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Author(s): Yoshiharu Nishiyama, Henri Chanzy and Paul Langanc

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

Yoshiharu Nishiyama,[a] Henri Chanzy,[b] and Paul Langan[c]

[a]Department of Biomaterials Science
Graduate School of Agriculture and Life Sciences
The University of Tokyo
Tokyo 113-8657, Japan
[b] Centre de Reserches sur les Macromol■cules V■g■tale
CNRS, BP 53, 38041 Grenoble, France.
[c]Bioscience Division,
Los Alamos National Laboratory
Los Alamos, NM 87545, USA

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° along the polymer axis to form flat ribbon-like chains. 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. Through the development of new techniques we have been able to prepare fiber samples of cellulose with exceptionally high order. 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. 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

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 and the new neutron Protein Crystallography Station at Los Alamos National Laboratory. X-ray data were collected at beamlines ID2 and ID13 at the European Synchrotron Radiation Facility, Grenoble. 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.

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

Polymorph	Source
Cellulose Iβ[1]	*Halocynthia roretzi*
Cellulose Iα	*Glaucocystis*
Cellulose Iα+Iβ[1]	*Cladophora*
Mercerized Cellulose II [1,2]	Flax
Mercerized Cellulose II [3]	Ramie
Regenerated Cellulose II	Fortisan
Cellulose III_I [4]	*Cladophor*
Cellulose III_{II}	Ramie

An Example of the neutron data is shown in **figure 1**. This is the first fiber diffraction pattern from a sample of pure cellulose Iβ to be reported. The patterns, which are typical, display substantial resolution: up to the 11th 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. 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.

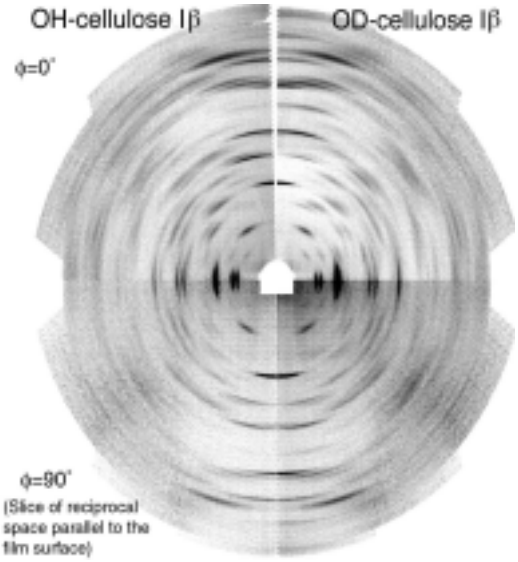

Figure 1, Neutron diffraction data collected on D19 at the ILL, Grenoble, France, from cellulose Iβ. The image has been remapped into cylindrical reciprocal space with the fiber axis vertical. The left-hand side corresponds to hydrogenated cellulose and the right-hand side to deuterated cellulose.

Results and Discussion

Our most advanced analyses concern revised crystal structures and hydrogen bonding arrangements for cellulose II and Iβ. 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. 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 and also new synchrotron data collected from mercerized ramie fibers. In both cases although the chains are antiparallel they have different backbone and sugar conformations and similar hydroxymethyl group conformations; *gt* for both chains. 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.

Previous X-ray fiber diffraction studies on cellulose I have not provided a definitive crystal structure. New data resulting from the development of ^{13}C CP/MAS solid sate NMR revealed unexpected details in highly crystalline cellulose I that could only be explained by a system consisting of two distinct crystal phases, designated cellulose Iα and Iβ. Our fiber diffraction studies on cellulose I are the first on isolated Iα and Iβ phases. A recent proposal for the structure of cellulose Iβ, based on data from mixed phase cellulose I has two parallel chains in a monoclinic unit cell, with the conformation of each chain essentially the same and each hydroxymethyl group in the *tg* conformation.[5] Our synchrotron X-ray studies differ in detail with the two chains having small differences in the conformations of the hydroxymethyl groups and glycosidic links that are associated with differences in hydrogen bonding geomtetry.

Despite their quality, the X-ray data do not allow location of hydroxyl H atoms for either cellulose II or Iβ. Neutron data have been used to reveal the hydrogen bonding arrangements. Figure 3 shows a schematic representation of the hydrogen bonds in cellulose II. 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.

Each figure should have a number and caption, formatted as follows below the figure:

Figure 1. The figure number should be boldface, but the figure caption should be regular typeface, below the figure, with full justification. (8 pt)

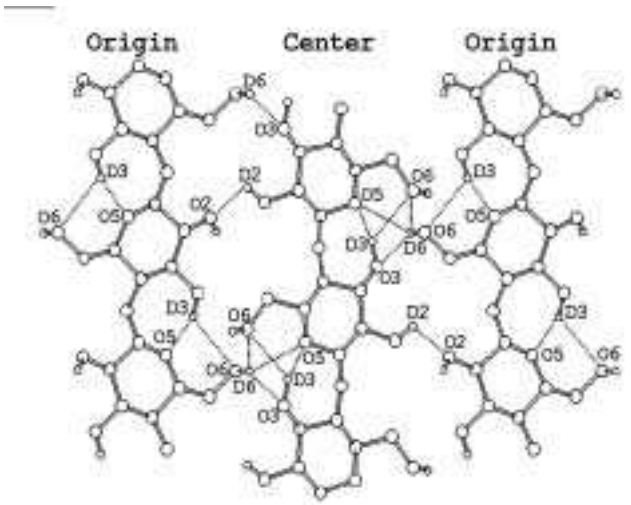

Figure 3. Hydrogen bonds in cellulose II as determined by neutron fiber diffraction. Only atoms involved in hydrogen bonding are labeled.

Conclusions

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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