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BAND ASSIGNMENTS IN THE RAMAN SPECTRA OF CELLULOSES

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ABSTRACT

Our investigations of the vibrational spectra of celluloses have been extended by using the Raman microprobe to study the spectra of native celluloses. The microprobe allows spectra to be recorded from domains as small as 1 μm, so that, for fibers of simple morphology, the polarization of exciting and scattered radiation can be defined relative to molecular orientation. Series of spectra in which the polarization of the incident light was varied relative to the fiber axis were recorded from oriented fibers. Analysis of band intensities as a function of polarization revealed new information about the directional character of the vibrational displacements. In addition, a limited study of deuterated celluloses was conducted in order to identify the modes that involve hydrogen motions. The information from the studies of intensities and deuterated celluloses aided in the interpretation of the vibrational spectrum of cellulose. Although a complete assignment of the spectrum was not possible, this new information provides a more thorough characterization of the bands than had been possible in previous studies, and establishes a foundation for future microprobe studies of native tissues.

INTRODUCTION

A variety of spectroscopic and diffractometric techniques has been used to study the structure of cellulose fibers. Vibrational spectroscopy has played a key role in this multifaceted approach. In order to derive structural information from the vibrational spectra of celluloses, the bands in the different spectral regions must be characterized. The large number of vibrational degrees of freedom and the asymmetric structure of the cellulose molecule make interpretation of the spectrum difficult. The group-frequency approach usually used in interpretation of vibrational spectra is not suited to interpretation of the molecular-chain modes of cellulose. With the exception of a few modes associated with highly localized vibrations involving hydrogen atoms, most of the modes are highly coupled and delocalized. This is not surprising, because the pyranose rings and the linkages between them consist of systems of C–C and C–O bonds. These bonds have similar reduced masses and bond energies, so that their frequencies are sufficiently close for a high degree of coupling to occur between their vibrations.

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The problem of interpreting the spectrum of cellulose had earlier been approached by us by conducting a series of normal-coordinate analyses of cellulose model-compounds, and using the resulting information as the basis of the analysis. We now report a closely related investigation utilizing a Raman microprobe[1–3] to study the dependence of band intensities on the polarization of the incident light relative to the orientation of the molecules. The study revealed the directional character of many of the vibrational modes, and it serves as a foundation for studies of morphologically complex aggregates of cellulose. In addition, a limited study of carbon-deuterated cellulose was undertaken, in order to identify the bands involving OH and CH motions.

BACKGROUND

Model-compound studies. — A series of normal-coordinate analyses has been undertaken in order to provide a basis for understanding the vibrational spectra of cellulose. The compounds chosen were the 1,5-anhydropentitols[4,5] the acyclic pentitols[6], the pentoses[7], the inositols[8,9] and the hexoses[10]. For each group of compounds, the force constants were refined against the observed frequencies until a satisfactory fit was obtained. The force fields that were derived enabled successful prediction of the spectra of compounds not included in the refinements, and the calculated potential-energy distributions were reasonable in comparison with the group-frequency literature on the carbohydrates. The analyses were thus successful in developing a physically meaningful force-field specifically tailored to the carbohydrates.

The hexose force-field was then used to extend the normal-coordinate method to the cellodextrins[11], whose vibrational spectra more closely resemble the spectrum of cellulose. Because the number of vibrational degrees of freedom greatly exceeds the number of observed bands in the spectra of these compounds, it was neither possible nor meaningful to refine the force constants. Although the calculations predicted many more bands than are actually observed, the distribution of calculated frequencies was in qualitative agreement with the observed spectra. The force field derived appears to provide a good model for understanding the vibrational spectra of the cellodextrins and cellulose.

The potential-energy distributions calculated for the model compounds were quite complex. Except for the internal vibrations of the methylene groups, the modes below 1500 cm^{-1} are delocalized motions involving several internal coordinates. In earlier assignments of the cellulose vibrational spectrum, the modes below 1500 cm^{-1} were assigned to localized group-vibrations[12–15]. Although the potential-energy distributions generally agreed qualitatively with the types of motions suggested in the earlier assignments, the calculations indicated that the motions are often more delocalized than had been recognized in the early assignments. Above 1500 cm^{-1}, the CH and OH stretching modes do behave as relatively pure, group modes.

Raman spectroscopy. — In cellulose, all of the vibrational modes are potentially both infrared- and Raman-active. Raman spectroscopy has, however, some important advantages for recording spectra from cellulosic samples. Highly polar bond systems, which result in intense infrared bands, have relatively low polarizabilities and, hence, weak Raman intensities. Water, therefore, has very weak Raman bands and does not interfere with the spectrum of cellulose. The low-frequency region, which is observed with difficulty in the infrared spectra, is readily observed in the Raman spectra. Finally, cellulosic materials are often optically heterogeneous substrates which scatter light intensely. In infrared spectroscopy, any processes other than absorption which cause attenuation of the incident beam are problematic. Because the refractive index of the sample will often go through large changes in the neighborhood of absorption bands, the scattering losses will vary with frequency over the infrared region. In Raman spectroscopy, refractive index variations are not a problem, as the excitation frequency is far removed from any absorption bands. Therefore, Raman spectra of samples such as cellulose, which scatter light strongly, are more accurate representations of the vibrational motions and the characteristic vibrational transitions.

Raman microprobe. — A recent innovation in Raman spectroscopy was the development of the Raman microprobe[1–3]. The microprobe is a specially designed, optical microscope coupled with a conventional Raman spectrometer. The microscope performs two key functions. It focuses the exciting light on the sample down to a diameter of 1 μm; then, it gathers the scattered light and transmits it to the entrance slit of the spectrometer. Because the microprobe acquires spectra from such small domains, the structural heterogeneity of the domains is greatly lessened relative to the domains examined in conventional Raman spectroscopy. The microprobe makes it possible to identify the morphological features from which spectra are recorded, so that orientation, composition, and structure can be related to morphology.

The spectral attributes of the microprobe make new information available. In the present investigation, the microprobe was utilized to record spectra from morphologically homogeneous, fibrillar domains. The polarization of the Raman scattered light was analyzed to aid in the assignment of the Raman spectrum of cellulose.

EXPERIMENTAL

Sample preparation. — *Valonia* fibers were extracted from the purified cell-walls of *Valonia macrophysa* that was grown in our laboratory. The alga was extracted with chloroform–methanol and boiled in a 1% solution of sodium hydroxide for 6 h under nitrogen, then it was bleached with sodium chlorite following the procedure in Browning[16]. Oriented fibrils were pulled from the cell wall with forceps and mounted on small washers for examination with the microprobe. Scanning electron micrographs of the fibrils showed that they had a high degree of parallel orientation[17].

Deuterated-cellulose fibers were prepared from purified filaments of *Cladophora glomerata* that had been grown in D_2O. The alga was adapted to growth in D_2O following Crespi's methods[18]. The procedure used to purify *Valonia* was followed, except that the bleaching step was omitted. Because the filaments were too small to be mounted on washers, they were stretched across copper specimen-support grids normally used in electron microscopy.

Ramie (*Boehmeria*) fibers were purified by the same method used to purify *Cladophora* cellulose. The fibers were dried under tension, and mounted on small washers. Scanning electron micrographs showed that the fibrils possessed a high level of parallel orientation.

A sample of deuterated, bacterial cellulose was kindly provided by Dr. H. L Crespi. It was prepared by growing *Acetobacter xylinum* in a deuterated growth-medium[18]. The sample was purified as described for *Valonia,* and then hydrolyzed with acid[17]. The residue was made into the form of a pellet for examination with the conventional Raman system. A detailed description of the growth conditions used for the algae and the purification procedures is given elsewhere[17].

Acquisition of spectra. — Spectra of the *Valonia, Cladophora,* and ramie fibers were recorded with a Raman microprobe developed by Instruments SA. The microprobe system consists of Jobin Yvon Ramanor HG2S coupled with a Nachet optical microscope. Since we wanted to compare the intensities in spectra recorded with different polarizations of the exciting light, special modifications were made to the microprobe in order to avoid problems arising from the dichroism inherent in the optical system of the microscope and the monochromator First, a polarization scrambler was inserted at the coupling between the microscope and the monochromator. Second, a rotating mechanical stage was installed so that, instead of changing the polarization of the incident light directly, we were able to rotate the sample relative to the plane of polarization of the incident light as shown in Fig. 1 The stage was so aligned that its axis of rotation coincided with the optical axis of the microscope. Therefore, it was possible to rotate the sample without changing the domain being examined.

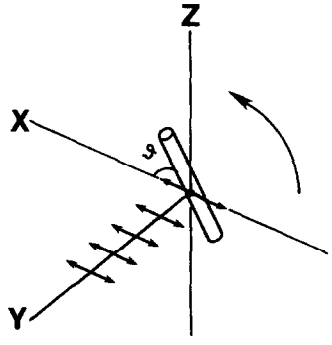

Fig 1 Representation of the experiment in which the angle between the electric vector of the incident light and the fiber axis was varied from 0 to 90° by rotating the fiber relative to the plane of polarization of the incident light

The exciting radiation was the 5145-Å line of an argon-ion laser. The power incident on the sample was ~7 mW. A 40-X Nachet objective with a numerical aperture of 0.75 was employed. The spectral slit-width was ~8 cm^{-1} The acquisition time required for each spectrum was 8 h. Multiple scans were recorded in order to lessen distortion of the relative intensities due to any drift in the laser power during a single scan.

The spectrum recorded from the deuterated, bacterial-cellulose pellet was acquired in the macro chamber of the same Raman spectrometer. The incident laser power was 150 mW. The spectral slit width was 3 cm^{-1}, and the acquisition time was 20 h.

RESULTS AND DISCUSSION

Analysis of band intensities. — Sets of spectra in which the angle between the electric vector of the incident light and the fiber axis (see Fig. 1) was varied from 0 to 90° in 15° increments were recorded from *Valonia* and ramie fibers, and are

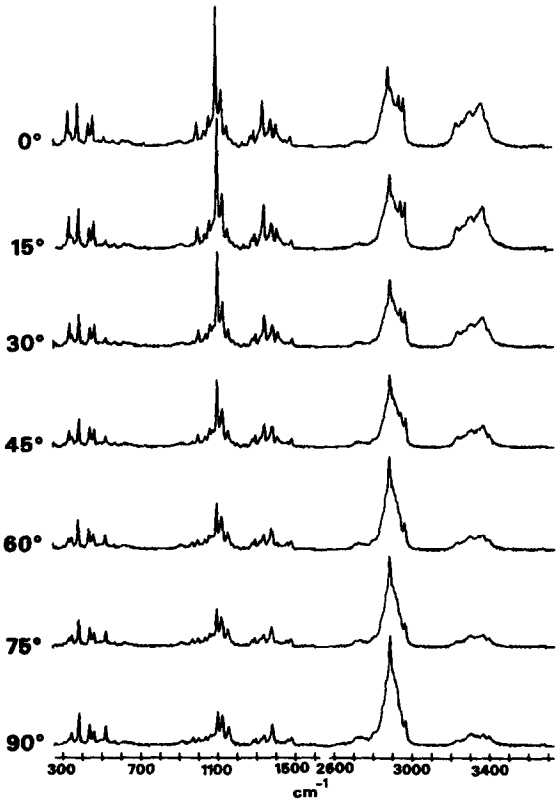

Fig 2. Polarized Raman spectra of a *Valonia* fiber [The angle between the electric vector of the incident light and the fiber axis was varied from 0 to 90° in 15° increments]

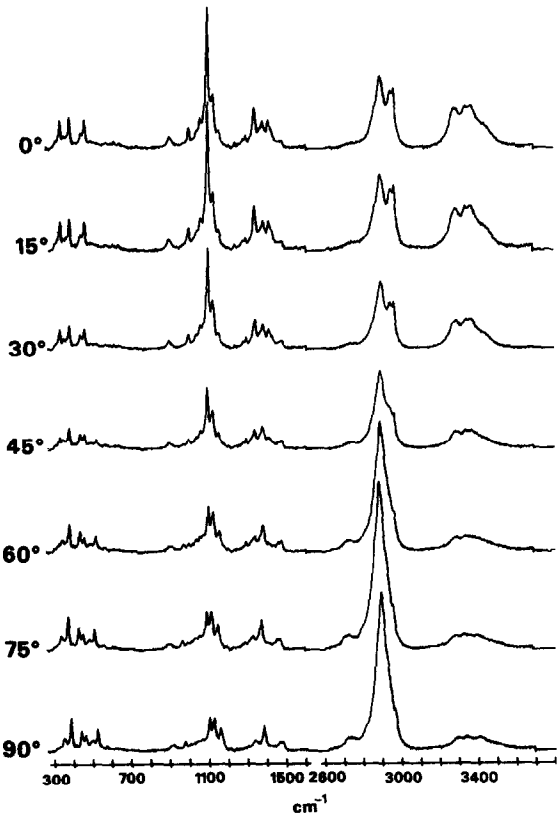

Fig 3 Polarized Raman spectra of a ramie fiber [The angle between the electric vector of the incident light and the fiber axis was varied from 0 to 90° in 15° increments]

shown in Figs. 2 and 3, respectively. Except for the band widths, the spectra of *Valonia* and ramie are very similar to each other below 3000 cm^{-1}, suggesting that the same vibrational modes occur in both celluloses. Above 3000 cm^{-1}, significant frequency differences are observed. The structural implications of these frequency differences will be discussed in a later section. Due to the similarity between the *Valonia* and ramie spectra, they can be compared to check the reproducibility of the band frequencies and the dependence of the band intensities on the polarization of the incident light.

From Figs. 2 and 3, it is clear that the band intensities are strongly dependent on the orientation of the incident electric-vector relative to the fiber axis. The dependence of the band intensities on θ, the angle between the incident electric-vector and the fiber axis, was modeled by the following equation:

$$I = a + b(\cos\theta)^2 + c(\cos\theta)^4, \qquad (1)$$

where a, b, and c are constants related to the derivatives of the polarizability

tensors with respect to the normal coordinates. The equation was derived according to Snyder's treatment of intensities for partially oriented polymers[19]. In the derivation, it was assumed that the cellulose chains are oriented parallel to the fiber axis, and that the microfibrils are oriented randomly around their axes.

Eq. *1* was fitted to the data in Figs. 2 and 3 by a linear-regression technique. The equation provided an adequate model for the dependence of the band intensities on θ for bands which were well resolved. Bands which were weak, or poorly resolved, or both, could not be fitted so well. This approach to classification of the bands in the spectra has been adopted because it is useful as a basis for future applications of Raman microprobe spectroscopy in investigations of molecular organization in native, plant tissue. The advantage of using ramie and algal cellulose spectra as reference spectra is that the organization of molecular chains within the fibrils is known to be simple, and parallel to the fibril axes.

Based on the relationships between the intensities and θ, the bands were divided into four groups. The classification of the bands is summarized in Table I. The first group of bands exhibits a single maximum and minimum in plots of intensity *vs* θ, as illustrated by curves a and c in Fig. 4. Such curves possess a single inflection point in the range of θ shown. This group of bands is designated group A in Table I. The second group of bands exhibits two maxima, at 0 and 90°, and a single minimum between 0 and 90° (see curves b and d in Fig. 4); these curves possess two inflection points. This group is designated group B in Table I. The multiple maxima may arise from accidentally degenerate modes which have maxima at 0 and 90° or from modes in which some elements of the polarizability tensor decrease during the vibration.

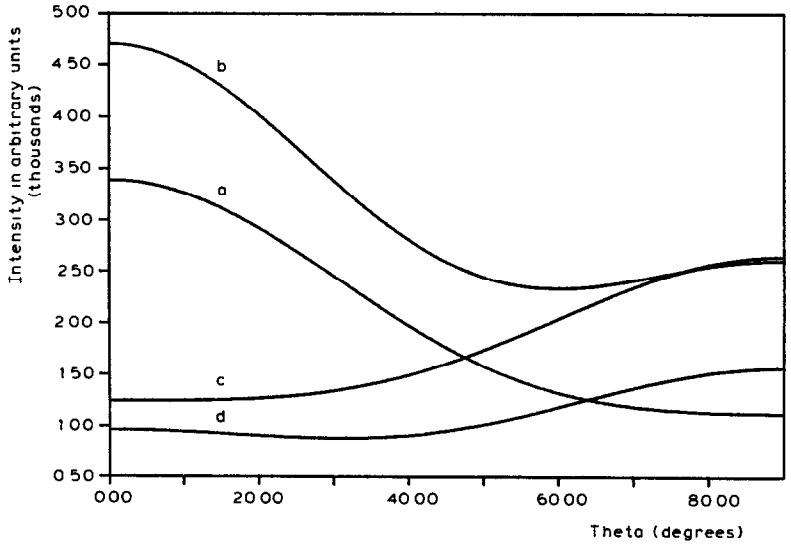

Fig 4 Plots of intensity *vs* the angle between the incident electric vector and the fiber axis (a) A_0 band, (b) B_0 band, (c) A_{90} band, and (d) B_{90} band

TABLE I

SUMMARY OF INTENSITY MAXIMA, DEUTERATION SENSITIVITIES, AND BAND ASSIGNMENTS FOR THE RAMAN SPECTRA OF *Valonia* AND RAMIE

Band frequency[a] *(cm^{-1})*		*Intensity*	*Deuteration classification*	*Assignment sensitivity*
Valonia	*ramie*			
331	331	A_0	weak	heavy atom bending,
344	344	$B_?$	weak	some heavy atom stretching
381	380	$B_?$	weak	some heavy atom stretching
437	437	$B_?$	weak	some heavy atom stretching
459	458	B_0	weak	some heavy atom stretching
520	519	A_{90}	weak	some heavy atom stretching
913	910	B_0	?	HCC and HCO bending at C-6
968	969	B_{90}	?	heavy atom (CC and CO)
997	995	A_0	?	stretching
1034	1037	A_0	?	stretching
1057	1057	A_0	?	stretching
1095	1095	A_0	weak	stretching
1118	1117	B_0	weak	stretching
1123	1121	A_0	weak	stretching
1152	1151	$B_?$	?	heavy atom stretching plus HCC and HCO bending
1279	1275	A_0	?	HCC and HCO bending
1292	1291	?	?	HCC and HCO bending
1334	1331	A_0	strong	HCC and HCO bending
1337	1337	A_0	strong	HCC, HCO, and HOC bending
1378	1378	$B_?$	strong	HCC, HCO, and HOC bending
1406	1407	A_0	strong	HCC, HCO, and HOC bending
1455	1456	B_{90}	strong	HCH and HOC bending
1477	1475	A_{90}	strong	HCH and HOC bending
2868	2866	B_{90}	strong	CH and CH_2 stretching
2885	2889	B_{90}	strong	CH and CH_2 stretching
2941	2943	$B_?$	strong	CH and CH_2 stretching
2965	2963	B_0	strong	CH and CH_2 stretching
3291	3286	B_0	strong	OH stretching
3334	3335	$?_0$	strong	OH stretching
3361	3363	$?_0$	strong	OH stretching
3395	3402	B_0	strong	OH stretching

[a]Only the bands resolved in both the *Valonia* and ramie spectra are included in this table

The bands were further categorized by whether they were most intense when θ was 0 or 90°. The bands that are most intense when θ equals 0° are designated by a subscript 0 in Table I. Example plots of intensity *vs.* θ for A_0 and B_0 bands are given by curves a and b, respectively, in Fig. 4. These bands result from vibrations in which the maximum change in polarizability is parallel to the chain axis. Those bands that are most intense when θ equals 90° are designated by a subscript 90. Example plots are given by curves c and d in Fig. 4. For these modes, the maximum change in polarizability is perpendicular to the chain axis. The direction in which the maximum change in polarizability occurs is related to the direction of the

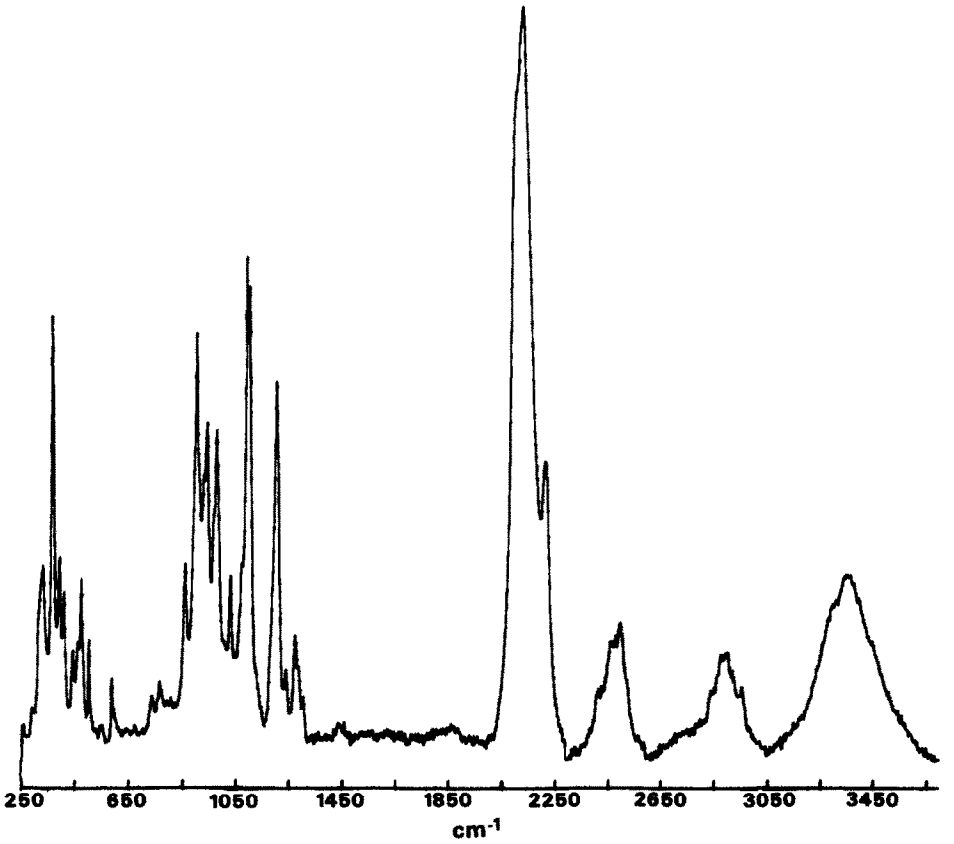

Fig 5. Raman spectra of a pellet of deuterated, bacterial cellulose.

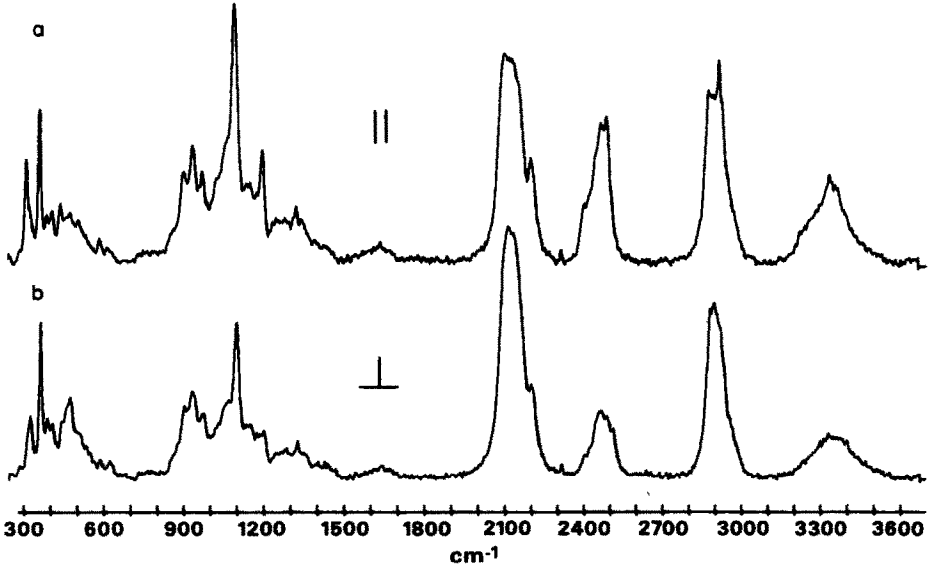

Fig 6. Raman microprobe spectra of a deuterated *Cladophora* fiber. (a) Electric vector parallel to the fiber axis, (b) electric vector perpendicular to the fiber axis

vibrational displacements The intensity study can, therefore, reveal information about the directions of the vibrations. In addition, it provides a more thorough characterization of the bands in the spectrum of cellulose than had been possible in previous studies.

Spectra of deuterated celluloses. — A limited study of deuterated celluloses was conducted in order to identify the modes which involve hydrogen motions. Fig. 5 shows a spectrum from a pellet of deuterated bacterial-cellulose, recorded in the conventional Raman mode. Fig. 6 includes spectra from an oriented sample of deuterated *Cladophora* cellulose recorded with the incident electric-vector parallel to and perpendicular to the fiber axis. The residual intensities in the CH (2800–3000 cm^{-1}) and OH stretching (3200–3500 cm^{-1}) regions indicate that the samples were not fully deuterated. Due to the residual hydrogen present in the samples, the full effect of deuteration on the spectrum of cellulose could not be determined. For most of the bands, however, we were able to determine whether the effect of deuteration was small or large. This information is listed in Table I.

Band assignments. — In previous assignments of the vibrational spectrum of cellulose, it has generally been assumed that the vibrational motions could be described in terms of simple, group motions. The normal-coordinate analyses demonstrated that assignments in the traditional sense are not meaningful. In the region below 1500 cm^{-1}, only the internal motions of the methylene groups can be adequately approximated as group motions. The rest of the modes are delocalized motions involving more than one group or site in the molecule. Furthermore, cellulose possesses many more vibrational degrees of freedom than the number of bands observed in the infrared and Raman spectra. Therefore, it is possible that some modes are accidentally degenerate. The observed bands may actually arise from a composite of several vibrational motions, all having approximately the same frequency.

The assignments to be described are not assignments in the traditional sense, but rather, are descriptions of the types of motions occurring in each region of the spectrum. The types of motions were identified from the potential-energy distributions for the hexoses[10] and the cellodextrins[11], and the study of deuterated cellulose. The assignments also involve a description of the directional character of the vibrations, based on the intensity study. For convenience, the spectrum has been divided into six regions.

Region of 250–550 cm^{-1}. — In the region between 250 and 550 cm^{-1}, several closely spaced, medium-intensity bands were observed in the Raman spectra of *Valonia* and ramie cellulose (see Fig. 2 and 3). A similar pattern had been observed in the infrared spectra of native celluloses[11–15]. The potential-energy distributions are to a large extent delocalized, indicating that the vibrational modes are quite complex. Displacement drawings based on the normal-coordinate calculations for the disaccharides[11] show that almost every atom in the molecule participates in these modes. The predominant motions are skeletal-bending modes involving the CCC, COC, OCC, and OCO internal coordinates. Small amounts of methine

bending (CCH and OCH) and skeletal stretching (CC and CO) contribute in the region. Torsional motions, which are out-of-plane bending about the C–O and C–C bonds, become significant below 300 cm^{-1}. The small sensitivity of the bands to deuteration is consistent with the small contribution of CH coordinates in the potential-energy distributions.

According to the classifications in Table I, the bands at 331, 459, and 520 cm^{-1} are types A_0, B_0, and A_{90}, respectively. The 344-, 381-, and 437-cm^{-1} modes all belong to the B category, but their distribution in groups B_0 and B_{90} is uncertain, due to divergences between the *Valonia* and ramie data. Since the 331- and 459-cm^{-1} modes are most intense when the incident electric vector is parallel to the chain axis, these modes are skeletal-bending modes, where the major change in polarizability is parallel to the chain axis. An accordion-like bending motion of the pyranose rings in the chain is a plausible description of this mode. Since the 459-cm^{-1} band falls in the B category, it may actually be a composite of a motion where the change in polarizability is parallel to the chain axis and a motion where the change in polarizability is perpendicular to the chain axis. Alternatively, some of the polarizabilities may decrease during the vibration. The 520-cm^{-1} mode is most intense when the incident electric-vector is perpendicular to the chain axis.

The normal-coordinate calculations for cellotetraose[11] showed that the frequency distribution below 700 cm^{-1} is sensitive to the dihedral angles at the glycosidic linkages. Raman spectra of various types of cellulose have indicated a sensitivity to the polymorphic form of the cellulose[20,21]. The observed spectral differences are very similar to the differences observed in the frequency distributions for the alternative structures of cellotetraose. This has reinforced the conclusion that cellulose I and II possess different orientations of the glycosidic linkages[20,21].

Region of 550–950 cm^{-1}. — In the region between 550 and 950 cm^{-1} of the Raman spectra of *Valonia* and ramie cellulose (see Figs. 2 and 3), the bands are weak and widely spaced. The region between 750 and 800 cm^{-1} is devoid of any significant features. Infrared spectra of native celluloses differ from the Raman spectra between 550 and 750 cm^{-1}, in that several medium-intensity peaks are observed[11–15]. Between 750 and 850 cm^{-1}, the i.r. spectra are also devoid of any significant features. Both the Raman and i.r. spectra possess a weak, poorly resolved, cluster of bands at ~900 cm^{-1}.

The potential-energy distributions[10,11] indicate that, between 550 and 750 cm^{-1}, the predominant internal coordinates are CCC, COC, OCO, CCO, and OH out-of-plane bending. The OH bending modes are observed in infrared spectra, but are absent from Raman spectra because of the large dipole moment and low polarizability associated with the OH bond No bands are calculated between 750 and 800 cm^{-1}, which is consistent with the frequency pattern observed. A cluster of peaks is calculated at ~900 cm^{-1} that involves HCC and HCO bending localized at the C-6 atoms.

The deuteration sensitivities of the bands in the Raman spectrum between

550 and 850 cm^{-1} are small. This observation is consistent with the dominance of the CCC, COC, OCO, and CCO internal coordinates in this region. Since the peaks around 900 cm^{-1} primarily involve methine bending coordinates, these bands should be strongly deuteration-sensitive. We were unable to identify a peak in the appropriate region of deuterated-cellulose spectra which could correspond to the 900-cm^{-1} bands. New peaks are shifted into the region at ~900 cm^{-1} by deuteration, so that it is difficult to tell if the 900-cm^{-1} band is still present. It appears, however, that the 900 cm^{-1} band is shifted by much less than would be the case were this a pure CH bending mode[22] Therefore, it is likely that the bands at ~900 cm^{-1} are more delocalized than was predicted by the cellodextrin calculations.

In assigning the modes at ~900 cm^{-1}, several empirical observations are useful. The band is significantly more intense in the spectrum of ramie than in that of *Valonia* (see Figs. 2 and 3). Below 3000 cm^{-1}, this is the most significant difference between the ramie and *Valonia* spectra. A comparison of the spectra of ramie, cotton, bacterial, algal, and amorphous celluloses suggested that the intensity of the 900-cm^{-1} band is related to the lateral size of the cellulose crystallites. The intensity of the 900-cm^{-1} band was also found to correlate in some instances with the intensity of the broad, upfield shoulders for the C-4 and C-6 atoms in the solid-state, ^{13}C-n.m.r. spectra of native celluloses[23]. The broad shoulders arise from cellulose chains on the crystallite surfaces and in the amorphous regions. These results suggest that the intensity of the 900-cm^{-1} peak is proportional to the amount of disorder in the cellulose. Since the likely sites of disorder in the cellulose molecules are the glycosidic linkages, the C-6 atoms, and the hydroxyl groups, the 900-cm^{-1} band is likely to involve one or more of these sites.

Region of 950–1180 cm^{-1}. — In the region between 950 and 1180 cm^{-1}, several closely spaced, intense bands are observed in both the Raman spectra of *Valonia* and ramie celluloses (see Figs. 2 and 3) and in the infrared spectra of native celluloses reported in the literature[11–15]. The normal-coordinate calculations[11] show that the band density in the 950–1180-cm^{-1} region is very high. The potential-energy distributions are dominated by CC and CO stretching motions. Small amounts of HCC, HCO, and skeletal atom bending also contribute to the bands. The motions are highly coupled, often involving coupling between the D-glucose rings. The high Raman and infrared intensities of the bands are consistent with the large band density and the dominance of CC and CO stretching motions predicted by the normal-coordinate calculations. It is difficult to determine the deuteration sensitivities, because many new bands appear in the region due to deuteration. The 1071-, 1095-, 1118-, and 1123-cm^{-1} bands exhibit very little sensitivity to deuteration, which is consistent with the negligible contribution of CH, CH_2, and OH coordinates to the modes responsible

The 997-, 1034-. 1057-, 1095-, and 1123-cm^{-1} modes all fall in the A_0 category. The band at 1118 cm^{-1} is a B_0 mode. Since these bands are most intense when the electric vector of the incident light is parallel to the fiber axis, they must result from CC and CO stretching motions which are parallel to the chain axis. The

968-cm^{-1} band is a B_{90} mode. It must result from skeletal stretching motions that are predominantly perpendicular to the chain axis. In addition to 1118- and 968-cm^{-1} bands, the band at 1152 cm^{-1} also belongs in the B category. The ramie and *Valonia* data are not consistent, however, as to whether the 1152-cm^{-1} band is a B_0 or a B_{90} mode. The directionality of the 1152-cm^{-1} mode is, therefore, uncertain.

Region of 1180–1500 cm^{-1}. — Between 1180 and 1270 cm^{-1}, the Raman and infrared spectra of *Valonia* and ramie celluloses exhibit only weak and widely spaced bands. The potential-energy distributions for the cellodextrins indicate that the 1180–1270-cm^{-1} region is a transition region[11]. Below 1180 cm^{-1}, CC and CO stretching coordinates dominate the potential-energy distributions, while, above 1270 cm^{-1}, HCC, HCO, HCH, and COH bending coordinates are most significant. Between 1180 and 1270 cm^{-1}, the modes involve significant amounts of skeletal stretching, as well as methine bending.

The cellodextrin calculations showed that the frequency distribution is sensitive to the orientation of the glycosidic linkages in the 1200–1300-cm^{-1} region[11]. The differences in the frequency distributions correspond closely with the differences observed between the spectra of celluloses I and II. A medium-intensity band is observed in the Raman spectrum of cellulose II at 1261 cm^{-1} that is not observed in the spectrum of cellulose I. This observation lends support to the proposal that celluloses I and II possess different molecular conformations. The sensitivity of the bands to conformation may arise from the nature of the potential-energy distributions. Since the 1180–1270-cm^{-1} region is a transition region, many different types of internal coordinates contribute to the modes, thereby increasing the amount of delocalization.

In the 1270–1500-cm^{-1} region, several closely spaced, medium-intensity bands are observed in both the Raman and infrared spectra of native celluloses. The bands are strongly sensitive to deuteration. The normal-coordinate calculations also predict a high density of bands in the region[11]. The predominant, internal coordinates in the potential-energy distributions are CCH, OCH, COH, and HCH bending. Between 1430 and 1500 cm^{-1}, the major internal coordinate is HCH bending; from 1430 to 1350 cm^{-1}, it is COH bending; and from 1350 to 1270 cm^{-1}, it is HCC and HCO bending. Except for the internal modes of the CH_2OH groups, the motions are quite delocalized. The dominance of CH and OH bending coordinates is consistent with the strong, deuteration sensitivity of the bands.

The Raman bands at 1279, 1334, 1337, and 1406 cm^{-1} are all A_0 modes. Since these modes are most intense when the electric vector of the incident light is parallel to the chain axis, they must result primarily from HCC and HCO bending motions, where the change in polarizability is parallel to the chain axis. Although COH bending coordinates also contribute to the potential-energy distributions above 1300 cm^{-1}, OH bending is very weak in Raman spectra, so that the intensities will be dominated by the motions of CH groups.

The Raman bands at 1455 and 1479 cm^{-1} fall in the B_{90} and A_{90} categories, respectively. The potential-energy distributions show that these bands are HCH

bending modes which contain a very small proportion of COH bending[11]. The bands are most intense when the electric vector of the incident light is perpendicular to the fiber axis. Therefore, the vibrations must be so oriented that the change in polarizability accompanying the vibration is also perpendicular to the chain axis. This can only occur in the so-called *gt* and *tg* rotational orientations for the methylene groups[24]. In the *gt* orientation, the C-6–O-6 bond is *gauche* to the C-5–O-5 bond and *trans* to the C-4–C-5 bond, whereas, in the *tg* orientation, it is *trans* to the C-5–O-5 bond and *gauche* to the C-4–C-5 bond. The Raman spectra do not provide a basis for discriminating between the two forms. Since the 1455-cm^{-1} band is a B mode, it might also be a degenerate vibration, or result from a vibration where elements of the polarizability decrease during the motion.

Region of 2800–3000 cm^{-1}. — Between 2800 and 3000 cm^{-1}, the Raman spectra of *Valonia* and ramie contain several closely spaced, very intense bands. In the infrared spectra, the band structure is very similar, but the bands are not so intense[12,14,15,25]. The bands are strongly deuteration-sensitive The normal-coordinate calculations predict that the CH and CH_2 stretching vibrations occur in this region. These modes are isolated from the other motions in the molecule and, therefore, behave as group vibrations

The 2868- and 2885-cm^{-1} bands fall into the B_{90} category in the Raman spectra of both ramie and *Valonia*. The 2965-cm^{-1} band is a B_0 band in both sets of spectra. Although it is difficult to assign the bands in this region, due to the overlapping of the bands and the possibility of Fermi resonance, the most intense band, at 2885 cm^{-1}, is most likely due to the methine protons. The methine CH bonds are perpendicular to the chain axis, and hence would result in stretching bands that are most intense when the electric vector of the incident light is perpendicular to the chain axis. Also, there are more methine protons than CH_2 protons, so that the methine stretch should be the most intense CH band.

The CH_2 group should exhibit both symmetric and antisymmetric stretching bands. The antisymmetric stretching band will be at higher frequency than the symmetric stretching band. Since the symmetric, methylene bending mode is most intense with the incident electric vector perpendicular to the fiber axis, the symmetric methylene stretch is also expected to be most intense in the perpendicular mode, while the antisymmetric stretch is expected to be most intense in the parallel mode. The 2965-cm^{-1} band is most intense with the incident electric vector parallel to the fiber axis, and is a plausible frequency for a CH_2 antisymmetric stretching mode.

Because there are several modes other than the methine stretching band that are most intense when the incident electric vector is perpendicular to the chain axis, the symmetric CH_2 stretching mode is more difficult to identify. As already mentioned, the 2868-cm^{-1} band is a B_{90} mode. The 2848- and 2904-cm^{-1} bands were classified as B_{90} bands based on the *Valonia* data, but they were not resolved in the ramie spectra. The 2941-cm^{-1} band was classified as a B_0 band, based on the *Valonia* data, and as a B_{90} band, based on the ramie data. Since the symmetric CH_2

stretching frequency is usually at least 100 cm^{-1} lower than the antisymmetric stretching frequency[26], either the 2848- or the 2868-cm^{-1} band is most likely to be the CH_2 symmetric stretching mode.

Region of 3200–3500 cm^{-1}. — Between 3200 and 3500 cm^{-1}, the Raman spectra of *Valonia* and ramie contain several closely spaced, medium-intensity bands. In the infrared spectra of native celluloses, the band frequencies are the same as in the Raman spectra, but the bands are much more intense. The bands are strongly deuteration-sensitive. The normal-coordinate calculations[10,11] predict that the OH stretching vibrations occur in this region. As was the case with the CH motions, the OH motions are isolated from the other internal motions of the cellulose molecule. Hydroxyl stretching motions, however, can couple with lattice modes due to their involvement in intermolecular hydrogen-bonds. Since the normal-coordinate calculations are based on an isolated-molecule approximation, they cannot predict the coupling of lattice modes with internal modes in this region.

All of the bands were found to be most intense when the electric vector of the incident light was parallel to the fiber axis, suggesting that the OH groups are predominantly oriented parallel to the chain axis. The bands are not clearly resolved, however, so it is possible that some of the bands might be more intense with the electric vector perpendicular to the chain axis.

Although the OH bands in both the *Valonia* and ramie spectra appear to be most intense with the incident electric vector parallel to the fiber axis, the band frequencies differ significantly. The spectra of *Valonia* have a peak at 3231 cm^{-1} that is not observed in the spectra of ramie. The spectra of ramie, on the other hand, have a peak at 3429 cm^{-1} that is not observed in *Valonia* spectra. The frequency differences suggest that the hydrogen-bonding pattern in *Valonia* cellulose differs from the hydrogen-bonding pattern in ramie cellulose. These differences in the hydrogen-bonding patterns are related to the structural differences between the I_α and I_β forms of native cellulose, which are discussed in more detail elsewhere[17,27].

CONCLUSIONS

Based on the relationships between band intensities and the polarization of the incident light, the bands in the Raman spectrum of cellulose were classified into four groups. The classification of the bands in this manner revealed information about the direction of the vibrational motions in cellulose. The directions of the vibrations are such that the major change in polarizability associated with the motions is either parallel or perpendicular to the chain axis. Raman spectra recorded from deuterated celluloses allowed the vibrational modes involving CH and OH motions to be identified. These spectra demonstrated that most of the modes are complex coupled vibrations. Results from normal-coordinate analyses of cellulose model-compounds were used in order to determine the types of motion most likely to occur in each region of the spectrum. These calculations also

suggested that the vibrational motions are very complex. The information from the normal-coordinate calculations, intensity studies, and spectra of deuterated celluloses aided in the interpretation of the vibrational spectrum of cellulose. The importance of these results, even though they are not complete assignments, lies in the foundation that they establish for microprobe studies of native tissues by providing a thorough characterization of the bands in the vibrational spectrum of cellulose.

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