Experimental report

17/12/2015

Proposal: 8-01-454 **Council:** 4/2015

Title: Reinvestigation of hydrogen bonds using perdeuterated cellulose Iβ

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: Yoshiharu NISHIYAMA

Experimental team: Daisuke SAWADA
Trevor FORSYTH
Yoshiharu NISHIYAMA
Yu OGAWA

Local contacts: Estelle MOSSOU
Trevor FORSYTH

Samples: perdeuterated cellulose

Instrument	Requested days	Allocated days	From	To
D19	3	3	08/12/2015	12/12/2015

Abstract:

The hydrogen bonding of cellulose I structure will be revisited using perdeuterated samples. Previously we proposed a hydrogen bond disorder in native cellulose based on neutron diffraction on samples selectively deuterated on the hydroxyl groups. However, recent calculatoions based on density functional theories predicts the minor hydorgen bonding pattern to have quite high energy. The hydrogen bond pattern should thus be revisited using a better dataset. In our previous study, the sample still contained a large amount of hydrogen (7 out of 10) resulting in an incoherent scattering cross-section 24 times higher than coherent one. We prepared a perdeuterated cellulose sample with similar crystallinity and alignment and expect to improve the data quality by now getting a signal dominated by coherent scattering.

Reinvestigation of hydrogen bond disorder using perdeuterated cellulose I_β

Yoshiharu Nishiyama[1], Daisuke Sawada[2], Estelle Mossou[3], V. Trevor Forsyth[3]
[1]Centre de Recherches sur les Macromolecules Vegetales, Centre National de la Recherche Scientifique; [2]Biology & Soft Matter Divisions, Oak Ridge National Laboratory, Oak Ridge TN 37831; [3]Institut Laue Langevin, Grenoble, Cedex 5, France.

Introduction

Cellulose, the most abundant organic renewable material on earth, has been studied intensely for over 160 years. This research has driven the development of many analytical methods broadly applied in polymer and carbohydrate chemistry. We now know with atomic precision that the naturally occurring cellulose I_α and cellulose I_β phases of cellulose, collectively referred to as cellulose I, are crystalline fibrous materials that consist of long parallel chains of beta-1,4 linked-D-glucose (1, 2). The structure of native cellulose is important since it is the structure found in biomass. All research efforts to convert biomass into useful materials or fuels are dealing with the native structure.

In 2002, we have refined the cellulose I_β structure using neutron diffraction data based on atomic resolution X-ray structure (1), but we could not fit the data with one hydrogen bonding pattern. We proposed a co-existing model of two hydrogen bonding patterns. Another neutron diffraction experiment under 15-Kelvin condition essentially gave the same results and thus this hydrogen bonds disorder was presumed to be static (3). However, theoretical calculations (3) based on our atomic coordinates indicated that one of the hydrogen bonding patterns was significantly more stable compared to the other one. On the other hand, the calculation also indicated the presence of many different hydrogen bond patterns, which was not considered in our first structure refinement.

A better neutron data set is necessary in order to investigate the potential hydrogen bonds disorder in cellulose I_β. The data quality in the previous works was essentially limited by the presence of large amount of hydrogen that gave high level of incoherent scattering. Recently we have grown acetobacter by feeding perdeuterated glycerol and expressed highly crystalline perdeuterated cellulose (4). In this experiment, we used the perdeuterated cellulose to collect neutron fiber diffraction data set.

Experimental summary

G*luconacetobacter xylinus* was grown in deuterated media to express deuterated cellulose. Hydrogen atoms attached to carbon was completely exchanged to deuterium atoms in the cellulose, but hydroxyl groups were partially remained as hydrogenated. In order to obtain perdeuterated cellulose, the cellulose samples were annealed 30 minutes in D_2O at 260 °C in pressure vessel. Partial cellulose I_α components in bacterial cellulose were also converted into cellulose I_β during the annealing process. The obtained suspension of perdeuterated cellulose I_β was mixed with fibrinogen solution in D_2O. Then, concentrated thrombin was added into the mixture to form a gel matrix. The gel was cut into specimens which had approximately 10 mm (W) x 20 mm (H) x 5 mm (D) dimensions. The gels were stretched to approximately 2.5 times as compared to the original lengths. Dried stretched fibers were immersed into 1N NaOD/D2O for a minute, and relaxed fibers were again stretched up to 1.5 times. The fibers were inserted into soda-glass thin-walled capillaries. The capillaries were bundled to form arrays of approximately 50 mm (W) x 10 mm (H) x1.5 mm (D). Neutron diffraction data were

collected using the four-circle D19 diffractometer at the Institut Laue-Langevin. The diffractometer is equipped with a large multiwire gas detector (30×120° angular aperture), a versatile monochromator and takeoff-angle assembly. A data set was collected at ambient conditions, using a neutron beam of wavelength 1.46 Å, using several different sample goniometer settings, and over a measuring time of about 18 hours. For each sample setting, the recorded data were corrected for spatial distortion using the D19 in-house program DCD19. The variation in response over the area of the detector was corrected for by dividing each data frame by a data frame collected from an isotropic incoherent scattering from vanadium rod. An affective absorption correction was determined by fitting a correction function that re-established a smooth incoherent scattering background.

Results

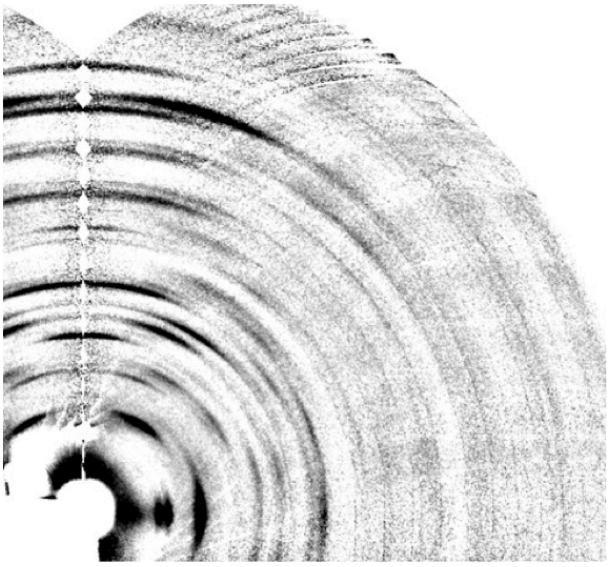

Figure 1. 2D Neutron fiber diffraction pattern of perdeuterated cellulose I_β.

The 2D neutron fiber diffraction pattern was shown in Figure 1. The diffraction peaks were observed up to approximately 1 Å resolution for meridional direction and 2 Å resolution for equatorial direction. The neutron intensity will be extracted using the method previously described (5). The neutron intensity will be used to refine the deuterium positions to determine the hydrogen bonds.

References

1. Nishiyama, Y.; Langan, P.; Chanzy, H. *J. Am. Chem. Soc.* **2002**, 124, 950–957.
2. Nishiyama, Y.; Sugiyama, J.; Chanzy, H.; Langan, P.; *J. Am. Chem. Soc.* **2003**,125, 14300-14306.
3. Nishiyama, Y.; Johnson, GP.; French, AD.; Forsyth VT.; Langan, P. *Biomacromolecules*. **2008**, 9, 3133–3140
4. He, J.; Pingali, S.V.; Chundawat, S.P.S; Pack, A.; Jones, A.D.; Langan, P.; Davison, B.H.; Urban, V.; Evans, B.; O'Neill, H. *Cellulose* **2014**, 21, 927-936.
5. Sawada, D; Nishiyama, Y; Langan, P; Forsyth, VT; Kimura, S; Wada, M. *Biomacromolecules*. **2012**, 13, 288-291.