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Crystal distortion of monoclinic hen egg-white lysozyme crystals using X-ray digital topography

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ABSTRACT

The crystal distortion of monoclinic hen egg-white lysozyme (HEWL) crystals was observed by the X-ray digital topography. The local rocking curves were obtained by the series of the topographic images. The single, double, triple, and multiple peaks of the local rocking curves were observed. The mapping of the full width of the one tenth maximum (FW10M) of the local rocking curves was obtained. It was found that the magnitude of FW10M depended on the distance of the peak splitting. The distribution of the distortion in the monoclinic HEWL crystals was investigated by the analysis of the local rocking curves.

1. Introduction

Crystal quality or perfection of protein crystals has been mainly investigated by a half width of the X-ray rocking curve. In rocking curve measurements, the intensity of the diffracted X-ray beam is recorded with rotation around Bragg angle. For examples the mosaicity, i.e. angular distribution of the strain in the crystal, is provided by the rocking curve measurement [1–10]. The perfection of protein crystals has been observed by digital topography with X-ray CCD camera [11–13]. In our previous study, the defects, mainly dislocations, in large protein crystals of tetragonal, orthorhombic and monoclinic hen egg-white lysozyme (HEWL) crystals were investigated by the traditional X-ray topography by film [14–16] and the new digital topography [13]. In these studies, we found curious dislocation structures only in monoclinic HEWL crystals. They were the curved and loop dislocation structures. In addition, it is well-known that the monoclinic HEWL crystals have hollow opening due to the Berg effect and that the growth rate of the +b face is much higher than that of the −b face [14,17]. Thus, it is expected that the regions of high and low perfection coexist in the identical crystals for the monoclinic HEWL crystals.

Therefore, in the present study, the perfection of monoclinic HEWL crystals was observed by the X-ray digital topography. The local rocking curves were obtained by the series of the topographic images. The crystal distortion in the monoclinic HEWL crystals was investigated by the mapping of a full width of the one tenth maximum (FW10M) of the local rocking curves.

2. Experimental procedure

Monoclinic HEWL crystals were grown by the liquid–liquid interfacial precipitation method [18]. A HEWL solution containing 3 mg/mL HEWL and 2.5% $NaNO_3$ at pH 4.5 was used. The crystal growth was carried out at 23 ± 0.1 °C. After about 2 weeks, large crystals up to 2 mm were grown. The crystals were monoclinic with space group $P2_1$, lattice constants of $a=28.0$ Å, $b=62.5$ Å, $c=60.9$ Å, $\alpha=\gamma=90°$, $\beta=90.8°$, and four molecules per unit cell [17]. The crystals were bounded by the habit crystallographic faces of $(0\ \bar{1}\ 0)$, $(10\ \bar{1})$, and $(\bar{1}0\bar{1})$.

Synchrotron monochromatic-beam X-ray topography was performed in BL15B1 at the Photon Factory (PF) of the High Energy Accelerator Research Organization (KEK). All experiments were carried out at 295 K. A monochromatic beam of 1.2 Å was selected by adjusting the double-crystal monochromator. The beam intensity was 1×10^{11} photons/s mm^2 at 10 keV. The vertical and horizontal beam divergences were calculated to be 5 and 15 μrad, respectively, and $\Delta\lambda/\lambda$ was 2×10^{-4}. The details of the X-ray topography experiments have been described in previous papers [13–15].

The topographs were recorded by an X-ray CCD camera (Photonic Science, X-FDI 1.00:1). The distance between the specimen and the CCD camera was 35 cm. The CCD camera had the following specifications: the scintillator is gadolinium oxysulphide doped with terbium; the pixel size is approximately 6.45 μm^2; the pixel resolution is 1392 × 1040 and the input active area is approximately 9.0 × 6.7 mm^2. The specimens were placed in the beam on a goniometer head that allowed them to be rotated about an axis perpendicular to the beam. Diffracted beam topographs were recorded by the CCD camera. The specimen was rotated and

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successive digital X-ray topographs were obtained. Sequential slice images were obtained by rotating the specimen. The CCD camera output the sequential digital topographic images as 12-bit grey-scale TIFF files.

In order to process the large amount data in these images, we developed new computer software. This software was used to analyze rocking curve peaks on a single pixel. In order to deal with the rocking curves at all pixels by the computer software, the full width of one tenth of maximum intensity (FW10M) of the rocking curves was estimated by the manner as shown in Fig. 3 [9,10,12]. For split peaks (see Fig. 4), the FW10M was defined as the width across multiple peaks. These analyses were performed and mapped to the original image positions by our computer software.

3. Results and discussion

The digital topography was carried out by using a dozen or so monoclinic HEWL crystals. A typical monoclinic HEWL crystal was analyzed to comprehensive the distortion of the monoclinic HEWL crystals. Fig. 1 shows the optical photograph of the typical monoclinic HEWL crystal. The black lines are drawn to guide the eye. The wedgelike region is observed in the right side in this figure. The wedge corresponds to a hollow opening which is developed on the (010) surface of the crystal. The hollow opening is considered to be due to the Berg effect, resulting in an enhanced growth rate at the crystal edge [17].

Fig. 2 shows monochromatic-beam X-ray digital topograph in 020 reflections, taken with the incident beam almost normal to (110) face. This topographic image is taken at the rotation angle of 0.033°. The boundaries of hollow opening are shown by white lines in Fig. 2. The sequential topographic images are recorded using the CCD camera by rotating specimens. The total number of images is 513 and the rotation angle per image is 0.0002°. The exposure time per image is 2 s, giving a total exposure time of

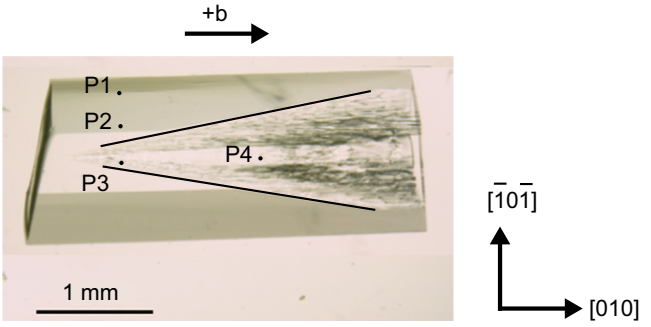

Fig. 1. Optical photograph of the monoclinic HEWL crystal. The wedgelike region was observed in the right side. The black lines were drawn to guide the eye. The four points (P1, P2, P3, and P4) were marked as guide corresponding to Fig. 2.

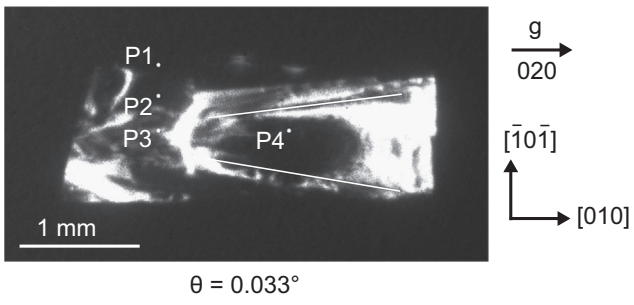

Fig. 2. Typical image of the digital topography of the monoclinic HEWL crystal. Typical local rocking curves were observed at P1, P2, P3, and P4.

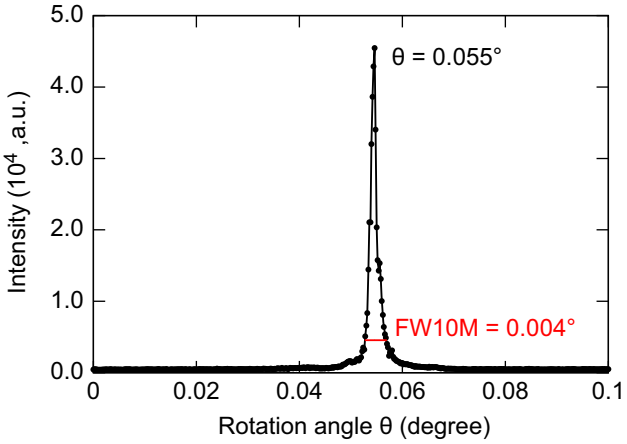

Fig. 3. Local rocking curve at P1 in Fig. 2.

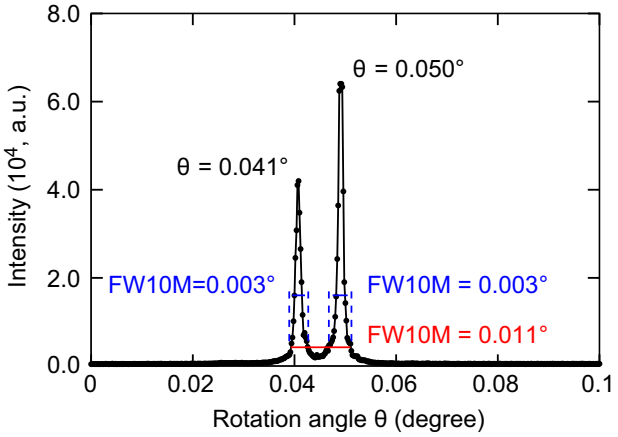

Fig. 4. Local rocking curve at P2 in Fig. 2.

1026 s. Fig. 2 shows extracted images from these sequential images. The typical local rocking curves are obtained at P1, P2, P3, and P4. The region around P4 corresponds to the inside of the hollow opening. The single, double, triple, and multiple peaks are observed at P1, P2, P3, and P4, respectively.

Fig. 3 shows the local rocking curve at P1 in Fig. 2. The narrow single peak is observed, and the FW10M of this curve is 0.004°. The angular position of the peak top is 0.055°. It is reported that high-quality tetragonal HEWL crystals have 0.006°–0.013° of the FW10M [10]. The FW10M of this specimen is narrower than that of those crystals. Therefore, it is shown that the specimen had high perfection locally.

Fig. 4 shows the local rocking curve at P2 in Fig. 2. The peak splitting is observed, and the FW10M of this local rocking curve is 0.011° as defined. The FW10M of each peak is 0.003°. Such splitting is observed by Lovelace et al. [12]. The FW10M of both single peaks is almost same as that of P1 in Fig. 3. The peak splitting occurs in this local rocking curve, but each peak is quite sharp. The angular positions of the peaks are 0.041 and 0.050°.

Fig. 5 shows the local rocking curve at P3 in Fig. 2. The peak splitting is observed, but each peak is also quite sharp. The FW10M among these peaks is defined by 0.024° as shown in Fig. 5. The angular positions of the peaks are 0.025, 0.037, and 0.045°. The maximum of the distance among the peaks is 0.024°. The FW10M corresponds to the maximum distance among the peaks.

Fig. 6 shows the local rocking curve at P4, where is the hollow opening as shown in Fig. 2. The peak splits into multiple peaks, but each peak is still sharp. The FW10M of this curve is defined by