the grinding of the cellulose during the making of the disc causes minor changes in structure. The absence of the band at 3240 cm^{-1} from the spectra of tunicin cellulose, randomly oriented in the disc and in the film, suggests the absence of a particular type of hydrogen bond rather than a change in orientation.

The assignment of bands in the CH stretching region is complicated by the opportunities for coupling. Evidence for such coupling has been obtained from the dichroic behaviour of the bands and the simpler spectrum found in the CD stretching region following deuteration of bacterial cellulose[12], a cellulose which also consists of the two phases[8]. The sensitivity of bands near 2900 cm^{-1} to changes in orientation and in the hydrogen-bond system supports Dechant's

assignment[12] of this band for bacterial cellulose to the symmetric stretching vibration of CH_2 groups rather than to the CH stretching vibrations of the methine groups[2,13].

Sugiyama et al.[9] cited the assignment by Blackwell et al.[14] of the band near 750 cm^{-1} in the spectrum of *Valonia* cellulose to a CH_2 rocking mode, but noted the absence of a corresponding Raman absorption band[14] and left the assignment open. Dechant[12] found that a band at 745 cm^{-1} was present in the spectrum of bacterial cellulose irrespective of the number of OD groups included in the crystal lattice and assigned this band to the ring-breathing vibration and the 710 cm^{-1} band to a CH_2 rocking mode. The latter mode should be sensitive to differences in hydrogen bonding between the two phases. It was found[12] also that the spectrum of bacterial cellulose in the region 700–400 cm^{-1} was changed extensively by the deuteration of CH and CH_2 groups, which led to the postulation of extensive coupling with the spectra being structure-dependent. Nonetheless, as mentioned above, good correspondence was found between the bands in the spectra of the *Valonia* and tunicin celluloses in this region down to 480 cm^{-1} with only small deviations near 650 cm^{-1}. This finding provides further evidence for the conformational similarity of the two native cellulose phases.

The differences found in bands with links to hydrogen bonding in the spectra of the *Valonria* and tunicin celluloses, and the strong similarities between bands related to the backbone and conformational structure, support the postulates of other workers that the two cellulose phases differ in the disposition of their hydrogen bonds rather than in their molecular conformations.

Differences found between the spectra of the celluloses recorded for films and discs can be attributed to the differences in orientation in the presentation of the samples, but the grinding to which the celluloses are subjected during preparation of the discs may also cause some changes.

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