X-ray crystal structure analyses of magnetically oriented microcrystalline suspensions

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Single-crystal X-ray diffraction (XRD) is most frequently and widely used for determining crystal structures. This method can be the best way to determine crystal structures, but in many circumstances materials cannot be grown large enough to be analyzable by single-crystal XRD. We may imagine, though, that if the crystallographic axes of all microcrystals in a sample are aligned in the same direction, then their diffraction will be equivalent to that from a single crystal. It has been proposed that a magnetically oriented microcrystal array (MOMA)—a polymer composite containing three-dimensionally aligned microcrystals—can produce XRD data equivalent to that measured from a single crystal, from which the crystal structure can be determined. The magnetic susceptibility tensor of biaxial crystals—including the orthorhombic, monoclinic, and triclinic crystal systems—has three principal values, which we refer to as χ_1, χ_2, and χ_3, assuming $\chi_1 > \chi_2 > \chi_3$. The principal axes corresponding to χ_1 and χ_3 are referred to as the easy and hard magnetization axes, respectively. Special rotating magnetic fields can cause simultaneous alignments of the χ_1 and χ_3 axes, results in a three dimensional alignment of biaxial crystals. Special magnetic fields are, for example, frequency-modulated (FM) field, the rotation speed switches between ω_{fast} and ω_{slow} ($\omega_{\mathrm{fast}} > \omega_{\mathrm{slow}}$) every 90° during one revolution, and a stop-and-go (S&G) field, the rotation stops every 180° or 360° during one revolution. We prepared a MOMA as follows. Microcrystals were suspended in a liquid in which they were allowed to rotate freely. After the microcrystals were magnetically aligned by exposing them to an FM or stop-and-go (S&G) magnetic field, the suspension was solidified. Thus prepared MOMAs for some

microcrystals led to the structure determination using conventional XRD measurement. The MOMA method has shown promise in determining the crystal structure of microcrystalline powders. However, it has some drawbacks: (i) The choice of suspending liquid is limited. The suspending liquid is necessary to form a good suspension of the microcrystals to be investigated, and it must be solidified by some means such as ultraviolet light irradiation, gelation, or temperature decrease. There is a limited number of liquids that satisfy both of these requirements. (ii) The microcrystals' alignment partially deteriorates during solidification. (iii) The microcrystals, once fixed in a solid matrix, are difficult to recover.

To solve these problems, we have proposed the magnetically oriented microcrystal suspension (MOMS) method. In this method, magnetically oriented microcrystals are subjected to *in-situ* XRD without consolidating the suspending liquid.

In this thesis, three MOMS methods have been developed. To maintain the microcrystals' three-dimensional magnetic alignment, the MOMS had to be rotated in a magnetic field. However, the sample rotation is required to collect the single-crystal diffraction data. Thus, the time scales between the MOMS rotation and the XRD oscillation measurement needed to be adjusted.

In Chapter 2, we collected *in-situ* XRD data from a MOMS of L-alanine microcrystals with a conventional in-house diffractometer by introducing an X-ray shutter, which shielded the impinging X-ray beam in synchronization with the time-varying sample rotation. The shutter was a disc with a 10° slit, rotating synchronously with the rotation of the MOMS. This slit width corresponded to the oscillation of the same angle. Using the 22 XRD images obtained, covering data over a range of 220°, we determined the crystal structure from a sample of L-alanine microcrystals of 20 to 45 μm, with $R_1 = 0.0421$. Also,

we determined the crystal structure with R_1 = 0.0991 using L-alanine microcrystals with sizes less than 5 μm.

In Chapter 3, we used S&G sample rotation, which did not require the shutter. In this method, the rotation of the MOMS was ceased for a certain duration, during which the XRD data was collected by the oscillation method. We theoretically estimated the changing orientation of the microcrystals during this interval and proved that they would cause no significant fluctuations. With this method, the time required to collect the XRD data was greatly reduced to a level comparable to that required for the single-crystal measurement. The crystal structure was determined from L-alanine microcrystals with sizes of less than 20 μm.

In Chapter 4, we attempted to apply the MOMS method to biomacromolecules. Because biomacromolecules exhibit weaker diffraction than inorganic and small organic compounds, a synchrotron X-ray source was required. A magnetic attachment was placed in the beam line of Spring-8, and the suspension sample was subjected to time-varying rotation. The X-ray beam continuously impinged on the rotating suspension, and XRD data were continuously recorded with a CMOS detector. We investigated the condition required to match the time scales of the MOMS rotation and the readout speed of CMOS. This method was applied to L-alanine and lysozyme MOMSs. We could not yet determine the crystal structures of these biomacromolecules, but we did show the MOMS method to be useful.