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SYNCHROTRON X-RAY AND NEUTRON FIBER DIFFRACTION STUDIES OF CELLULOSE POLYMORPHS

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ABSTRACT
We are determining the precise crystal structures and hydrogen-bonding systems of naturally occurring and processed cellulose polymorphs by fiber diffraction. X-rays are used to determine the positions of carbon and oxygen atoms. Neutrons, in combination with isotopic substitution of labile hydrogen atoms in the fibers by deuterium, are used to determine the positions of hydrogen atoms involved in hydrogen bonding. Methods have been developed for obtaining oriented polycrystalline fibers that diffract X-rays and neutrons to atomic resolution. For the first time, data have been collected from naturally occurring pure cellulose Iα isolated from *Glaucocystis* (a microalgae) and from pure cellulose Iβ, isolated from *Tunicate* (a sea creature). We have also collected data from the polymorphs resulting from cellulose processing; II (both mercerized and regenerated), III(I) and III(II).

INTRODUCTION
Although the crystalline nature of cellulose has been one of most studied structural problems in polymer science there remain many open questions. Cellulose is a polymer formed by (1-4)-linked β-D-glucosyl residues that are alternately rotated by 180^o along the polymer axis to form flat ribbon-like chains.[1] Each glucosyl unit bears three hydroxyl groups, one an hydroxymethyl group. It has been long recognized that these hydroxyl groups and their ability to bond via hydrogen bonding not only play a major role in directing how the crystal structure of cellulose forms but also in governing important physical properties of cellulose materials. The fact that cellulose exists as a crystalline aggregate of these chains has been long established by X-ray fiber diffraction.[2,7] X-ray fiber diffraction has become a standard tool for studying the crystalline nature of cellulose, allowing classification into a number of polymorphs; naturally occurring cellulose I, and processed celluloses II, III and IV.[3, 4] Despite advances made using this technique, the resulting crystal structures have not been definitive.[5-16] Indeed many aspects of these structures have been contradicted by results from other studies.

Recently, the applications of new techniques to this problem have resulted in new insights. High resolution ^{13}C solid-state NMR studies have shown that cellulose I is composed of species specific compositional ratios of two crystalline allomorphs, cellulose Iα and cellulose Iβ.[17, 18] Electron diffraction[19] has been used to determine the unit cells of cellulose Iα and Iβ, and a combination of electron microscopy and biochemical techniques has shown that the cellulose chains pack in a parallel-up arrangement.[20] Synchroton X-ray diffraction studies of microcrystals of cellulose oligomers [21-23] and Molecular Dynamics studies [24] have provided model structures of cellulose II that differ from those determined in earlier X-ray fiber diffraction studies and are in better agreement with the results from ^{13}C solid-state NMR studies. These results have clarified many ambiguities arising from X-ray fiber studies. However they have not provided any new precise crystal structures or direct information about hydrogen bonding for cellulose.

We are involved in a long-term study to determine the precise crystal structures of the various cellulose polymorphs. Through the development of new techniques we have been able to prepare fiber samples of cellulose with exceptionally high order. [25] The quality of these samples is allowing us to exploit the unique properties of synchrotron X-ray and neutron sources in order to collect diffraction data to near atomic resolution. Synchrotron X-rays are used to provide accurate crystallographic parameters for C and O atoms. However, because of the relatively weak scattering power of H atoms for X-rays, neutrons are used to determine H atom parameters. We have developed methods for replacing labile H atoms with D, without any loss in crystalline perfection. [26] Deuterated fibers can diffract neutrons with intensities that are substantially different from the intensities diffracted from hydrogenated fibers. These differences, along with the phases calculated from the C and O positions determined in our X-ray studies, are used to calculate Fourier difference syntheses in which density associated with labile hydrogen atoms is imaged. The unprecedented high resolution of these data is revealing new information on cellulose structure and hydrogen bonding.

EXPERIMENTAL METHODS

Table 1 lists the cellulose polymorphs that we have investigated so far. Neutron data were collected on diffractometer D19 at the Institut Laue Langevin, Grenoble. X-ray data were collected at beamlines ID2 and ID13 at the European Synchrotron Radiation Facility, Grenoble. We are currently involved in commissioning another neutron diffraction station at LANSCE, Los Alamos, which will allow these, and similar, fiber diffraction studies to be carried out in the USA.[27] Sample preparation methods varied depending on the source of cellulose, the polymorph studied, the deuteration state and whether the sample was intended for X-ray or neutron diffraction. However specific details are given in table 1 references.

Examples of the neutron data are shown in figure 1. These are the first fiber diffraction patterns from samples of pure cellulose Iα and cellulose Iβ to be reported. The patterns, which are typical, display substantial resolution: up to the 11^{th} layer line along the fiber direction, indicating a resolution of the order of 0.9A. Significant differences can be observed in the diffracted intensity from deuterated and hydrogenated samples. An

example of the X-ray data is shown in figure 2. The left-hand side of this figure corresponds to the actual data and the right-hand side to a 2D fit of the background and Bragg intensities using program LSQINT, part of the BBSRC funded CCP13 project. Although we have used CCP13 software extensively we have found limitations in its application to high-resolution data and to data from textured samples. We have written our own data analysis software to address these limitations.[32]

Table 1 Cellulose polymorphs that have been investigated using neutron and X-ray fiber diffraction in this project.

Polymorph	**Source**
Cellulose Iβ[28]	*Halocynthia roretzi*
Cellulose Iα	*Glaucocystis*
Cellulose Iα+Iβ[28]	*Cladophora*
Mercerized Cellulose II [28,29]	Flax
Mercerized Cellulose II [30]	Ramie
Regenerated Cellulose II	Fortisan
Cellulose III_I [31]	*Cladophora*
Cellulose III_{II}	Ramie

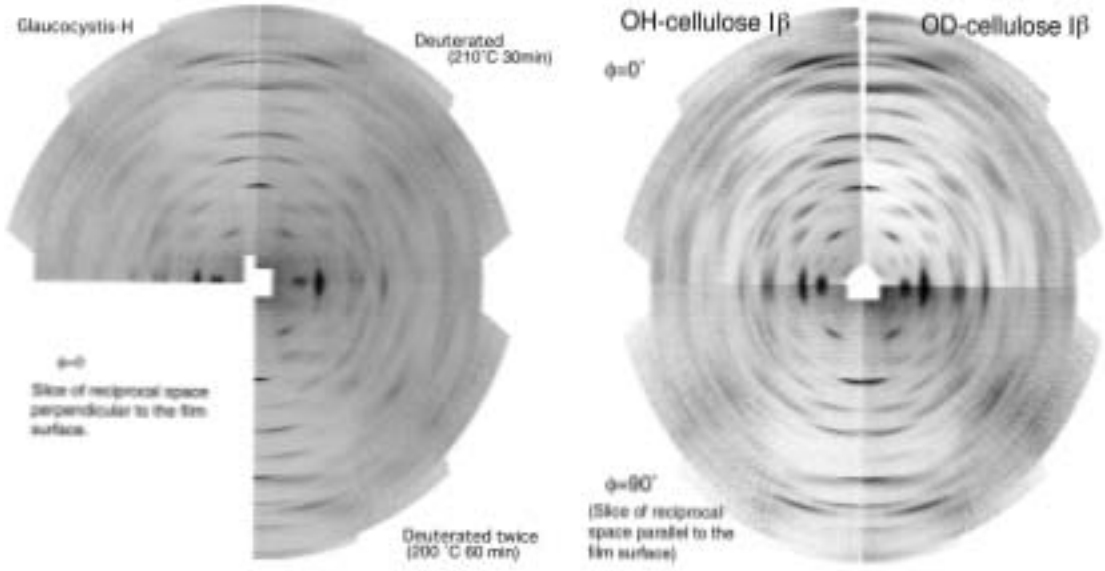

Figure 1. Neutron diffraction data collected on D19 at the ILL, Grenoble, France, from cellulose Iα (left-hand side) and cellulose Iβ (right-hand side). The images have been remapped into cylindrical reciprocal space with the fiber axis vertical. For both images the left-hand side corresponds to hydrogenated cellulose and the right-hand side to deuterated cellulose.

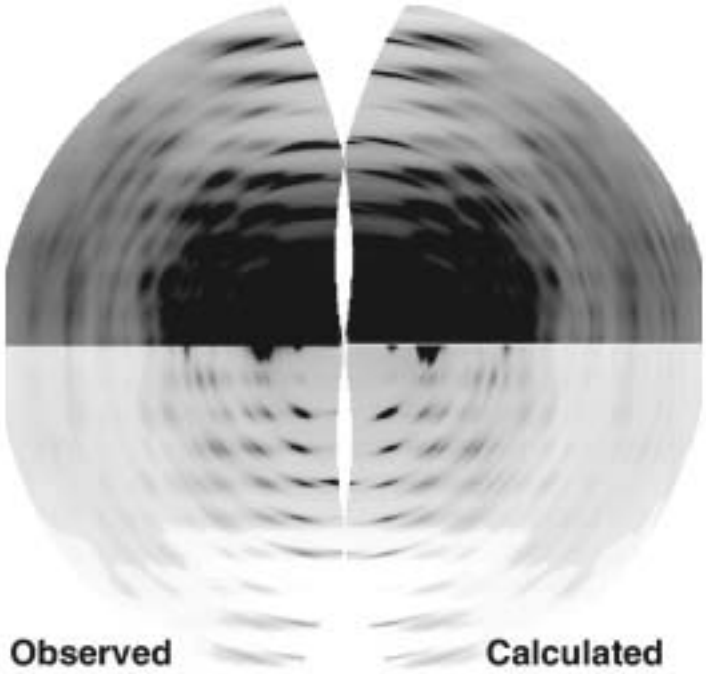

Figure 2. Left-hand side: Synchrotron X-ray diffraction data collected on an online MAR image plate at the ESRF, Grenoble. Right-hand side: A 2D fit of the background and Bragg intensities using program LSQINT, part of the BBSRC-funded CCP13 project, Darebury, UK. The images have been remapped into cylindrical reciprocal space with the fiber axis vertical and reproduced at low display thresholds (top) and high display thresholds (bottom) so that the full dynamic range of the reflections can be seen.

RESULTS AND DISCUSSION

Our most advanced analysis concerns a revised crystal structure and hydrogen bonding arrangement for cellulose II. Cellulose II can be prepared from cellulose I by two distinct processes, regeneration and mercerization. Previous X-ray fiber diffraction studies have shown that both preparations result in essentially the same structures.[11, 33] Two antiparallel chains with equivalent backbone and sugar conformation but different hydroxymethyl group conformations pack in a monoclinic unit cell; *gt* for the chain located at the cell origin and *tg* for the center chain. We have re-examined the structure of cellulose II using both the published X-ray data from regenerated Fortisan fibers [11] and also new synchrotron data collected from mercerized ramie fibers[30]. In both cases although the chains are antiparallel they have different backbone and sugar conformations and similar hydroxymethyl group conformations; *gt* for both chains.[34] A small observed difference between the mercerized and regenerated structures may be related to a large observed difference in the amount of hydroxymethyl group disorder on the central chain: ~30% for regenerated cellulose and ~10% for mercerized cellulose. The structure of mercerized cellulose II is shown in figure 3.[30] The exceptional resolution of the X-ray data allowed individual atomic thermal parameters to be refined using SHELX97, a program normally used in small molecule crystallography.[35]

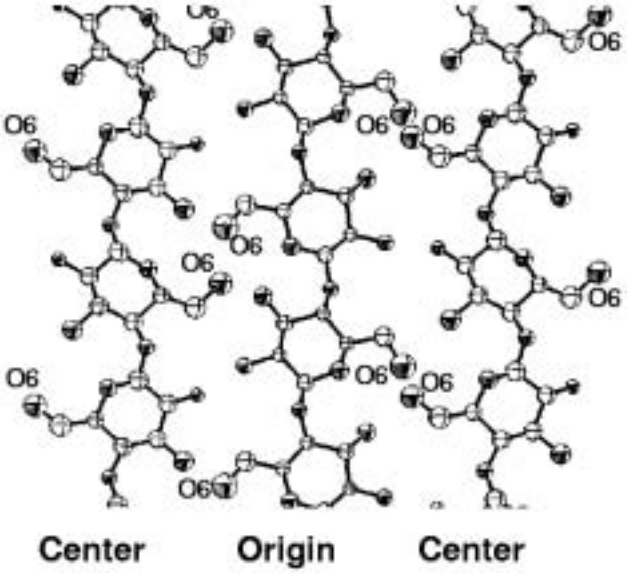

Figure 3. Structure of mercerized ramie cellulose II showing thermal ellipsoids with 50% probability surfaces enclosing the atom centers. Only the hydroxymethyl group oxygen atoms have been labeled for clarity.

Despite their quality, the X-ray data do not allow location of hydroxyl H atoms. Neutron data have been used to reveal the hydrogen bonding arrangement. In the neutron study fibers of mercerized flax were used. Figure 4 shows a schematic representation of the hydrogen bonds in cellulose II.[29] The putative intrachain hydrogen bonds are found to be bifurcated with a major component between O3 and O5 and a minor component between O3 and O6. The O6 atom of the corner chain and its bound hydrogen atom are in positions to donate to three possible acceptor atoms of the center chain (a major component O6…O6, and two minor components, O6…O5 and O6…O3). One explanation for this four-centered hydrogen bonding arrangement is related to hydroxymethyl group disorder. When O6 of the corner chain is in a *gt* conformation, it accepts from O6 of the center chain, but when it is in the *tg* position, O3 of the center chain accepts from O6 of the corner chain. A crystallographic averaged position of the hydrogen atom attached to O6 would correspond to the hydrogen atom's observed position. The four-centered arrangement is then a statistical effect rather than a true bonding arrangement. We are presently investigating whether the observed differences in hydroxymethyl group disorder result in differences between the hydrogen bonding arrangements adopted by mercerized and regenerated cellulose II.

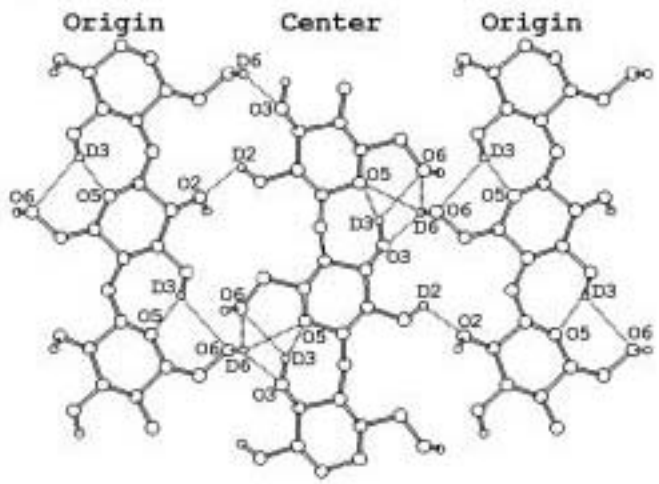

Figure 4. Hydrogen bonds in cellulose II as determined by neutron fiber diffraction.[29] Only atoms involved in hydrogen bonding are labeled.

Similar analyses for cellulose Iα, cellulose Iβ are underway and will provide revisions of currently accepted structures. These studies illustrate how advances in sample preparation methods in combination with complementary X-ray and neutron diffraction techniques are providing complete and precise crystallographic information. This information is crucial for understanding the structural basis for the physical, chemical and biological properties of cellulose.

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