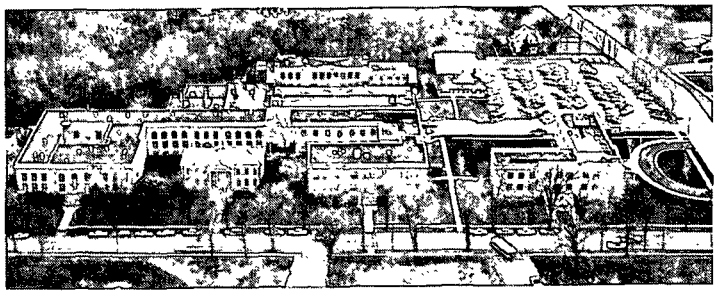

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PATTERNS OF AGGREGATION IN NATIVE CELLULOSES: IMPLICATIONS OF RECENT SPECTROSCOPIC STUDIES

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PATTERNS OF AGGREGATION IN NATIVE CELLULOSES: IMPLICATIONS OF RECENT SPECTROSCOPIC STUDIES

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ABSTRACT

The patterns of aggregation of native celluloses are considered in terms of secondary and tertiary structures. The spectra of native celluloses are taken to indicate that they all possess the same secondary structure, with a basic repeat unit consisting of the dimeric anhydrocellobiose unit, with nonequivalent anhydroglucose units. The different crystalline forms, I_α and I_β, are different tertiary structures of aggregates of molecules possessing the same secondary structure. Representative Raman spectra are presented, which support the view that the primary differences between the tertiary structures corresponding to the I_α and I_β forms arise from different patterns of intermolecular hydrogen bonding. Furthermore, they indicate that the I_α form can be transformed into the I_β form by processes that involve intermediate states with different secondary structures. The observations of structural transformations invite the speculation that the tertiary structures of celluloses isolated from higher plants may be determined as much by the processes of isolation as by the native structure; preliminary results suggest that the tertiary structures are indeed influenced by history as well as native order.

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INTRODUCTION

In previous reports we have proposed models of the structures of different celluloses which were developed in order to reconcile spectroscopic observations with diffractometric data and conformational energy computations [1-4]. We have also discussed in detail both the complexities and the uncertainties which are inherent in the pursuit of structural models [5]. In this report we will focus on native celluloses, with particular attention to the highly ordered aggregates of molecular chains commonly regarded as crystalline. Our purpose, in part, is to address the relationships between secondary and tertiary structures which occur in the native celluloses, as these relationships are reflected in spectroscopic observations.

In the following we begin by discussing the levels of structure at which questions can arise, with particular attention to secondary and tertiary structures. We then review briefly the primary conclusions derived from Raman spectroscopy and solid state ^{13}C NMR measurements about each of these levels of organization. Next we examine some spectra representative of recent results and their implications concerning structure at both secondary and tertiary levels. Finally, we consider some transformations of structure, particularly at the tertiary level, and the issues they raise about the structures of celluloses isolated from higher plants, and the degree to which they reflect the native state.

LEVELS OF STRUCTURE

It is useful to begin by examining the three levels of organization that must be considered for a complete definition of the structure of a crystalline aggregate of cellulose. The first level is that of the chemical structure reflected in the pattern of covalent bonds. Though some questions have again been raised recently with respect to the possibility of a very limited degree of covalent linkages to other cell wall polysaccharides [6], the dominance of the β-1,4 linked anhydroglucose structure is no longer in question, and is here taken for granted.

The next level of organization is that of the secondary structure or the conformations of individual chains, and involves the definition of the location in space of the individual repeat units relative to each other. It also includes the internal organization of individual repeat units where the covalent structure allows variation. In the case of cellulose, this would comprehend variation of the dihedral angles defining the position of the primary alcohol group at C6, the dihedral angles defining the glycosidic linkages, and any internal variations of the dihedral angles of the pyranose rings.

The conformational level of structure is the one most readily probed by spectroscopic measurements. The energy levels between which transitions are observed in the Raman spectra are quite sensitive to the internal coordinates which define molecular conformations. The ^{13}C NMR measurements are also sensitive to the internal coordinates which define molecular conformations, but in addition they are sensitive to site equivalences within the repeating structures of the molecular chains.

The third level of organization is the tertiary structure which reflects the arrangement of the molecular chains relative to each other within the ordered three-dimensional domains. This is the level of structure probed by diffractometric measurements, which are inherently most sensitive to regular three-dimensional arrays or lattice structures.

The secondary and tertiary levels of structure are clearly not independent of each other, for the shapes of the individual chains are primary determinants of the manner of their packing, and the forces involved in intermolecular interactions are among the factors which determine the equilibrium conformations of the individual chains. Because of this interdependence, the effects of three dimensional organization are also manifested in spectral measurements, both in observations reflecting variations in the internal coordinates and in measurements sensitive to intermolecular hydrogen bonding patterns.

Consideration of the symmetries associated with different levels of structure is helpful in clarifying their relationships to each other. The symmetries associated with molecular conformations are the axial symmetries allowable with translation in one dimension, parallel to, and coincident with, the chain axis. The symmetries associated with the three dimensional structure are those of a space lattice. The relationship between the symmetries at the two levels of structure requires that the aggregation of the chains in a crystalline domain must be such that the symmetry elements of the individual chain are a subset of the symmetry elements of the lattice.

In crystallographic studies of cellulose a twofold screw axis has generally been assumed to be coincident with the molecular chain axis. The spectroscopic measurements have indicated departures from this symmetry sufficient to result in nonequivalence of adjacent anhydroglucose rings and a correlated nonequivalence of successive glycosidic linkages in each molecular chain. These departures, however, appear to be small enough so that the diffractometric reflections associated with them are quite weak, sufficiently weak, in fact, that they have been neglected in crystallographic analyses. But while the nonequivalences have been regarded as negligible in crystal-

lographic studies, neither the Raman spectra nor the solid state ^{13}C NMR spectra can be rationalized without taking the nonequivalences into consideration. Therein lie the grounds of the apparent conflicts between the results of diffractometric and spectroscopic studies.

SPECTROSCOPIC STUDIES OF STRUCTURE

Raman spectroscopic investigations undertaken over the last decade initially focused attention on questions of secondary structure and were in part directed at interpretation of the Raman spectra of a wide range of celluloses. Later, complementary studies of solid state ^{13}C NMR spectra were added, and questions of both secondary and tertiary structures were addressed. In the earlier studies, presented here in brief overview, issues of structure were not posed explicitly in terms of secondary and tertiary structures as in the present report; we believe the present approach adds clarity to the discussion.

I. Molecular Conformations

In the earliest Raman spectroscopic studies it was found that the spectra of different allomorphs of cellulose could not be accounted for on the basis of the then available structures derived from diffractometry [1]. Particularly in the conformation sensitive low frequency region, the variations in the spectra pointed to a structural model in which the chain conformations depart from the twofold helical symmetry generally assumed to prevail in all the structures accepted at that time. Further consideration of packing in the unit cells, of the spectra and structures of the two model disaccharides, cellobiose [7] and beta-methyl cellobioside [8], and of some conformational energy calculations, led to the proposal that the glycosidic linkages in a chain of cellulose alternate in conformation between small left- and right-handed departures from the conformation that would define a twofold helix [2-5].

In studies of the solid state (CP/MAS) spectra of cellulose, the resonances for C1 and C4 were clearly shown to be split into multiplets [9,10]. 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 [9].

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, at values of the dihedral angles representing

small lefthanded and right-handed departures from the twofold helical structure, was confirmed in the study by Perez et al. [11]. The plausibility of alternating glycosidic linkages has also been supported by the investigations of French [12] and of Sakthivel et al. [13].

II. Crystallinity

In a collaboration with VanderHart the origins of the multiplicities in the solid state (CP/MAS) ^{13}C NMR spectra of native celluloses were investigated [14,15]. The multiplicities which arise from physically nonequivalent environments for chemically equivalent carbons could not be accounted for in terms of different sites in a unique unit cell. A comprehensive analysis of the spectra led to the proposal that native celluloses are composites of two distinct crystalline forms, identified as I_α and I_β. A decomposition of the spectra based on such a model has been described in the earlier reports [14, 15]. Further comprehensive investigations based on analysis of relaxation processes provided additional support for this model [16]. Continuing surveys of a wide variety of native celluloses have shown them to possess varying proportions of the two forms, with the I_α form dominant in celluloses from primitive organisms while the I_β form is dominant in celluloses from higher plants. The most recent studies have suggested that the I_α and I_β forms possess cellulose molecules with the same conformations of the heavy atom skeleton, but with different hydrogen bonding patterns. In terms of the present discussion, this implies the same secondary structure with different tertiary structures.

In summary then, the spectral studies have shown that the basic repeat unit of secondary structure is the dimeric anhydrocellobiose unit, and that the tertiary structures reflect different patterns of aggregation of molecular chains possessing the same secondary structure. In the following section we present two representative sets of spectra which reflect some of the variability in patterns of aggregation.

REPRESENTATIVE SPECTRA

The spectra presented here were selected because they possess features which are sensitive to tertiary structure. The first set are taken from a study of the Raman spectra of fibrillar celluloses in which comparisons were made between spectra of fibrils of ramie and Valonia celluloses taken as representative of the higher plant and primitive celluloses respectively. The second set are from a study of an algal cellulose that was treated with liquid ammonia to convert it to cellulose III, followed by treatment with boiling water to recover the cellulose I form. It is of particular interest here because both Raman and solid state ^{13}C NMR spectra show clear evidence of

changes in tertiary structure.

The spectra in Figure 1 are taken from the work of Wiley and Atalla [17]; they show Raman microprobe spectra of fibrils of ramie and Valonia ventricosa fibrils. The microprobe incorporates a microscope which has been modified to permit focusing of the exciting laser, through the objective, onto the sample; the manner is not unlike that for illumination in a reflecting metallographic microscope. The light scattered from the illuminated point on the sample is gathered also by the objective and imaged on the entrance slit of the spectrometer. Use of the microprobe allows spectroscopic examination of domains as small as 1 μm from individual fibrils; it also allows precise control of the orientation of the electric vector of the exciting laser beam relative to the axis of the fibril. The spectra in Figure 1 were acquired with the electric vector oriented at 0 and 90° to the fibril axes. Because both celluloses used are known to have the molecular chains parallel to fibril axes, the spectra can be characterized as having the orientation of the electric vector as parallel and perpendicular to the molecular chain axes, respectively.

Two comparisons of spectral features are relevant to the theme of this report, one relating to secondary structure the other to tertiary structure. In the region between 250 and 1500 cm^{-1} the bands, particularly those in the lower range, are associated with deformations of the internal coordinates which define the secondary structure; significant changes in the equilibrium values of these coordinates result in changes in the spectra. In Figure 1 it is seen that the spectra of ramie and Valonia celluloses are very similar in this region, the only noticeable differences being slightly lower resolution and a correlated increase in linewidth of bands in the spectrum of ramie; the differences reflect the smaller lateral dimension of crystalline domains in ramie. These features in the spectra point to very similar if not identical secondary structures in the two celluloses.

In contrast with the lower frequency region, a comparison of the spectra in the OH stretching region reveals significant differences between the spectra of Valonia and those of ramie. Indeed it appears that there are few bands in common, and this is true of both parallel and perpendicular spectra. In assessing the differences it must be kept in mind that the bands associated with the intramolecular hydrogen bond systems are likely to be very similar because of the similarity in secondary structure. It would follow, therefore, that the considerable difference in the observed OH stretching bands reflects a very dissimilar pattern of intermolecular hydrogen bonding, and hence different tertiary structures for the two celluloses.

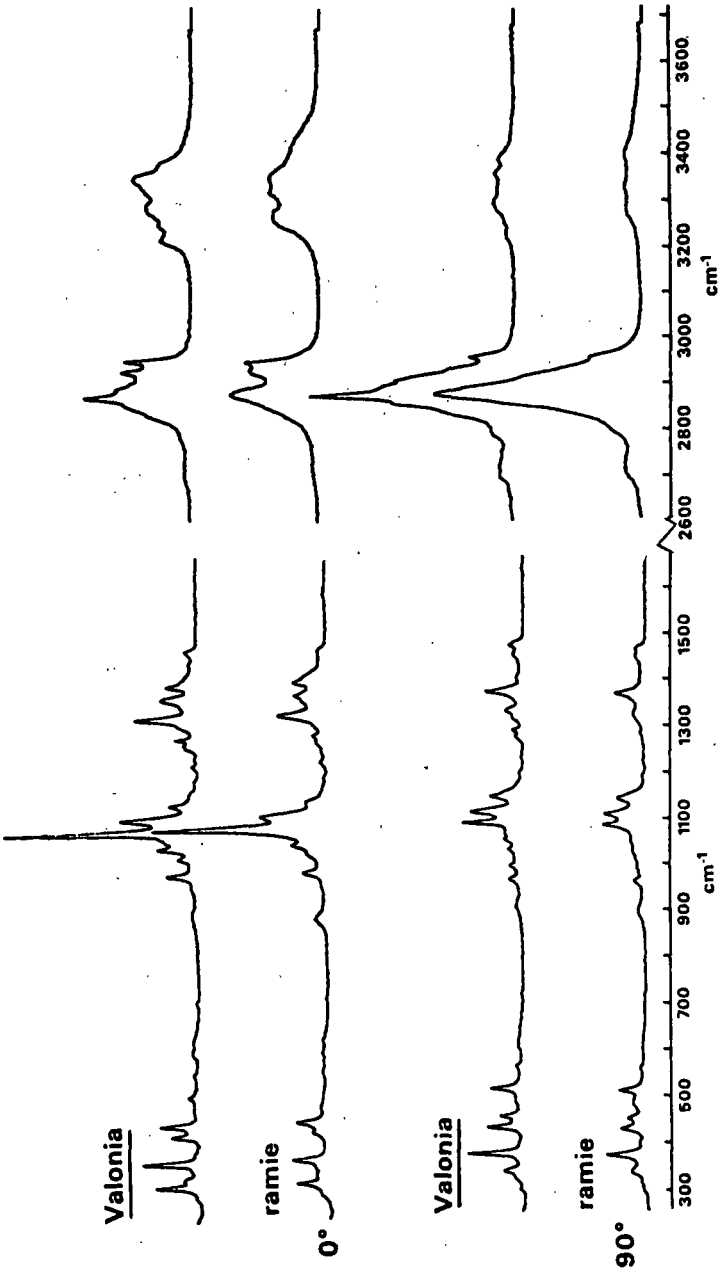

FIG. 1. Raman microprobe spectra of Valonia and ramie fibrils recorded with the electric vector of exciting radiation at 0 and 90 degrees to the fibril axes.

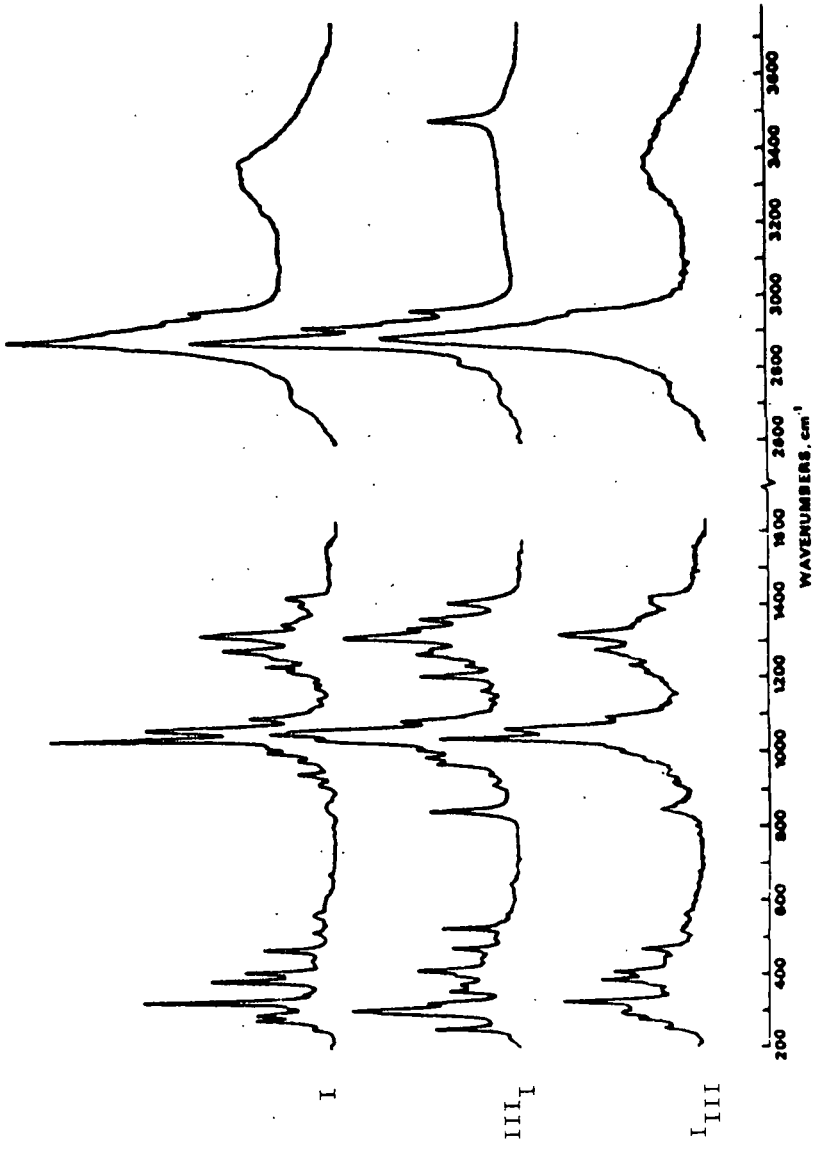

FIG. 2. Raman spectra of Cladophera cellulose, in the native form, after conversion to cellulose III, and after reversion to the cellulose I lattice upon boiling in water.

The spectra in Figure 2 are part of an investigation of cellulose from a fresh water alga, Cladophera glomerata, which generates celluloses very similar to those from Valonia ventricosa. The three spectra shown in Figure 2 are from the cellulose purified in its native form, from the cellulose after conversion to cellulose III by treatment with liquid ammonia, and from the cellulose I recovered from the cellulose III by boiling, this last sample designated as I_{III}. These spectra were acquired from macroscopic samples of the celluloses pressed into pellets, and thus are not sensitive to orientation. The spectra are of particular interest because they suggest that the transformation to cellulose III results in alteration of secondary and tertiary structure, while the subsequent recovery of cellulose I results in recovery of the same secondary structure as in the starting material, but this time with a different tertiary structure.

In assessing the spectra in Figure 2 it is in order to examine the same two spectral regions as in the spectra of ramie and Valonia. In the low frequency region we find that the spectra for samples I and I_{III} have the same relationship to each other as those in the same region of Valonia and ramie, that is, the same bands are observed, with approximately similar intensity patterns, but with the bands in the spectrum of I_{III} somewhat less clearly resolved and slightly broadened. In contrast, the spectrum of cellulose III indicates a significantly different secondary structure, because of the many new bands and the variations in the intensity patterns.

In the OH region the bands are not as well resolved as in the oriented samples of ramie and Valonia, but it is nevertheless clear that the bands in the spectrum of the I sample are different from those in the spectrum of the I_{III} sample. Furthermore, it is not at all unlikely that summation of the bands in the parallel and perpendicular spectra, respectively, of Valonia and ramie would produce summation spectra similar to those of celluloses I and I_{III}. These observations suggest that the tertiary structure of I_{III} is similar to that of ramie, while that of the original I is similar to that of Valonia. Such relationships are indeed suggested by the solid state ^{13}C NMR spectra which have been reported earlier [18].

While the spectra of celluloses I and I_{III} in Figure 2 at first seem to duplicate, in a composite form, the same information as that in the spectra of Figure 1, the spectrum of cellulose III, as that of an intermediate state, alters their significance. It clearly indicates a major reorganization of both secondary and tertiary structure, which is partially reversed, at the secondary level in the recovery of cellulose I_{III}. The key observation, in relation to the theme of the present report, is that the pattern of aggregation within the tertiary structure can be, and has been transformed.

It is to be noted that the results of the solid state ^{13}C NMR studies of the celluloses discussed above are consistent with the interpretation presented here. The distribution of the resonances of chemically equivalent carbons can be taken as reflecting the secondary structure, while the multiplicities within these resonances reflect the tertiary structure. The spectra of ramie and Valonia ventricosa are consistent with two distinct tertiary structures corresponding with the same secondary structure [15]. For the celluloses represented by the Raman spectra in Figure 2, their ^{13}C NMR spectra also are consistent with alteration of both secondary and tertiary structures in the transition to cellulose III, and recovery of the secondary structure of I, but with a different tertiary structure in I_{III}. Furthermore, the tertiary structure of I_{III} is very similar to the tertiary structure of the celluloses from the higher plants as represented by ramie or cotton linters celluloses.

STRUCTURE FORMATION, TRANSFORMATION, AND RE-FORMATION

In an earlier report it was noted that a correlation appears to exist between the crystalline form of the native cellulose and the organization of the synthesizing complexes in the organism producing it [18]. Organisms which have the complexes organized in rosettes or arrays of rosettes produce native cellulose in which the I_β form is dominant, while the more primitive organisms which have the complexes organized in linear or less ordered patterns produce cellulose in which the I_α form is dominant. In terms of the levels of structure set forth above, the secondary structure appears to be common to all native forms, while the tertiary structure appears to be sensitive to some cooperative characteristic of the the arrays of complexes. This observation does suggest that the secondary structure associated with native celluloses is more stable than generally assumed.

The secondary structure of native celluloses is clearly more stable than that of cellulose III. Since both the Raman spectra and the ^{13}C NMR spectra indicate a change in the secondary structure in the transition to III, the reappearance of the cellulose I upon boiling is a clear instance of the re-formation of this structure from another structural state. This interpretation is supported by the results of a study of heterogenous acetylation followed by heterogeneous deacetylation carried out by Horii and coworkers [19]. When the procedure was applied to Valonia ventricosa cellulose in which the I_α tertiary structure is dominant, the cellulose recovered was found to have the I_β tertiary structure dominant. When the same process was applied to cotton cellulose, no change in the tertiary structure was observed. It seems very likely that the acetylation disrupts or at least significantly perturbs the secondary structure, yet here again the secondary structure of

cellulose I was re-formed.

A more comprehensive discussion of the implications of our observations must remain for future reports, yet it is necessary to raise some question concerning assumptions frequently implicit in discussions of the structures of celluloses. The studies of structure which we have reviewed elsewhere, and to which we alluded earlier in this report are based primarily on observations of celluloses which occur in relatively pure form and possess simple morphologies in their native states. In relating the results of such observations to studies of celluloses in other plants, the assumption has always been implicit that, no matter what else occurs in the cell walls with the cellulose, the cellulose itself is present in the form of elementary fibrils that possess the same pattern of aggregation, and, hence, the same tertiary structure as the celluloses which occur in pure form. This assumption must now be questioned, and indeed such questions have become central to our investigations, because the tertiary structures which they address are important in determining many of the properties of the cellulosic materials which are of practical importance.

Preliminary results of solid state ^{13}C NMR relaxation studies suggest that the tertiary structures of celluloses in wood pulp are significantly influenced by the specific procedures used to separate the cellulose from the other cell wall constituents. Thus, celluloses in wood pulps can be regarded as possessing the native structure of cellulose at the secondary level only, while the tertiary structure appears to be as much a function of the procedure of isolation as of the native molecular architecture. This fundamental point may provide the most plausible rationale yet for the differences between the properties of pulps prepared by the different commercial processes.

CONCLUDING SUMMARY

In summary, we have found it helpful to consider the patterns of aggregation of celluloses in terms of secondary and tertiary structures. The secondary structures are those which are characterized by the internal coordinates which define molecular conformation, while the tertiary structures specify the spatial relationships between molecular chains within an ordered aggregate. In these terms our spectral observations of native celluloses are taken to indicate that all of the native celluloses possess the same secondary structure, with a basic repeat unit consisting of the dimeric anhydrocellobiose unit, with nonequivalent anhydroglucose units. The different crystalline forms, I_α and I_β, are viewed as representing different tertiary structures of aggregates of molecules possessing the same secondary structure.

Representative Raman spectra have been presented, the patterns

of which are most simply understood in terms of the secondary and tertiary structures they represent. They support the view that the primary differences between the tertiary structures corresponding to the I_α and I_β forms of native cellulose arise from different patterns of intermolecular hydrogen bonding. Furthermore, they indicate that the I_α form can be transformed into the I_β form by processes that in some cases involve intermediate states with different secondary structures.

Analysis of the states of aggregation of cellulose in terms of secondary and tertiary structures, when taken together with the observations of structural transformations, invites the speculation that the tertiary structures of celluloses isolated from higher plants, wherein they occur together with hemicelluloses and lignins, may be determined as much by the processes of isolation as by the native structure. Preliminary results of studies which address this question suggest that the tertiary structures are indeed influenced by history as well as native order.

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