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Improvement of Crystal Homogeneity of Tetragonal Hen Egg White Lysizmye Crystals under Application of an External Alternating Current Electric Field

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X-ray diffraction rocking-curve measurements were carried out on tetragonal hen egg white (HEW) lysozyme crystals grown with and without the application of an external alternating current (AC) electric field. The crystal quality was investigated by the full width at half maximum (FWHM) value for each rocking curve. For two-dimensional maps of the FWHMs measured on the 440 and the 12 12 0 reflection, the crystal homogeneity was improved under application of an external electric field at 1 MHz, compared with that without. In particular, the significant improvement of the crystal homogeneity was observed for the 12 12 0 reflection.

1 Introduction

High-quality single crystals of proteins are necessary in order to achieve structure-guided drug design and controlled drug delivery, because the crystal quality governs the refinement of the 3D structure of protein molecules obtained from X-ray and neutron structure analysis. In particular, large protein crystals of more than 1 mm size are required in order to perform neutron structure analysis. This is attributed to the weakness of the brightness of neutron beam. Large protein crystals often include significant strain due to dislocations, impurities etc., which results in non-homogeneity of the crystal quality. In general, non-homogeneity of crystal quality leads to lowering of the resolution for structural analysis of protein molecules. Therefore, the homogeneity of protein crystals is also important to obtain the 3D structure of protein molecules with high resolution for neutron structural analysis. However, it is difficult to grow high-quality homogeneous single crystals of proteins with large size. Therefore, the establishment of a crystallization technique that can achieve this is required.

We have demonstrated that the nucleation rate [1, 2] and nucleated phases [3] of proteins can be regulated under application of an external alternating current (AC) electric field, by focusing the electrostatic field added to the chemical potentials of the liquid and solid phases. More recently, we have indicated that the crystal quality of a part of protein crystals (212.85 μm diameter) is improved by applying an external AC electric field at 1 MHz, by considering the effect of an electrostatic field on the entropy of the solid [4]. This result is useful for X-ray structure analysis that can thinly focus a beam diameter by using high intensity synchrotron radiation. However, this improvement in the crystal quality of a part of protein crystals implies that of the whole protein crystals, which leads to a precise 3D structure of protein molecules for neutron structure analysis. In this paper, we report an improvement in the crystal homogeneity of the whole tetragonal hen egg white (HEW) lysozyme crystals by the application of an external AC electric field at 1 MHz, as verified by X-ray diffraction rocking curve measurements.

2 Experiment

HEW lysozyme was purchased from Wako Pure Chemical Industries, Ltd., and was used without further purification. Solutions of 57 mg/mL HEW lysozyme and 0.5 M NaCl at pH 4.3, were used for the crystallization experiments. Under these conditions, the obtained crystals were tetragonal with the $P4_32_12$ space group, and lattice constants of a = 79.1 Å and c = 37.9 Å.

Crystallization experiments were conducted at 21±0.2 °C using the batch method. HEWL crystals grown on the sides of electrodes were used to obtain X-ray diffraction rocking-curve profiles. The distance between the electrodes was 12 mm, and the solution volume was 2.7 mL. An external AC electric field of 400 V/cm was applied at 1 MHz. Crystals were grown with and without application of the external electric field for 9 days.

X-ray diffraction rocking-curve measurements were conducted at room temperature in the beamline BL15B1 at the Photon Factory (PF) of the High Energy Accelerator Research Organization (KEK) in Japan. In these measurements, the reflected images of entire crystals for the 440 and the 12 12 0 reflections were detected using a high spatial resolution, two-dimensional digital CCD camera (effective pixel: 6.45 mm × 6.45 mm). X-ray rocking curves for the 440 and the 12 12 0 reflections were reconstructed from the reflected intensities in the region with a beam spot size of 387 mm (60 pixels). Therefore, the instrumental resolution function (*IRF*') [8] which takes into account the dimensions of the sample and the horizontal beam divergence (0.178 mrad) can be calculated to be 1.75 $\times 10^{-3}$ °. X-ray rocking curves for tetragonal HEW lysozyme crystals prepared with an external electric field

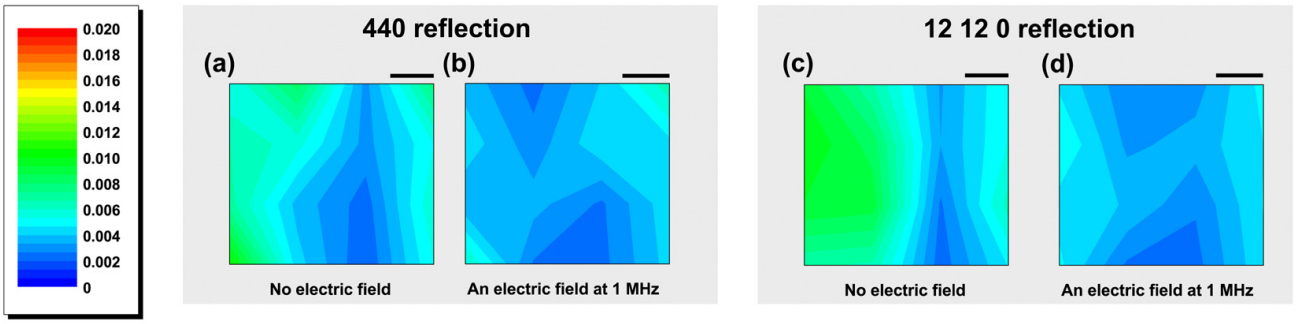

Fig. 1: Two-dimensional maps of FWHMs measured for the 440 and the 12 12 0 reflections from tetragonal HEW lysozyme crystals prepared with and without an external AC electric field. (a) and (c) No electric field, and (b) and (d) Applied fields at 1 MHz. The upper-right scale bar for each two-dimensional map represents 0.3 mm.

at 1 MHz were obtained by partitioning the whole crystal into nine or sixteen regions. Both single and multiple peaks were observed from each region of the crystal. In the case of multiple peaks, each peak was fitted using a Gaussian function, and the FWHM of the multiple peak was then evaluated as a sum of the FWHMs for each single peak.

3 Results and Discussion

Figure 1 shows an example of two-dimensional maps of the FWHMs measured for the 440 and the 12 12 0 reflections from tetragonal HEW lysozyme crystals prepared with and without an external electric field. The upper-right scale bar for each two-dimensional map represents 0.3 mm. We first focus on the two-dimensional maps of the FWHMs for the 440 and the 12 12 0 reflections from the crystal prepared without an external electric field. As shown in Fig. 1(a), it was found that the values of the FWHMs in the crystal gradually changed for the two-dimensional map of the 440 reflection from the crystal prepared without an external electric field. Moreover, the homogeneity of those on the 12 12 0 reflection was inferior to that on the 440 reflection, as seen in Fig. 1(c). It was previously shown that the values of the FWHMs obtained from the crystal prepared without an external electric field increase for diffraction peaks with order higher than the 440 reflection [4]. This is due to the sensitivity of high-order reflections to the strain in the crystals. In other words, the inferior homogeneity for the 12 12 0 reflection could reflect the fact that the crystals prepared without an external electric field have significant strain. Accordingly, the overall crystal quality was inhomogeneous for the crystals prepared without an external electric field.

Under a 1 MHz applied field, on the other hand, the homogeneity of the values of the FWHMs was improved for the 440 reflection compared with those without an external electric field, as shown in Figs. 1(a) and (b). This tendency was also the same for the 12 12 0 reflection, as shown in Figs. 1(c) and (d). Additionally, the two-dimensional map on the 12 12 0 reflection maintained almost the same homogeneity as that for the 440 reflection under application of the external electric field at 1 MHz. This suggests that the strain in the crystal significantly decrease by applying the external electric field at 1 MHz. Therefore, the crystal homogeneity of the whole tetragonal HEW lysozyme crystals was improved by the application of an external AC electric field at 1 MHz [9].

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