Structure analyses of cellobiose and cellulose using X-ray diffraction and solid-state NMR spectroscopy on oriented samples

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Chapter 1 Background of the study

Cellulose is synthesized by enzymatic polymerization, crystallized, organized to nano-crystals (CNC), and self-assembled in microfibers. It is further organized into cell walls, fibers, etc. Biosyntheses at terminal complexes are diverse depending on biological sources, resulting in diversities in subsequent morphologies of cellulose. Two crystal forms, \( \alpha \) and \( \beta \), determined by diffraction and spectroscopic methods are known. Size and shape of CNCs depend on sources. Organizations and orientations of CNCs in microfibers and fibers are diverse. All these hierarchical diversities in structure make it difficult to characterize the physical, chemical, and biological properties of cellulose. It is evident that structures should be elucidated at every level of hierarchies, however, structures exhibited are so complicated.

Chapter 2 Determination of anisotropic diamagnetic susceptibility of cellobiose crystals by performing X-ray diffraction measurement

In this chapter, we present a facile method for determining the anisotropic diamagnetic susceptibility of biaxial crystals by performing X-ray diffraction measurements. The proposed method is based on the fact that the amplitude of fluctuations in a crystallographic axis under a magnetic field depends on the direction of the field with respect to the susceptibility axes. By calculating the magnetic energies of a crystal under static and rotating magnetic fields, we determined the relationship of the half-width of the diffraction spots corresponding to the \( (hkl) \) planes with the anisotropic magnetic susceptibilities \( \chi_1 - \chi_2 \) and \( \chi_2 - \chi_3 \) for the static field and \( \chi_1 - \chi_2 \) and \( \chi_1 - \chi_3 \) for the rotating field, where \( \chi_1 > \chi_2 > \chi_3 \). We have shown that the absolute values of the anisotropic diamagnetic susceptibilities can be determined if the size of
the microcrystals is known, whereas only the ratio \((\chi_2 - \chi_3)/(\chi_1 - \chi_2)\) or \((\chi_1 - \chi_3)/(\chi_1 - \chi_2)\) can be determined if the microcrystal size is unknown.

The developed method was applied to cellobiose microcrystal. The ratio of diamagnetic anisotropy of cellobiose (monoclinic, space group \(P2_1\)) was determined using two X-ray fiber diffraction patterns that were obtained from its microcrystalline powder oriented in static and rotating magnetic fields. We first determined the directions of the magnetic axes with respect to the crystallographic axes to obtain that \(\chi_3\) is parallel to the \(b^*\) axis and the \(\chi_1\) axis makes angles of 67.9° and 22.8° with respect to the \(a^*\) and \(c^*\) axes, respectively. Following the analysis method proposed recently, azimuthal half widths of the X-ray diffraction spots for \((hkl)\) planes of the oriented samples were plotted as a function of \(\sin^2 \Phi\), where \(\Phi\) is an angle that characterizes the direction of the reciprocal vector of the \((hkl)\) plane in the \(\chi_1\chi_2\chi_3\) coordinates, with \(\chi_1\), \(\chi_2\), and \(\chi_3\) being the principal axes of the magnetic susceptibility tensor. The half width linearly depended on \(\sin^2 \Phi\) as predicted by the proposed method. From the values of the slope and intercept of the plot, the ratio of the diamagnetic anisotropy, \(r_\chi = (\chi_2 - \chi_3)/(\chi_1 - \chi_2)\) was determined, where \(\chi_1 > \chi_2 > \chi_3\). We estimated that \(r_\chi = 1.4\sim 1.7\).

**Chapter 3** Determination of the chemical shift tensors of cellobiose by using a MOMA sample

In this chapter, the single crystal rotation technique was applied to magnetically oriented microcrystal arrays (MOMAs) of cellobiose (monoclinic) microcrystals, and the principal values and principal axes of the chemical shift tensors of the C1 and C1′ carbons were determined. Rotation was performed about the magnetic \(\chi_1\), \(\chi_2\), and \(\chi_3\) axes.
of MOMA, and the measurements were performed at six different orientations with respect to the applied magnetic field. With this choice of rotation, crowded peaks were reduced and the peaks for the C1 and C1´ carbons were resolved by comparing with simulation results. Six components of the chemical shift tensor expressed with respect to the magnetic \( \chi_1,\chi_2,\chi_3 \)-frame were determined. The tensors thus obtained were transformed into those relative to the molecular frame.

**Chapter 4 Structure analysis of cellulose microfiber (Cotton, Ramie and Wood) via the anisotropic diamagnetic susceptibility**

In this chapter, the orientational distribution of cellulose nanocrystals (CNCs) in a cellulose whisker (CW) was investigated by means of the X-ray diffraction of magnetically oriented samples of CWs. A cellulose sample (Cotton: Whatman CF11) was hydrolyzed and fractionated to prepare three different CW samples with a size ranging from ca. 10 \( \mu \)m to 100 \( \mu \)m. Each of the fractions that were suspended in a liquid matrix was aligned under a static or a rotating magnetic field, and the matrix was solidified to prepare magnetically oriented microcrystal arrays (MOMAs). Then, the MOMAs were investigated by X-ray diffraction measurements. By analysis of the diffraction patterns, it is concluded that the c-axes of the CNCs are uniaxially distributed within a CW and that the orientational order increases with decreasing CW size. The average magnetic susceptibility \( \langle \chi_a \rangle \) of the CWs was expressed in terms of their size and of the X-ray azimuthal half width. Using these expressions, a correlation length for the orientation of CNCs in a CW was determined. Then, this method developed by using Cotton CW was also applied to the Ramie and Wood CWs to determine the correlation lengths.
Chapter 5 Summary

In this thesis, attempts are made to develop methods to elucidating some aspects of cellulose structures. Techniques of magnetic orientation of crystals and fibers are used to prepare oriented samples that are analyzed by X-ray diffractions and solid-state NMR spectroscopy. By orientation, it becomes possible for the first time to obtain more information on structures. In this thesis, magnetically oriented cellobiose (a smallest repeating unit of cellulose) crystals and cellulose whiskers are studied. X-ray diffraction and solid-state NMR techniques are used to elucidate the solid state structure of these materials. These two techniques are powerful to analyze solid state structures and mutually complementary. In chapter 2, the diamagnetic anisotropy of cellobiose is determined, which provides the experimental conditions needed to prepare the oriented samples. In chapter 3, the chemical shift tensors of C1 and C1’ carbons of cellobiose crystals are determined using magnetically oriented samples. In chapter 4, magnetically oriented cellulose whiskers are analyzed by X-ray diffraction and the correlation lengths of CNC orientations are determined.