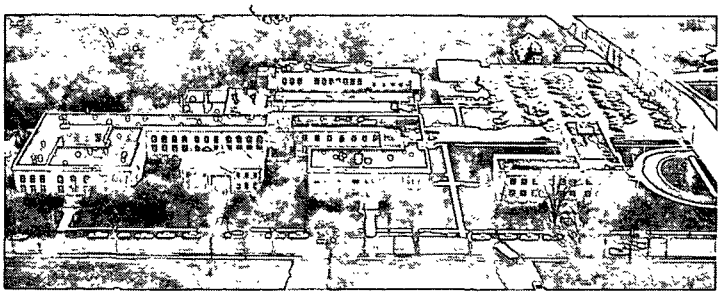

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STUDIES ON THE STRUCTURE OF CELLULOSE USING RAMAN SPECTROSCOPY AND SOLID STATE ^{13}C NMR

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STUDIES ON THE STRUCTURE OF CELLULOSE USING RAMAN SPECTROSCOPY AND SOLID STATE ^{13}C NMR.

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SYNOPSIS

An overview of earlier reports on Raman spectroscopic and solid state ^{13}C NMR studies is presented, together with the results of recent investigations extending earlier programs. The earlier studies have shown that there are two stable ordered conformations of the cellulose molecule; they are predominant in celluloses I and II respectively. They occur separately or with each other, and together with disordered conformations in most cellulosic materials. The earlier studies have also shown that the majority of native celluloses are composites of two crystalline forms, I_α and I_β, which

occur in different proportions depending on the source of the cellulose. The I_α form is dominant in celluloses produced by primitive organisms, while those produced by the higher plants have the I_β form dominant.

Further studies have shown that the I_α and the I_β forms possess cellulose molecules with the same conformations of the heavy atom skeleton, but with different hydrogen bonding patterns. These in turn result in slightly different lattices. Studies of the effects of perturbing treatments show that the I_α form is more susceptible to hydrolytic treatments, and that it can be transformed into the I_β form by a number of treatments which are known to disrupt the structure of the lattice without dissolution. All of these observations point to greater stability of the I_β form. The results of examination of celluloses from a variety of algal sources were found consistent with a speculation that the balance between the I_α and I_β content of a particular native cellulose is correlated with the pattern of organization of the terminal complexes at the site of biogenesis of cellulose fibrils on the surface of the cell wall membrane.

INTRODUCTION

Though the structures of cellulose have been the subject of investigation over many decades, probably more intensively than those of any other natural homopolymer, many questions remain unanswered [1]. No single proposal or hypothesis has reconciled all the data from different approaches to structural investigation. Over the past decade the difficulty has arisen anew as attempts were made to reconcile the results of spectroscopic investigations, using newly available instrumental methods, with the results of diffractometric studies which have been used to refine the classical models of structure. In recent years, however, a measure of convergence has emerged.

In past reports we have described the results of our investigations of the structures of cellulose, based on use of both Raman spectroscopy and solid state (CP/MAS) ^{13}C NMR. In this report we present an overview

of the results of our earlier studies, and then extend our understanding of the nature of the structures by incorporating recent findings from our continuing studies.

BACKGROUND

Studies of Molecular Conformations

In the first comprehensive report on Raman spectroscopic studies of cellulose, one of us concluded that the differences between the spectra of celluloses I and II could not be accounted for on the basis of the then available structures derived from diffractometry [2]. It was argued that the differences between the spectra of celluloses I and II, particularly in the conformation sensitive low frequency region, could only be accounted for by a structural model in which the chain conformations are different, and in which they depart from the two-fold helical symmetry generally assumed to prevail in all the structures accepted at that time. It was speculated that the conformations of celluloses I and II might be represented as slight right-handed and left-handed departures from the two-fold symmetry, respectively. These speculations were informed, in part, by examinations of the structures of cellobiose [3] and β-methyl cellobioside [4], as well as some of the conformational energy calculations available at that time.

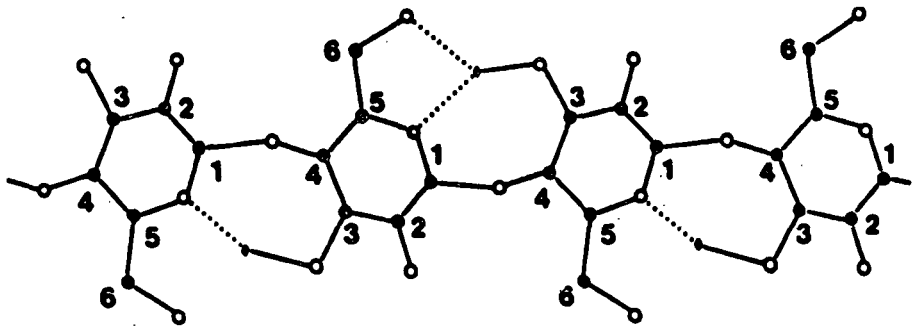
Further consideration of packing in the unit cells, and further examination of the structures and spectra of the two model disaccharides led to revisions of the proposed conformational differences [5]. The new proposal was based, in the first instance, on the infrared and Raman spectra of the two disaccharides in the OH stretching region, and on the proximity of the dihedral angles of their glycosidic linkages to those associated with the double minima observed in conformational energy maps. These minima occur on either side of the line which defines all structures possessing two-fold helical symmetry, though they are quite close to the line [6,7]. The revised proposal envisioned the glycosidic linkages in a chain of cellulose as alternating in conformation

between small left- and right-handed departures from a two-fold helix conformation. The differences in conformations between celluloses I and II were viewed as resulting from differences between the internal organization, in space, of the anhydrocellobiose unit which is taken to be the basic repeat unit of physical structure.

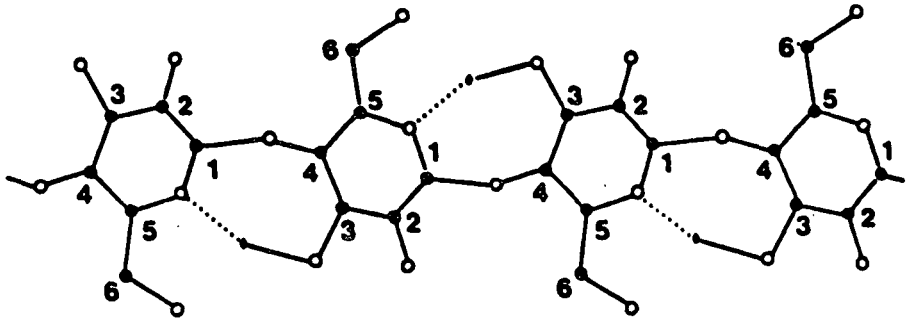
In the first studies of the solid state (CP/MAS) spectra of cellulose, the resonances for C1 and C4 were clearly shown to be split into multiplets [8,9]. These observations were taken to support the proposal that the disaccharide unit, consisting of anhydrocellobiose with nonequivalent anhydroglucose units, was the basic repeat unit of structure. Such a structure inherently implies glycosidic linkages which alternate in conformation along the cellulose chain [8]. Particularly in the case of cellulose II, where only the resonances of carbons 1 and 4 are split, the spectra imply that neither lattice packing nor hydrogen bonding patterns can account for the splitting, but rather a nonequivalence of adjacent anhydroglucose units, centered at the glycosidic linkage is the most plausible explanation.

Finally, these interpretations were brought together with the results of studies of the vibrational spectra of celluloses I and II, for the OH stretching regions, in the proposal that the key difference between the two conformations characteristic of the two forms of cellulose, is the presence in cellulose I of a bifurcated intramolecular hydrogen bond analogous to that observed in β-methyl cellobioside. This hydrogen bond involves the proton from the hydroxyl group on C3 of one ring, and both the ring oxygen and the oxygen of the primary hydroxyl on the adjacent ring [10]. Such a bond was assumed to occur in association with the alternate glycosidic linkages that have internal coordinates similar to those of the β-methyl cellobioside, and which therefore would have interatomic distances, between the three oxygen atoms involved, which are short enough to allow the bifurcated intramolecular hydrogen bond system to form.

Schematics of the two proposed conformations are shown in Figure 1. They have been identified as k_I and k_{II} because of their predominance in celluloses I and II respectively. The key difference between the conformations depicted in Figure 1, is the presence of the



k_I



k_{II}

Legend:
- ● = Carbon
- ○ = Oxygen
- • = Hydrogen
- —— = Covalent bond
- ····· = Hydrogen bond

FIG. 1. Schematic representation of conformations k_I and k_{II}. Skeletal atoms are depicted in approximately the same positions in both conformations; the key difference lies in disposition of the intramolecular hydrogen bonds.

bifurcated intramolecular hydrogen bond associated with every other glycosidic linkage in conformation k_I. The association of distinctive Raman spectra with each of these conformations, particularly in the low frequency region, allowed the development of procedures for quantifying the varying degrees of conformational distributions in samples of cellulose which are only partially converted from one form to another. One of the key practical consequences of this work was the realization that most processes which convert cellulose I to cellulose II, whether based on mercerizaion or regeneration, result in only partial conversion of the cellulose; a residual amount of cellulose in the conformation characteristic of cellulose I is always present, even though it is usually not detected by x-ray diffractometry.

Since these proposals were presented, a number of their elements have received support from the results of other investigations. The occurrence of the double minima in the conformational energy maps was confirmed in the study by Perez et al. [11], who also found that nonequivalent glycosidic linkages are to be expected in cellotetraose. The plausibility of alternating glycosidic linkages has been supported by the investigations of French [12] and of Sakthivel et al. [13] both reported in the present proceedings.

Studies of Crystallinity

The first major undertaking in our joint effort was an investigation of the origin of the multiplicities in the solid state (CP/MAS) ^{13}C NMR spectra of the celluloses, particularly those of native celluloses [14,15]. The multiplicities in the spectra of pure samples of cellulose II could be rationalized in terms of physically nonequivalent environments for chemically equivalent carbons, but within a single unit cell. For the native celluloses, however, the multiplicities could not be accounted for in terms of different sites in a unique unit cell. The sharper components of the C-1, C-4, and C-6 resonances possess multiplicities that suggest magnetically nonequivalent sites within crystalline domains. The narrow lines observed are different within the spectra of different native forms; the relative intensities are not constant, and they are not in the

ratios of small numbers as would be expected if they arose from different sites within a single unit cell.

We have found the proposal that native celluloses are composites of two distinct crystalline forms, identified as I_α and I_β, the most plausible basis for interpretation of the spectra. A decomposition of the spectra based on such a model has been described in our earlier reports. The spectra were obtained by taking appropriate linear combinations of the spectra of a regenerated cellulose I and of a cellulose from Acetobacter xylinum; these two celluloses were judged to be the closest to the two extremes on the basis of a two component model.

In further investigations of the solid state ^{13}C NMR spectra, relaxation measurements on both protons and carbon nuclei were made [16]. They confirmed that unit cell inequivalences, rather than crystal surface chain resonances, determine the profiles of the sharp multiplets. It was also found that the higher plant celluloses contain a smaller proportion of the I_α form than had previously been proposed.

Experiments based on weak ^{13}C - ^{13}C spin exchange were also conducted. These probed the spatial environment, within a radius of 0.7 to 1.0 nm, around carbons identified with individual multiplet components, assumed to belong exclusively to the I_α or I_β forms. The spectra of "nearest neighbours" were isolated for three different multiplet lines in an algal cellulose, and for two different lines in a higher plant cellulose. The results rule out the possibility that tertiary morphology can give rise to any multiplicity in these spectra. The results also provide strong support for the hypothesis of multiple crystalline forms in the algal cellulose; however, no additional evidence for multiple crystalline forms in the higher plant celluloses was developed from these measurements.

FURTHER STUDIES OF NATIVE CELLULOSES

Raman Spectral Studies

In light of the earlier proposals concerning the conformational states of cellulose chains, the question naturally arose whether the two forms, I_α and I_β, represent true solid state allomorphs, with molecules possessing identical conformations, or whether, like celluloses I and II, they contain molecules with different conformations as well as different lattices [17].

Comparison of the Raman spectra in the conformation sensitive region revealed a great deal of similarity in the spectra of the native celluloses used in the ^{13}C NMR studies. In the OH stretching region, on the other hand, there were significant differences. These observations have been described in detail by Wiley and Atalla [18,19], and led to the conclusion that the I_α and I_β forms of cellulose represent lattices with nearly identical conformations of the heavy atom molecular skeletons, but with different hydrogen bonding patterns.

In an extension of these studies, an effort was made to resolve the the OH stretching bands into those of the I_α and I_β components. The original spectra used in the resolution were those of a regenerated cellulose I, which was known to be essentially of the pure I_β form, and of a cellulose from Cladophera glomerata, which is similar to celluloses from Valonia ventricosa, and equally rich in the I_α form. Beginning with the proportions of the two forms determined from the solid state ^{13}C NMR spectra, it was possible to resolve the Raman spectra in the OH stretching region into two component spectra correspondiong to the two pure forms.

The two spectra are shown in Figure 2, which displays the Raman spectra of the two forms in the OH stretching region. It is clear from Figure 2 that the I_α spectrum possesses bands which do not occur in the I_β spectrum, and vice versa. Though in the studies by Wiley and Atalla it was not possible to distinguish the

bands seen in Figure 2, it was clear that the lowest frequency band in the OH stretching region of the spectrum of the I_α component does not occur in the spectrum of the I_β component. Similarly, the high frequency shoulder that is pronounced in the spectrum of the I_β component does not occur in the spectrum of the I_α component. These spectra, when taken together with observations noted earlier, provide further support for the view that the key difference between celluloses I_α and I_β lies in the patterns of hydrogen bonding.

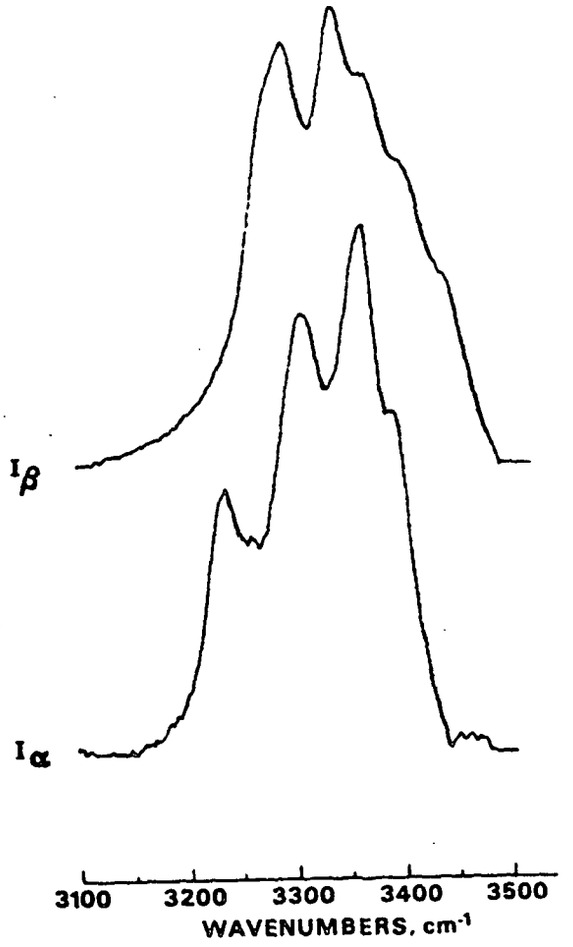


FIG. 2. The resolved spectra of the I_α and I_β components of native celluloses in the OH stretching region of the Raman spectra.

Susceptibility to Structural Peturbation

In an important extension of the characterization of the structure of cellulose, the relative susceptibilities of the different forms to perturbation have been explored. We have carried out studies of the response to acid hydrolysis, and to the partially reversible transformations associated with treatment with liquid ammonia. Others have carried out different transformative treatments which are similar in effect. We review these here.

Studies of hydrolysis

In our first exploration of the effects of acid hydrolysis [20] we reported that in the highly crystalline cellulose from Rhizoclonium heiroglyphicum, both components of the original material were equally susceptible to acid hydrolysis. We have since applied a more severe hydrolytic treatment corresponding to boiling in 4 Normal HCl for 44 hours. In this instance the algal source was Cladophera glomerata, which produces a cellulose equal to that of Rhizoclonium in crystallinity and I_α content. This treatment resulted in a yield of 22% and did indeed show a greater susceptibility of the I_α form to the hydrolytic environment. The ^{13}C NMR (CP/MAS) spectra are presented in Figure 3, and clearly show a significant loss of the I_α component. This can be interpreted as the result of greater susceptibility of the I_α form to acid hydrolysis, or of some transformation of the I_α form to the I_β form.

Horii and coworkers have reported a transformation of the I_α form to the I_β form as the result of treatment in steam at high temperatures, up to 280°C [21]. The conditions they used are expected to be highly hydrolytic. They report, however, that their yield was approximately 85% [22], so that a substantial amount of the I_α form must be transformed to the I_β form.

Both our results and those of Horii, et al. indicate that the I_β form is more stable than the I_α form,

whether its disappearance is attributed to hydrolysis or to conversion.

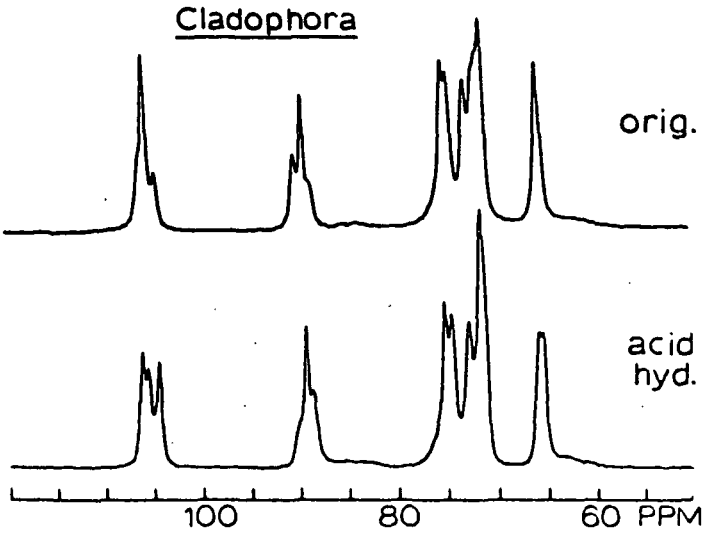


FIG. 3. Solid state CP-MAS ^{13}C NMR spectra of Cladophera glomerata cellulose before and after acid hydrolysis.

Liquid ammonia treatment

In yet another study of the response of the structure of native forms to perturbation, we treated some of the cellulose derived from Cladophera glomerata in anhydrous liquid ammonia, under conditions that result in transformation to high crystallinity cellulose III [23]. We then recovered some cellulose I by boiling a portion of the cellulose III in water. We have labelled this sample I_{III} to indicate that it is regenerated from cellulose III. The ^{13}C NMR (CP/MAS) spectra are shown in Figure 4; they are quite similar to spectra reported by Chanzy and coworkers for cellulose III and I_{III} prepared from Valonia macrophysa cellulose [24]. The Raman spectra we have recorded are included in Figures 5 and 6, together with spectra of samples of celluloses I and II, for purposes of comparison.

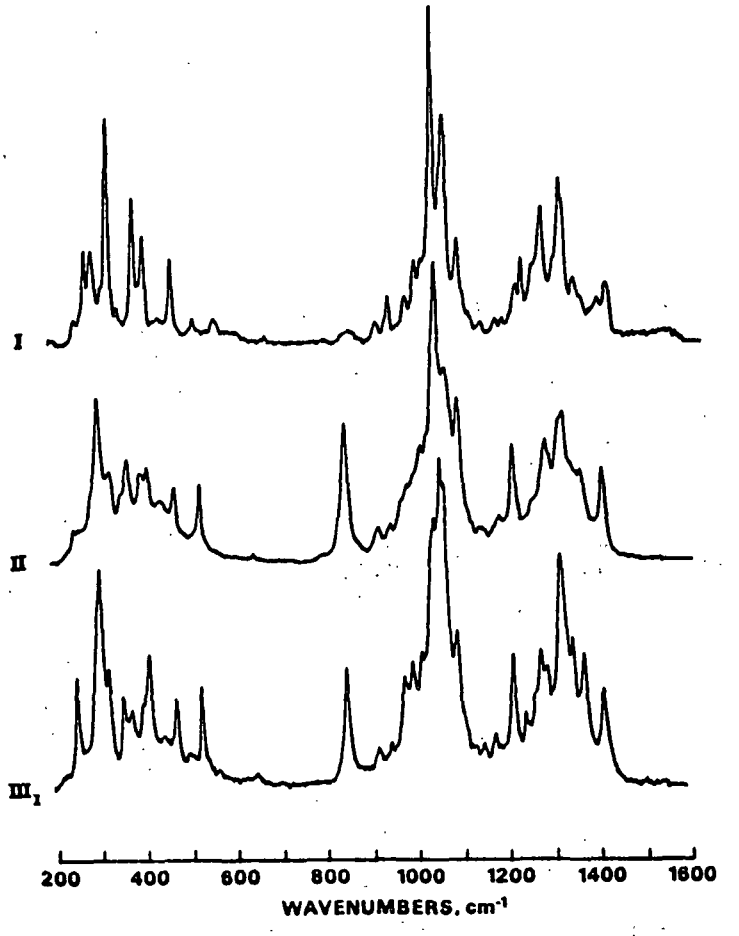


FIG. 5. Raman spectra of celluloses I, II, and III, in the conformation sensitive region.

(CP/MAS) and Raman spectra of cellulose I_{III}, shown in Figures 4 and 6, respectively, are typical of those of celluloses derived from the higher plants, where the I_β component is dominant. Thus, the treatment in anhydrous liquid ammonia followed by boiling in water, results in transformation of I_α cellulose to I_β cellulose. Here again there is a clear implication that the I_β form is more stable than the I_α form.

It should be noted that in the course of electron microscopic examination Chanzy and coworkers observed that the transformation from I to III to I_{III}, is

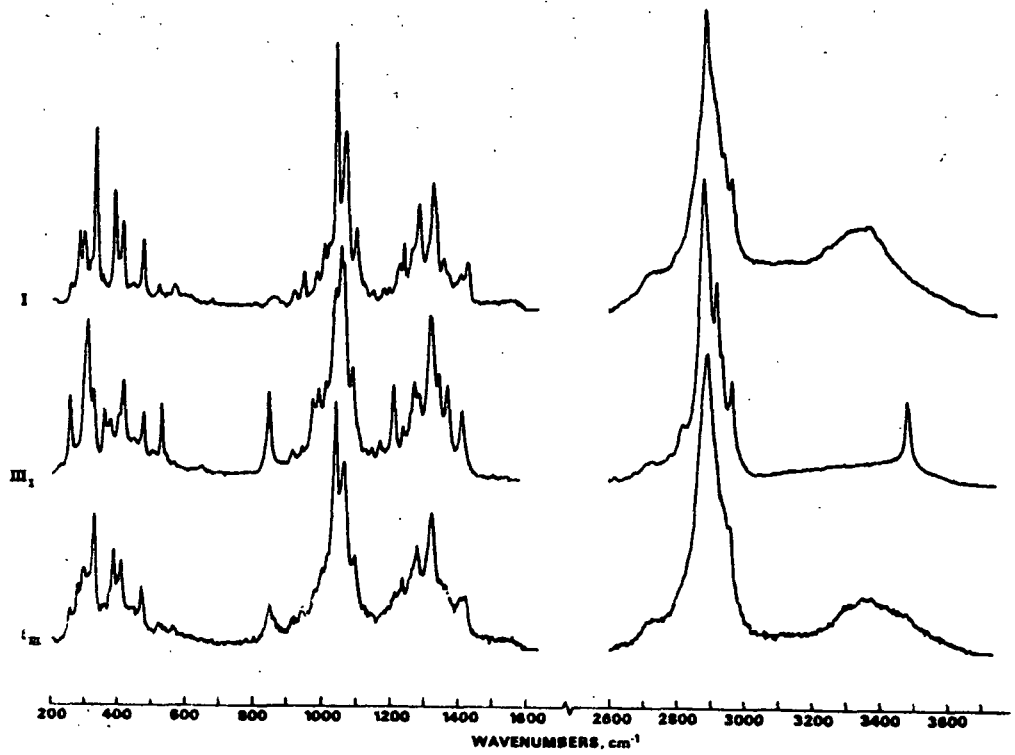


FIG. 6. Raman spectra of Cladophera cellulose, in the native form, after conversion to cellulose III, and after reversion to the cellulose I lattice upon boiling in water.

accompanied by a reduction of the lateral size of the fibrils from dimensions typical of Valonia celluloses to ones characterisitic of higher plant celluloses. This led them to the speculation that the differences in the NMR spectra are associated with differences in morphology.

Acetate transformation

In their study of the acetylation of Valonia cellulose, Horii and coworkers [25] investigated the effects on the (CP/MAS) spectra of heterogeneous acetylation, followed by heterogeneous deacetylation. They observed an effect almost identical to that reported above for the transformation of Cladophera cellulose when it was converted to cellulose III and then to cellulose I_{III}. That is, it was changed from a cellulose of quite high crystallinity and with the I_α form dominant, to a cellulose of somewhat lower crystallinity, with the I_β form dominant. An analogous experiment with a cotton cellulose showed little or no change in the form of the cellulose recovered when it was compared with the starting material. These results also are consistent with greater stability of the I_β form.

STRUCTURE AND THE PATTERNS OF BIOGENESIS

In further extension of our studies of the two forms of cellulose I, we have considered the possibility that the distribution of the two forms in different native celluloses may reflect patterns of biogenesis; we have previously noted that the more primitive forms of cellulose producing organisms are the ones which produce the native forms with higher I_α content. It occurred to us that the balance between the I_α and I_β forms may be associated with the organization of the assembly of the elementary fibrils during biogenesis [26]. In pursuit of this possibility we have examined celluloses from a variety of primitive plant forms. These will be reported in detail elsewhere, but we present here two preliminary observations. They are based on examination of celluloses from the two algae, Chara excelcius and Laminaria japonica.

Chara excelcius is a member of the order Zygnemateles, which is thought to be the algal precursor to the higher plant forms; it appears to be the most primitive plant form possessing arrays of rosettes as the primary sites of cellulose biosynthesis. The organization of biosynthetic complexes has been investigated most comprehensively by Brown and his coworkers and presented in overview by Brown [27]. Laminaria japonica is a brown alga, and is of particular interest because it is one of the primitive algae which possess linear terminal complexes as the sites of cellulose biosynthesis, but, unlike the other algae of this type examined by us so far, it produces cellulose with crystalline lateral dimensions more akin to those of the higher plants.

The (CP/MAS) spectra of the cellulose from Chara excelcius show it to be similar to the higher plant celluloses, in that it is predominantly of the I_β form. Thus, it appears that the indication of a correlation between the predominance of the I_β form and the organization of biosynthetic sites into rosettes, is indeed supported by all of our observations so far.

The (CP/MAS) spectra of cellulose from Laminaria show it to be very much like those of other primitive algae wherein the I_α form is dominant. As noted earlier, it is of interest here because the X-ray diffractogram of this cellulose shows it to be more like the celluloses of the higher plants with respect to lateral dimension. The observation that it is predominantly of the I_α form even though it is limited in lateral dimension, excludes the possibility that the differences between the two forms, I_α and I_β, are primarily manifestations of morphological differences.

These observations, when taken together with pattern of variation of the organization of synthesizing complexes described by Brown, lead us to the suggestion that the native celluloses synthesized by the organisms which possess arrays of rosettes as the primary sites of biosynthesis are predominantly of the I_β form, while the celluloses synthesized by organisms which possess linear or otherwise arranged terminal complexes as the synthesizing sites are predominantly of the I_α type. Thus, it appears that the differences between the two forms are related to the architecture and organization

in space of the synthesizing complexes. The implications of this conclusion are quite broad, but we will discuss them in greater detail elsewhere.

In an interesting extension of the question of the relation between biogenesis and structure Chanzy and his coworkers [28] recently investigated the (CP/MAS) spectra of tunicin, which is an animal cellulose derived from a marine organism. They observed that the spectra were of the pure I_β form. That is, there were no indications in the spectra of any of the spectral features normally associated with the I_α component. There findings are consistent with the proposal of distinct crystalline forms of cellulose, as well as with our conclusion that the I_β form is the more stable one.

CONCLUDING SUMMARY

In summary then, the Raman spectra of the many celluloses investigated over many years support the conclusion that there two stable ordered conformations of the cellulose molecule, which occur most often in the majority of samples. The two conformations are predominant in celluloses I and II, respectively, and occur separately or together with disordered conformations in most cellulosic materials.

The ^{13}C NMR (CP/MAS) spectra indicate that the majority of native celluloses are composites of two crystalline forms, I_α and I_β, which occur in different proportions depending on the source of the cellulose. The celluloses produced by primitive organisms have the I_α component dominant, while those produced by the higher plants have the I_β form dominant.

Further Raman spectral studies have shown that the I_α and the I_β forms possess cellulose molecules with the same conformations of the heavy atom skeleton, but with different hydrogen bonding patterns. These in turn result in slightly different lattices.

Studies of the effects of perturbing treatments, both by ourselves and by others, leave little question

that the I_β form is the most stable one. Studies of the effects of boiling in acid and in water at high temperatures and pressures, resulted in preferential hydrolysis of the I_α form or the transformation of the I_α form to the I_β form. Processes which disrupt the structure of the native cellulose, but nevertheless allow regeneration of cellulose I, were also found to result in transformation of cellulose from the I_α form to the I_β form. These processes included treatment with anhydrous ammonia followed by regeneration in boiling water, and heterogeneous acetylation followed by heterogeneous deacetylation.

Examination of celluloses from a variety of algal sources confirmed a speculation that the celluloses with the I_β form dominant arise from organisms that possess arrays of rosettes at the primary sites of assembly of the cellulose molecules, while those with the I_α form dominant arise from organisms that possess the geometrically more simple arrangements of terminal complexes.

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REFERENCES

[1] R. H. Atalla, In Structures of Cellulose, R. H. Atalla, Ed., ACS Symposium Series No. 340, American Chemical Society, Washington, DC, 1987, p. 1.
[2] R. H. Atalla, Appl. Polym. Symp. 1976, 28, 659.
[3] S. S. C. Chu and G. A. Jeffrey, Acta Crystallogr. 1968, B24, 830.

[4] J. T. Ham and D. G. Williams, Acta Crytallogr. 1970, B29, 1373.
[5] R. H. Atalla, In Hydrolysis of Cellulose, R. D. Brown, Jr. and L. Jurasek, Eds., Advances in Chemistry Series No. 181, American Chemical Society, Washington, DC, 1979, p.55.
[6] D. A. Reese and R. J. Skerrett, Carbohyd. Res. 1968, 7, 334.
[7] S. Melberg and K. Rasmussen, Carbohyd. Res. 1979, 71, 25.
[8] R. H. Atalla, J. C. Gast, D. W. Sindorf, V. J. Bartuska, and G. E. Maciel, JACS 1980, 102, 3249.
[9] W. L. Earl and D. L. VanderHart, JACS 1980, 102, 3251.
[10] R. H. Atalla, Appl. Polym. Symp. 1983, 37, 295.
[11] B. Henrissat, S. Perez, I. Tvaroska, and W. T. Winter, In Structures of Cellulose, R. H. Atalla, Ed., ACS Symposium Series No. 340, American Chemical Society, Washington, DC, 1987, p.38.
[12] A. D. French, Proceedings of the 10th Cellulose Conference, May 1988, this volume.
[13] A. Sakthivel, A. F. Turbak, and R. A. Young, Proceedings of the 10th Cellulose Conference, this volume.
[14] R. H. Atalla and D. L. VanderHart, Science 1984, 223, 283.
[15] D. L. VanderHart and R. H. Atalla, Macromol. 1984, 17, 1465.
[16] D. L. VanderHart and R. H. Atalla, In Structures of Cellulose, R. H. Atalla, Ed., ACS Symposium Series No. 340, American Chemical Society, Washington, DC, 1987, p.88.
[17] R. H. Atalla, In Structure, Function and Biosynthesis of Plant Cell Walls, W. M. Dugger and S. Bartinicki-Garcia, Eds., American Society of Plant Physiologists, Rockville, MD, 1984, p.381.
[18] J. H. Wiley and R. H. Atalla, In Structures of Cellulose, R. H. Atalla, Ed., ACS Symposium Series No. 340, American Chemical Society, Washington, DC, 1987, p.151.
[19] J. H. Wiley and R. H. Atalla, Carbohyd. Res. 1987, 160, 113.
[20] R. H. Atalla, R. E. Whitmore, and D. L. VanderHart, Biopolymers 1985, 24, 421.
[21] F. Horii, H. Yamamoto, R. Kitamaru, M. Tanahashi, and T. Higuchi, Macromol. 1987, 20, 2949.
[22] F. Horii, personal communication.

[23] J. Hayashi, personal communication.
[24] H. Chanzy, B. Henrissat, M. Vincendon, S. Tanner, and P. S. Belton, Carbohyd. Res. 1987, 160, 1.
[25] A. Hirai, F. Horii, and R. Kitamaru, Macromol. 1987, 20, 1440.
[26] W. Herth, personal communication.
[27] R. M. Brown, Jr., J. Cell. Sci. 1985, Suppl. 2, 13.
[28] H. Chanzy, personal communication.