The Structures of Native Celluloses, and the Origin of their Variability

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The structures of native celluloses have traditionally been presented in terms of two-domain models consisting of crystalline and non-crystalline fractions. Such models have been of little help in advancing understanding of enzyme-substrate interactions. In this report we first address issues that complicate characterization of the structure of native celluloses particularly with respect to information that may be relevant to understanding susceptibility to enzyme action. Key among these is the fact that crystallographic models define structure primarily at the level of the unit cell while susceptibility to the action of enzymes appears to be influenced more by aggregation at the next higher level of structure, that of the elementary fibrils. Recent developments in structural characterization, based primarily on the use of spectroscopic methods, have revealed that native celluloses are species specific composites of two forms of cellulose, I_α and I_β. They appear to possess the same secondary structure but distinctly different tertiary structures. Their aggregation appears to reflect an inherent tendency of the cellulose molecules to self-assemble into fibrils at a hierarchy of levels. This is most clearly revealed through observations of the biogenesis of cellulose in model systems incorporating cultures of *Acetobacter xylinum.* Since the induction of enzyme systems seems to be sensitive to subtle differences in tertiary structure, advances in characterizing the variability of structure at these levels will be central to future explorations of the dynamics of enzyme-substrate interactions.

INTRODUCTION

Review of the literature on biodegradation of cellulose reveals an increasing diversity among the enzyme systems which have been identified as active in the degradation of cellulose as well as related plant cell wall polysaccharides. It is also evident that the process of induction of the enzymes involves complex interactions between the cellulose containing substrates and the micro-organisms producing the enymes. While characterization of the enzyme systems has advanced considerably through induction of modem methods of protein chemistry, our understanding of the variabilily of the substrates is much more limited. Yet a deeper understanding of the nature of native celluloses is a prerequisite for advances in characterizing the dynamics of the enzyme-substrate system. Native celluloses are of particular importance because they are the substates that have guided the evolution of the wide variety of cellulolytic enzyme systems produced by different organisms and because the structures of the native celluloses are complex and diverse. The diversity of the structures is important because, perhaps more than any other natural substrate, the susceptibility of cellulose to enyme action is as much a function of its state of aggregation as it is of its primary structure. And the state of aggregation of each native cellulose seems to be specific

to the species within which it is formed. This has not been generally recognized, in part, because of the tendency of studies of cellulose structure to view cellulose within the conceptual framework of polymer science without adequate attention to its biological character. Thus, studies of structure often seek to establish an ideal crystal lattice for native cellulose and to regard all real celluloses as departing from this ideal form primarily with respect to the degree of disorder. The disorder is then regarded as imparting an amorphous character to some of the cellulose and methods are sought to quantify the "amorphous" fraction.

In contrast, recent studies have shown that all celluloses that are relatively pure in their native states are composites of two distinct forms that are intimately blended in a manner and in proportions that are unique to the species producing them. The far wider category of celluloses that, in their native states, are intimately blended with other polysaccharides and with lignin, are even more diverse with respect to their patterns of aggregation. This report will discuss efforts to characterize the nature of native celluloses and their states of aggregation beyond simply categorizing their degree of order and in a manner that may allow more comprehensive investigations of the nature of the interaction between the cellulosic substrates and the microrganisms that disassemble them. One intent of the discussion is to make the case that, at least in the context of biological studies, there is a need for a significant shift in the manner in which cellulose is viewed. In particular, it is no longer adequate to regard it simply as the β-1,4-linked polymer of anhydroglucose and to apply to it most of the usual principles and methodologies of polymer science. While these are indeed helpful in many contexts, particularly those based on the industrial utilization of cellulose and cellulose derivatives, the traditional principles of polymer science do not begin to capture the distinctive features of the molecule which are associated with its entry into the majority of biological processes.

In this report we first address the issues that complicate characterization of the structure of cellulose particularly with respect to information that may be relevant to understanding enzyme-substrate interactions. We then review recent developments concerning our understanding of structure in native celluloses. Information developed from spectroscopic investigations that have been undertaken in recent decades is presented first. The patterns of self-assembly of native cellulose are then considered on the basis of observations of its biogenesis in model systems incorporating cultures of *Acetobacter xylinum*, the cellulose producing bacterium. Finally, future directions are considered briefly.

BACKGROUND

The view of cellulose presented here is not entirely novel for it was at the center of some thought at the turn of the last century. This is reflected in the writings of Cross and Bevan, among the pioneers in the investigations of cellulose, when they first confronted the proposal that cellulose possesses characteristics of a crystalline material. They wrote "*The root idea of crystallography is identical invariability; the root idea of the world of living matter is essential individual variation*" (1). Their comments were in response to proposals of crystallinity in cellulose based on observations of its birefringence fully a decade before the first x-ray diffraction patterns had been recorded for cellulose.

In recognition of the many biological functions of cellulose, there is a need to reclaim it from the field of polymer science and to recognize that it is a unique biological molecule with many distinctive properties that are key to its entry into biological processes. Over the past 100 years much of the research on cellulose has been undertaken in relation to its utilization in industral processes. It is perhaps not surprising that the paradigms of polymer science, developed initially in relation to cellulose, but then extended considerably in relation to synthetic polymers, would be used as the basis for investigations of cellulose. The perspective they represent, however, is not adequate to the native celluloses. The majority of crystallizable synthetic polymers, as well as cellulose regenerated from solution, can be viewed in terms of the notions of crystallinity and the occurrence of separate domains that are amorphous or crystalline, depending on the past history of the sample. For native celluloses, in contrast, it is increasingly obvious that they must be regarded as self-assembling naturally occurring substances, and that the self-assembly occurs at a hierarchy of levels that cannot be understood in terms of the simplistic notions of crystallization and separate phases, both of which are rooted in the classical thermodynamics of macroscopic systems.

When considering the structures of native celluloses it is important to distinguish two categories of native forms. The first category includes the celluloses that occur in relatively pure form in their native state and which can be isolated using procedures that are relatively mild and that do not perturb or alter the state of aggregation to any significant degree. These are also the celluloses that have been the subject of most structural studies. They will be identified as the *Pure Celluloses.* Examples would be cotton, bacterial cellulose and some of the algal celluloses. The second category, which includes the vast majority of naturally occurring celluloses, consists of the celluloses that are an integral part of the complex architecture of the cell walls of higher plants and which, in the vast majority of instances, occur intimately blended with the other cell wall constitutents; to distinguish them from the first category they will be described as *Complex Celluloses.* They are most intimately blended with other cell wall polysaccharides, which are primarily other b-1,4-linked pentosans or hexosans with varying degrees of limited branching, collectively identified as the hemicelluloses. The celluloses and the other cell wall polysaccharides are together also quite intimately integrated with cell wall lignins. The celluloses in the second category undergo significant changes in their state of aggregation during traditional isolation procedures (2). However, these changes have not been well recognized or characterized in most instances. One of the major challenges yet to be overcome in future studies is the definition and characterization of the native state of such celluloses prior to the application of isolation procedures that, in most instances, are severe and disruptive of structure. In studies of the action of cellulalses and cellulosomes, examination of the transformations of higher plant celluloses during isolation is particularly relevant when commercial microcrystalline celluloses are used as the substrates. Most such celluloses are prepared from dissolving pulps, which are the residues of the secondary walls of woody plants after they have been exposed to the very severe thermal and chemical conditions involved in pulping and bleaching commercial pulps.

The usual practice in most prior discussion of higher plant celluloses has been to view the structures of the pure celluloses as adequate models of the more complex forms of cellulose. Until recently, it has generally been assumed that the cell wall structure in higher plants is adequately represented as a two phase system, one consisting of the microfibrils of pure cellulose, the other a blend of all of the other constituents (3). It is implicit in this view that the cellulose from such cell

walls can be isolated by removing the other components, leaving behind the cellulose in a condition that approximates its native state. Furthermore, it is also generally assumed that the microfibrils, both in their native state and after isolation, are not unlike those that occur in the pure native celluloses. There are now reasons to reassess the assumptions, and the reassessments are at two levels (4). The first follows from observations that it is not possible to remove the other cell wall constituents without altering the aggregation of the cellulose at the microfibrillar level. The second follows from evidence that raises questions concerning validity of the two phase model. While for some applications the adoption of two phase models of the architecture of plant cell walls may be useful, it is now clear that the models must be revised to incorporate recent findings that indicate that they can no longer rationalize the organization of plant cell walls or their cellulosic constituents. All of these considerations are central to the many questions associated with enzyme-substrate interaction.

Much of the discussion of the pure celluloses has concentrated on secondary and tertiary structure at the level of the unit cell (with characteristic dimensions of the order of 1 nm), and on the equivalence or non-equivalence of different monomeric or dimeric units within the unit cell (4). What are more relevant with respect to interactions with enzymes, however, are the implications of organization in cellulose at the level next above that of the unit cell; for native celluloses that level is the most elementary of fibrillar structures. And it is at this level that departures from the organization of an ideal lattice first manifest themselves. They are most clearly obvious in the curvature and twisting that is seen in most electron micrographic images of native celluloses. The key point is that because they represent departures from an ideal lattice they influence the results of all measurements that are sensitive to lattice order. Measurements carried out on a substance with an ideal lattice would result in diffraction patterns that are very sharp and spectral lines that are very narrow. Departures from such a lattice result in broadening of both diffraction and spectral measurements, and such broadening is frequently used in efforts to quantify order in materials or arrive at measures of the degrees of their crystallinity. Such measurements have also been adopted in attempts to quantify the degee of order in cellulosic samples and they have been used to explore correlations between order in the cellulosic substrates and their susaptibility to enzymatic action. The difficulty with this approach is that the traditional interpretations of broadening in diffractometric patterns or spectral lines have been in terms of the occurrence of domains of homogeneously disordered matter. While assumptions that such domains occur are reasonably valid approximations in the case of most synthetic polymeric materials, and even for regenerated cellulose, they are at best very questionable in the instance of native celluloses.

The reality is that most native celluloses are not at all disordered but rather highly organized, often in a hierarchy of morphological features that are defined at the different scales of observation of the native tissue within which the cellulose occurs. The most elementary level beyond the unit cell is the microfibril, with characteristic lateral dimensions of the order 4 to 6 nm, and with longitudinal dimensions typically considerably in excess of 10 nm. With the exception of selected algal celluloses and the celluloses from the tunicates, which can have microfibrils with lateral dimensions of the order of 20 nm, all native celluloses depart from the organization of the ideal lattice at the level of the microfibril. These departures from the ideal lattice represent features that are unique to a particular native cellulose, and it is these futures that must be understood before the complex interaction of enzyme with substrate can be fully defined.

The curvature and the twisting of the fibrils necessarily result in departures from ideal lattice order. To facilitate characterization of these departures it is helpful to consider the different levels of structure in aggregated cellulose. The primary structure of cellulose has been well established for some time, of course, and is not in question here. The secondary level of structure, that is, the conformation, reflects the internal organization of individual monomeric units within the molecule as well as their relationship to each other in the chain structure. The tertiary level then is concerned with the arrangements of the individual molecules relative to each other in a particular aggegated form. In much of the literature on the structure of cellulose the distinctions between primary, secondary, and tertiary structure are not considered because specification of the coordinates of the atoms within a unit cell, which is the form in which crystal structures are usually presented, implicitly defines all three levels of structure. The recent findings alluded to above have been primarily based on spectroscopic methods, and in the interpretation of the results of such studies the distinctions between primary, secondary and tertiary structures is quite helpful.

The distinction between the different levels of structure is also likely to be helpful in future studies of the interactions of cellulose with any agents of chemical transformation acting on a cellulosic substance. The ability to distinguish between secondary and tertiary structures and to characterize them separately opens up the possibility of more precise interpretation of the results of observations. For example, mechanistic analyses of effects that are related to steric and conformational differences may be attributable to variability in secondary structures, while those that arise from differences in accessibility may he more directly related to tertiary structure. In the following discussions of recent advances in our understanding of the structures of native celluloses, questions associated with the different levels of structure will be examined.

RECENT DEVELOPMENTS

Spectroscopic studies

The characterization of order in cellulose using spectroscopic methods has been advanced along two lines in recent decades. Raman spectral studies revealed that there is greater diversity in the secondary structures of celluloses than had previously been recognized on the basis of diffractometric investigations. They also suggested that adjacent anhydroglucose repeat units in the chain structure are not symmetrically equivalent and that the basic repeat unit of structure must be recognized as anhydrocellobiose rather than anhydrogluocose. But perhaps of broader significance from the biological perspective was the finding, on the basis of solid state ^{13}C NMR invetigations, that native celluloses are composites of two distinct forms of aggregated cellulose and that the organization of the composite structures is distinctive of the particular species or tissue within which the cellulose is produced.

It is beyond the scope of this report to review the Raman spectral findings in full; a brief overview, however, may be helpful. The first key finding arising from the Raman spectral studies was that the crystallographic models propounded in the 1970s for cellulose could not rationalize the differences between the spectra of native celluloses (form I) and those of the regenerated or the mercerized celluloses (form II). The effort to rationalize the spectral observations included investigations of the vibrational spectra, both infrared and Raman, of many model systems. When the spectra of the celluloses were evaluated in light of the studies of model compounds it became

clear that sttructures described as twofold helical conformations of the chains represented approximations of the true structures but they ignored some of the key differences detected from the spectral information. Although the departures from twofold helical organization within the ordered domains were small, they were found to be at the heart of the distinctive features of the structures of many celluloses.

In summary, the conclusions were that the adjacent anhydroglucose units in a cellulose chain are nonequivalent and that the nonequivalences take different forms in cellulose I and II. In cellulose II, they are centered at the glycosidic linkages, while in native celluloses the departures from equivalence are somewhat less at the glycosidic linkages but greater at the primary alcohol group at C6 (5).

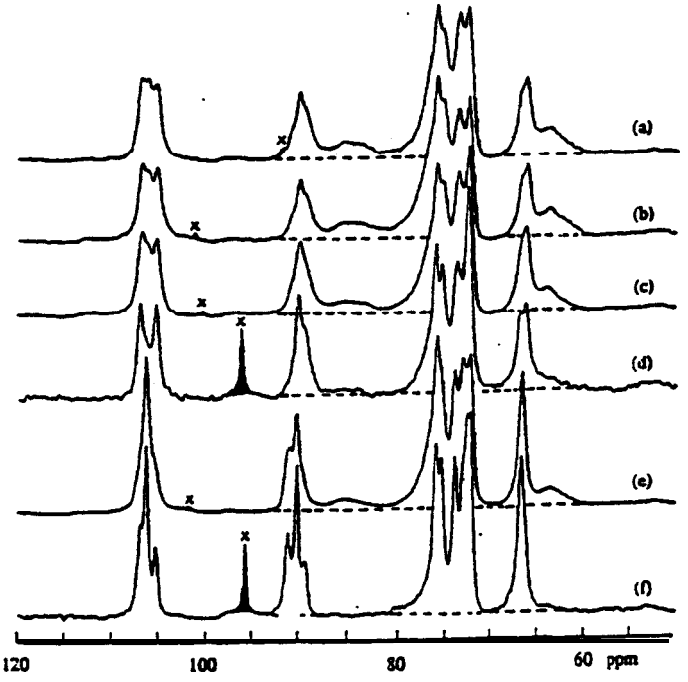

Figure 1. The solid state ^{13}C NMR spectra of a number of cellulose I samples: (a) ramie; (b) cotton; (c) hydrocellulose from cotton linters; (d) a low DP regenerated cellulose I; (e) *Acetobacter xylinum* cellulose; (f) *Valonia Ventricosa* cellulose. The (x) marks the first spinning side band of linear polyethylene, which was added as a chemical shift reference. Adapted from (7).

The inadequacy of the crystallographic models brought out by the Raman spectral studies were soon also encountered in the solid state ^{13}C NMR studies of the celluloses. The earliest of the NMR studies focused on the diverse forms of cellulose and attempted to resolve the question of nonequivalences among successive anhydroglucose units in the cellulose chain. It soon emerged,

however, that the solid state ^{13}C NMR spectra were revealing evidence of a structural diversity among the native celluloses that had only been hinted at by earlier results using other structure sensitive measurements (6, 7).

The diversity alluded to is well illustrated by the spectra shown in Figure 1 which includes the solid state ^{13}C NMR spectra of a variety of native celluloses, and one sample of cellulose I that had been regenerated from phosphoric acid solution at elevated temperatures (6, 7). The spectra clearly show a number of complex multiplet resonances associated with carbons that are chemically equivalent. The assignments of these resonances have been well established (8). Beginning at the downfield side, they are: Cl between 104 and 108 ppm; C4 with sharp resonances between 88 and 92 ppm, and broad components ranging down to about 83 ppm; a cluster assigned to C2, C3, and C5 in the range between 70 and 80 ppm; the resonances below 70 ppm are associated with C6.

After investigation of alternative interpretations of the diversity of the spectra of different native celluloses, the possibility of a composite structure was explored. It was found that the spectra in Figure 1 can in fact be resolved into linear combinations of spectra corresponding to two forms of cellulose I; these have been designated I_α and I_β to distinguish them from earlier efforts to categorize the diversity of native celluloses. The spectra of these two forms are shown in Figure 2, which also includes a spectrum of cellulose II in order to insure that the differences between the I_α and I_β forms cannot be confused with the clearly distinct cellulose II.

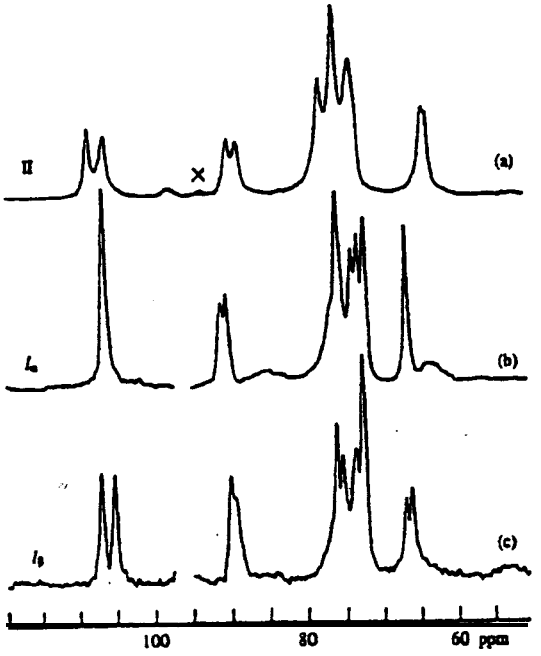

Figure 2. Solid state ^{13}C NMR spectra of: (a) low DP cellulose II; (b) the I_α form; (c) the I_β form. Spectra (b) and (c) were generated from linear combinations of spectra (d) and (e) in Figure 1. Discontinuities in spectra (b) and (c) occur where the spinning side bands of polyethylene would have occurred (7).

After investigation of a wide variety of native celluloses of the pure category, a number of patterns emerged. First, it became clear quite early in the investigations that all of the celluloses from plant sources are blends or composites of the two forms of cellulose and that the particular blend is specific to the species and tissue from which the cellulose is derived. The features distingtishing the spectra of a particular cellulose include the relative proportions of the I_α and I_β forms and the degree of resolution of the resonances associated with each of the carbon atoms. Next, it was noted that among the pure celluloses from higher plants the I_β form always appears to be dominant. In contrast, the bacterial celluloses as well as all the algal celluloses of high crystallinity are predominantly of the I_α form. Finally, it was obvious from examination of the spectra that those recorded from samples that had been shown to be highly ordered on the basis of diffractometry exhibit the sharpest resonances in the multiplets and have the smallest shoulders associated with resonances of C4 and C6. All of these results indicate that the solid state ^{13}C NMR spectra will be important complements to other methods directed at expanding our understanding of native celluloses.

In an effort to explore the differences between the I_α and I_β forms, two lines of investigation have been followed Electron diffractometric studies have indicated that the I_α form has a triclinic unit cell with one chain per unit cell and with nonequivalent anhydroglucose units. They have also Suggested that the I_β form has a monoclinic cell of space group $P2_1$ with two chains per unit cell and incorporating chains with twofold screw axis symmetry, requiting that adjacent anhydroglucose units be symmetrically equivalent (9). The other line of inquiry has been based on vibrational spectroscopy including both infrared (10) and Raman spectral measurements (11). Both sets of spectral measurements point to similar secondary structures in the two forms but with

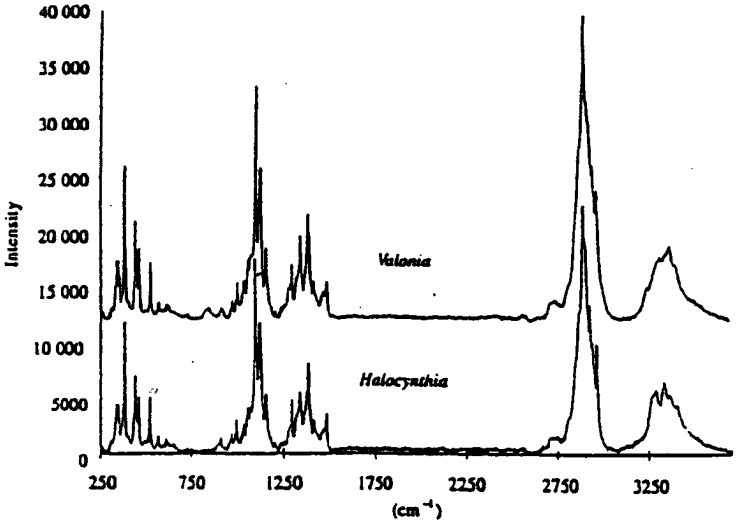

Figure 3. Comparison of the Raman spectra of I_α rich *Valonia* cellulose and I,, rich *Halocynthia* cellulose. Note the near identity of the spectra in the region below 1500 cm^{-1} and in the region between 2700 and 3000 cm^{-1} (C-H stretching). This is in contrast to the clear difference in the O-H stretching region above 3000 cm^{-1} (11).

distinctly different hydrogen bonding patterns. These findings are illustrated in Figure 3, which shows the Raman spectra of *Valonia,* an algal cellulose, which is predominantly of the I_α forms,

and *Halcynthia*, a tunicate cellulose, which is predominantly of the I_β form. Their comparison is particulary useful because the lateral dimensions of their fibrils are both of the order of 20 nm, and they, therefore, allow the generation of spectra of equal resolution. It is clear from the comparison that the spectra are essentially identical in the low frequency region, which is most sensitive to secondary structure. In the OH stretching region, in contrast, there are signifficant differences between the band structure for the two forms. The indication here is that the secondary structures of the tow forms are the same but the tertiary structures are different. This finding is in sharp contrast to the conclusions derived from the diffractometric data. Another important observation with respect to the Raman spectra is that there is no evidence of correlation field splitting in the spectra of either form; such splitting is expected in the spectra of polymeric systems that have more than one chain per unit cell. This observation is not consistent with the interpretation of the diffractometric data that ascribes a two chain unit cell to the I_β form. Thus the results of the inrfared and Raman spectral studies appear to be at variance with those of the diffractometric observations. It is clear that the nature of the differences between I_α and I_β forms is not fully understood at the present time.

Model System

A number of important advances with respect to understandmg native celluloses have emerged from investigations of the bacterium *Acetobacter xylinum* and the cellulose it can produce they are perhaps the best characterized among the native cellulose. Two aspects will be reviewed briefly. The first is the hierachic assembly of native celluloses. The second is the role of hemicelluloses in regulating the aggregation of cellulose. Both have implications with respect to the action of enzyme system on cellulosics substrates.

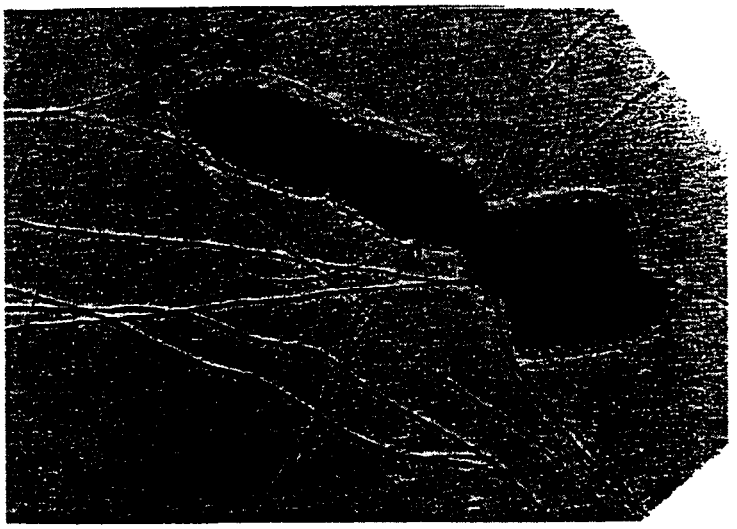

Figure 4. Several normal ribbons of cellulose synthesized by *Acetobacter* xylinum. Twists occur at several points, and in some regions smaller fibrillar subunits are visible. Adapted from (12) courtesy of Dr. C. Haigler.

It was noted earlier that native celluloses must be regarded as self assembling biological molecules that are organized hierarchically. This is most obvious from examination of the microfibrillar structure of bacterial celluloses have been produced under different conditions that can perturb the process of assembly at the different levels of hierarchic assembly. The most common form of the cellulose produced by *Acetobacter xylinum* is shown in Figure 4, which depicts the fibrils emerging from bacterial cells under conditions providing the least constraint on the assembly of the fibrils. The individual fibrils are of the order of 6 nm in width and they have the form of a ribbon with a regular twist to it (12). It has recently been demonstrated that the twist is right handed (13).

Figures 5 and 6 show ribbons that have experienced two different levels of perturbation. In Figure 5, the cellulose produced by *Acetobacter xylinum* was cultured in a medium containing carboxymethylcellulose (CMC). It clearly has resulted in some reduction of the coherence of order within the ribbons. In fact the micrograph reveals evidence of substructures that appear to be fibrillar and that are braided together as they might be in a rope. This points to the capacity of some molecules that are similar to cellulose to associate with it in its nascent state in a manner that results in limiting the degree of self assembly.

Figure 5. *Acetobacter xylinum* cellulose: substructures of the normal ribbon remain separate when synthesized in the presence of corboxymethyl cellulose. They may lie closer together, thereby resembling normal ribbons, but close inspection shows separated or sometimes highly splayed subunits. Adapted from (12) courtesy of Dr. C. Haigler.

A greater degree of limitation on self assembly is revealed in Figure 6, which shows the effects that can arise from the presence of an agent that can associate with the cellulose even more strongly than the CMC. The preparation of the sample shown in Figure 6 involved culturing the *Acetobacter xylinum* in a medium containing the fluorescent brightening agent (FBA) calcofluor,

followed by dilute acid washing to remove the FBA. The presence of the FBA usually results in the formation of a broad non-crystalline band of cellulose that is perpendicular to the cell axis. The micrograph shows that the most elementary of substructures can aggregate into individual fibrils after the acid wash, but they cannot self-assemble further to form a ribbon structure similar to those shown in figure 4. The fibrils are of the order of 1.5 nm in lateral dimension and appear to corerspond to the assemblies of cellulose molecules that emerge from individual assembly complexes as visualized on the plasma membranes of the cells.

If it is assumed that the pattern of fibrillar aggregation displayed by the ribbons of cellulose formed by *Acetobacter xylinum* reflect inherent self assembly characteristics of the cellulose molecule, it would follow that the most basic building blocks are the 1.5 nm fibrils emerging from a single assembly complex These then would assemble with similar fibrils emerging from adjacent complexes. In the absence of agents capable of associating with the nascent cellulose this would result in the normal ribbons shown in Figure 4. If the pattern of self assembiy is an inherent characteristic of the cellulose molecule in its nascent state it can be anticipated that the same influences would prevail during the formation of cellulose in higher plants, and that the basic building blocks would have similar characteristics. This is in fact what has been observed in much of the electron microscopic examination of celluloses from higher plants.

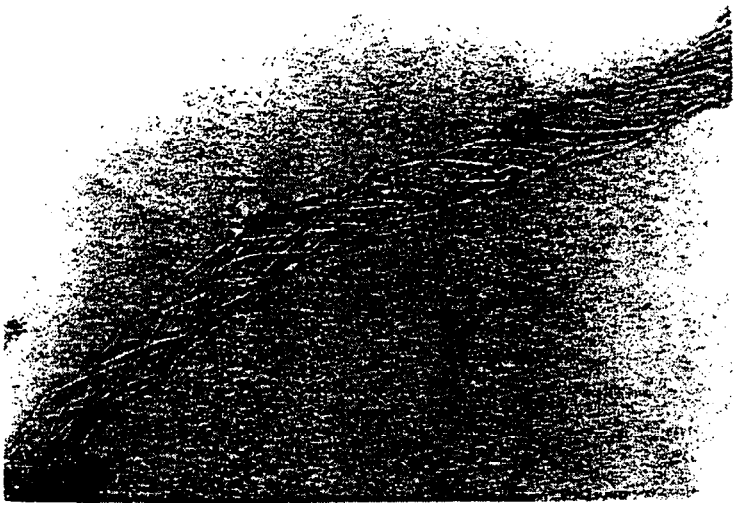

Figure 6. *Acetobacter xylinum* cellulose: a helix of microfibrils formed by gentle Washing of the FBA dye from extended sheets formed in its presence. The microfibrils contain cellulose I crystallites with smaller dimension than controls. All of these microfibrils would have been part of one normal ribbon. Adapted from (12) courtesy of Dr. C. Haigler.

In studies of the *Acetobacter xylimun* model system cited above, the modifying agents were added to the culture medium. In higher plants, in contrast, it is expected that during the formation of the cell wall matrix many agents will be present that can interact with cellulose to modify its assembly. The possible effects of matrix components have been investigated in

studies of the *Acteobacter xylinum* system wherein different hemicelluloses were added to the cultures at relatively low levels (14, 15, 16). It was observed that the hemicelluloses are indeed incorporated into the structures of the celluloses and that each of the hemicelluloses elicited a different pattern of modification of the cellulose produced in its presence. Furthermore, the changes induced by the hemicelluloses resulted in aggregation of the cellulose in a manner similar to that of higher plants. In particular, the solid state ^{13}C NMR spectra revealed a predominance of the I_β form as a result of the influence of the hemicelluloses. It can be anticipated on this basis that the patterns of self-assembly and the final tertiary structures of the native celluloses of higher plants are likely to be as divers as the plants within which they are formed.

on the basis of the observations of the model system it may be concluded that the celluloses in the category defined as pure the category defined as pure celluloses are formed in environments where in the other cell wall components cannot associate with the nascent cellulose sufficiently to modify its tertiary structures, while the complex celluloses are formed in cell wall matrices that include constituents that can modify the tertiary structure. A clearer definition of the diversity of the tertiary structures is essential to advancing our understanding of the interactions of enzyme systems with cellulosic substrates. Further elaboration of the diversity of native celluloses is beyond the scope of this report; the author has addressed many aspects in greater detail elsewhere (4).

FUTURE DIRECTIONS

One of the most significant conclusions that can be derived from the spectral studies is that the secondary and tertiary structures of native celluloses are, at a species and tissue specific. This requires that discussions of the entry of cellulose into biological processes begin to factor this reality into the analyses of data related to processes that can be influenced by the states of aggregation of the cellulose. The effort to characterize the species specific aspects of the variability of the structures of native celluloses is in its early infancy. As noted above, until recently it had seemed adequate to pursue the details of an ideal lattice structure. With the development of spectroscopic methods to complement the diffractometric methods there is now available a broader foundation upon which to base explorations of the species and tissue specificity of cellulosic structures.

A better characterization of the basis for species specificity is important for advancing understanding both with respect to the processes of biogenesis of cellulose as well as with respect to the processes at the heart of its disassembly by cellulases and cellulosomes. The relevance in regard to the latter field of study is highlighted by recent observations that certain micro-organisms capable of cellulolytic action release different proportions of the isozymes of a particular cellulase when cultured on different cellulosic substrates (17, 18). Clearly the action of the micro-organisms must involve an induction process that includes a yet unknown mechanism where by the nature of the secondary and tertiary structures of a particular cellulosic substrate is communicated to the site for the regulation of the expression of the cellulases. Explorations of the nature of this feedback mechanism will facilitate advancing a better understanding of the cellulolytic action of micro-organisms at the same time as it adds insight into the basis of species specificity of secondary and tertiary structure in celluloses.

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