Experimental Report

Proposal: **8-01-390** **Council:** 10/2011

Title: Neutron studies of alpha-chitin

This proposal is a new proposal

Researh Area: Biology

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Samples: polysaccharide

Instrument	Req. Days	All. Days	From	To
D19	7	3	12/11/2012	15/11/2012

Abstract:

Polysaccharides having simple molecular structure such as cellulose, chitin, and amylose represent the major part of biomass and have great potential as renewable resources. Determining their detailed structures and hydrogen bonding arrangements is a key to understanding and improving their properties and utilization. X-rays provide the positions of O and C atoms, whereas neutrons in addition provide the positions of H atoms. We would like to collect neutron data from alpha-chitin on D19 from both hydrogen and specifically deuterated samples. From these data we expect to determine a complete atomic resolution structure including the hydrogen bonding network.

ILL Ex. N0: 8-01-390

“Neutron studies of α-chitin”

Experimental Team: Nishiyama, Forsyth, Ogawa, Wada, Langan

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Introduction

Chitin is one of the most abundant and widespread polymers on earth. Its primary structure is a linear polymer of β1,4-linked *N*-acetyl glucosamine (GlcNAc), which is almost same with cellulose except for C2 position of glucopyranoside ring replaced by acetoamide group. As natural cellulose, chitin occurs naturally in a fibrous crystalline state, namely microfibril, and two natural crystalline polymorphs, α- and β-chitin have been identified. β-Chitin is found in some aquatic organism, such as squid, diatoms, and hydrothermal vent worms, etc. The crystal structure of b-chitin is characterized with a one-chain monoclinic unit cell with $P2_1$ where molecular chains are aligned as parallel chain arrangement. This overall features of β-chitin crystal was recently confirmed with X-ray and neutron diffraction analyses, and the structure was further refined at atomic resolution [1-3]. On the other hands, α-chitin is produced mainly as structural component of crustacean and insect cuticles and fungal cell wall. Its crystal structure has been proposed as a two-chains orthorhombic unit cell with $P2_12_12_1$ symmetry, which indicates an antiparallel chain arrangement of the crystal [4, 5]. However, this feature of α-chitin crystal raises a question about its biosynthesis. Since the fibrous crystal of a-chitin should be synthesized with unidirectional polymerization and crystallization as in cellulose and β-chitin crystal, the antiparallelism of a-chitin crystal does not seem to be compatible with the biosynthetic mechanism. In addition the X-ray fiber diffraction analysis was recently achieved on highly crystalline α-chitin crystal from a phytoplankton *Phaeocystis*, and the additional reflections were observed in the diffraction diagrams, which cannot be indexed as the proposed unit cell and, thereby suggested the existence of a larger unit cell of α-chitin [6]. This situation leads us to reconsideration of the crystal structure of α-chitin including a parallel arrangement of the molecular chains. In this experiment, therefore, we investigated the crystal structure of a-chitin using the neutron diffraction. With intracrystalline deuteration technique we could collect the high-resolution neutron fiber diffraction data from α-chitin in both the deuterated and hydrogenated states.

Experimental Section

Uniaxial-oriented fibers of α-chitin were obtained from tendons of snow crab, *Chionoecetes opilio*. The tendons were removed from its body after boiling in water and purified with successive treatments

with 5% KOH aqueous solution at room temperature for 16 hours and 0.3% NaClO aqueous solution at pH = 4.0, 80°C for 3 hours with rinsing with water after each step. Intracrystalline deuteration was carried out by hydrothermal treatment achieved in 0.1 N NaOD in D_2O at 190°C for 30 min.

Neutron fiber diffraction data was collected at D19 with multi-wire gas-filled detector. The wavelength was 1.46 Å. The samples weighing about 100 mg were cut to about 20 mm length and loosely packed into a cylinder of aluminum foil. The beam was collimated to 8 mm corresponding to the diameter of sample. The sample was mounted with the fiber axis parallel to the phi-axis. The cylindrically averaged reciprocal space was covered by using a discrete set of omega and chi angles of the 4-axis goniometer. The data set was collected at ambient condition using 1.46 Å over a measuring time of 36 hours for deuterated sample and about 12 hours for the hydrogenated sample.

Results

The neutron fiber diffraction diagrams are shown in Fig. 1a and b for deuterated and hydrogenated α-chitin, respectively. The diffraction extended to about 1.0 Å resolution. Significant difference in intensity can be readily seen between the deuterated and hydrogenated samples. The intensity of the innermost reflection on the equator is much weaker in the deuterated pattern than in hydrogenated pattern. In meridian, 0 0 1 reflection can be observed in the deuterated pattern while the corresponding reflection disappears in the hydrogenated pattern. The intensities of 0 0 2 and 0 0 4 reflections in deuterated patterns are much weaker than in hydrogenated pattern. The presence of relatively strong odd-order meridional reflections in the deuterated suggests an absence of two-fold symmetry along the fiber axis (*c*-axis) of α-chitin crystal.

The diffraction patterns were compared with simulated neutron diffraction pattern of currently accepted α-chitin structure but the intensity distributions were completely different. Apparently, the crystal structure has to be significantly revised.

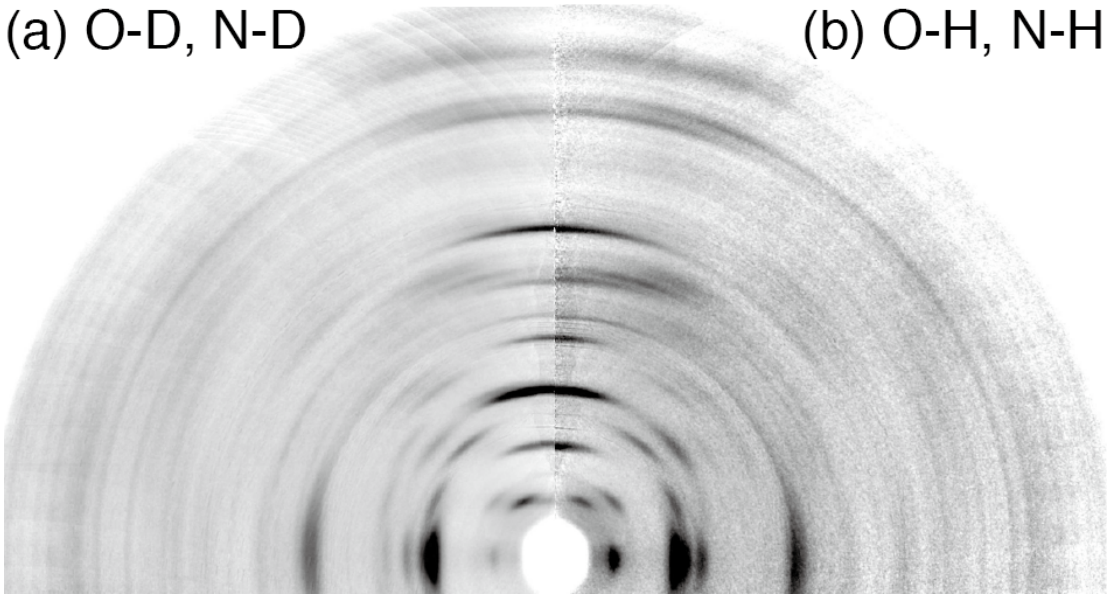

Figure 1. Neutron fiber diffraction patterns for (a) deuterated and (b) hydrogenated α-chitin from tendons of snow crab *C. opilio*.

References

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[4] Minke, R.; Blackwell, J. *J. Mol. Biol.* **1978,** *120,* 167–181.

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