Observation of Oscillatory Rocking Curves by Dynamical Diffraction in Protein Crystals

Rocking curves by X-ray diffraction for protein crystals such as glucose isomerase crystals have been investigated by digital X-ray topography with a charge-coupled device. The oscillatory profiles of the rocking curves were clearly observed by using different incident wavelengths and sample thicknesses. The observed oscillatory rocking curves were in good agreement with those predicted by dynamical theory of X-ray diffraction. This indicates that dynamical diffraction occurs even in protein crystals. This suggests the need for a dynamical diffraction model in protein-structure analysis by X-ray diffraction, which has not been used for conventional structural analysis so far.

High-quality protein crystals for structural analysis by X-ray diffraction have been grown by various methods [1]. The observation of dynamical diffraction in protein crystals is an interesting topic because dynamical diffraction generally occurs in perfect crystals such as silicon crystals. However, there has been no report on protein crystals showing clear dynamical diffraction, and it is not clear whether the perfection of protein crystals is still low compared with that of silicon crystals. In practice, protein crystallographers assume kinematical diffraction for low-quality crystals with defects such as dislocations [2].

Recently we observed dynamical diffraction in protein crystals such as glucose isomerase (GI) from *Streptomyces rubiginosus* crystals by X-ray topography as Pendellösung fringes and bead-like contrasts along dislocation images [3]. More interestingly, we succeeded in observing for the first time the oscillatory rocking curves by dynamical diffraction in protein crystals such as dislocation-free GI crystals [4]. These results demonstrate that dynamical diffraction occurs even in protein crystals. Here we present the investigation on oscillatory rocking curves by dynamical diffraction in GI crystals.

The rocking curves were measured by digital X-ray topography with a charged-coupled device in BL-20B. **Figure 1** shows a typical digital X-ray topograph of a dislocation-free GI crystal with orthorhombic form, taken with the 011 reflection, where the thickness of the GI crystal is 199 μm. By using such high-quality GI crystals, we succeeded in observing oscillatory rocking curves due to dynamical diffraction as shown in **Fig. 2**.

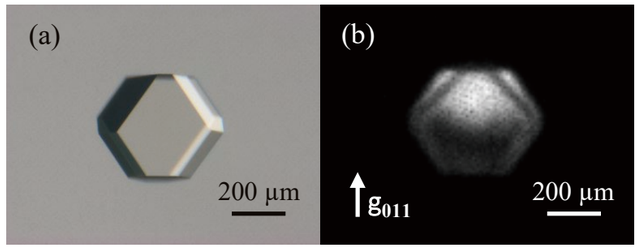

Figure 1: Typical GI crystal. **(a)** Optical micrograph, **(b)** digital X-ray topograph with CCD camera. No line contrasts corresponding to dislocations are observed in the X-ray topograph.

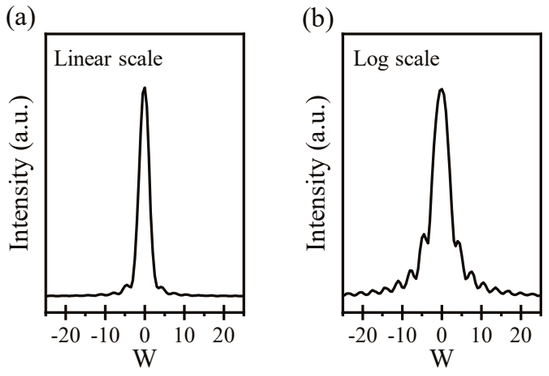

Figure 2: A typical rocking curve profile for the 011 reflection of a GI crystal with a thickness of 199 μm, taken with an incident beam with a wavelength of 1.2 Å. The horizontal axis is called the W scale, which is a parameter representing the deviation from the diffraction condition. In **(a)** and **(b)**, the intensities of the same rocking curves are shown on linear and logarithmic scales, respectively.

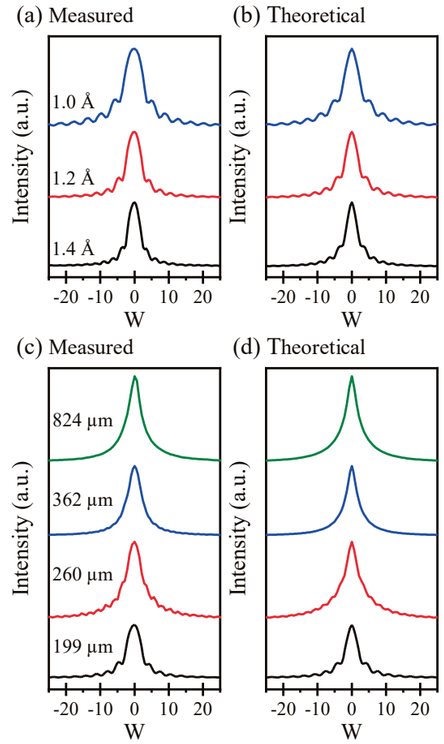

Figure 3: (a) Measured rocking curves, **(b)** theoretical rocking curves for 011 reflections of the same GI crystals with a thickness of 199 μm, taken with incident beams with different wavelengths of 1.0, 1.2 and 1.4 Å, respectively. **(c)** Measured rocking curves, **(d)** theoretical rocking curves for 011 reflections of GI crystals with different thicknesses of 824, 362, 260 and 199 μm, taken with an incident beam with a wavelength of 1.2 Å, respectively.

To clarify the dynamical diffraction further, the oscillatory rocking curves for GI crystals were measured by using different incident wavelengths and sample thicknesses, as shown in **Fig. 3**. All of them well-fitted those predicted by the dynamical theory of diffraction with absorption and angular dispersion of the beam [5]. Thus we conclude that dynamical diffraction occurs even in protein crystals such as GI crystals. These results suggest the need for a dynamical diffraction model in protein-crystal-structure analysis and model refinement.

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BEAMLINE

BL-20B

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