15B1/2010G605

Enhancement of Crystal Perfection for Tetragonal Hen-egg White Lysozyme Crystals under Application of an External AC Electric Field

Haruhiko Koizumi[1,*], Satoshi Uda[1], Kozo Fujiwara[1], Masaru Tachibana[2], Kenichi Kojima[3] and Jun Nozawa[1]
[1]Institute for Materials Research, Tohoku University, Sendai 980-8577, Japan
[2]Graduate School of Nanobioscience, Yokohama City University, Yokohama 236-0027, Japan
[3]Yokohama Soei University, Yokohama 226-0015, Japan

1 Introduction

High quality single crystals of proteins are required to achieve structure-guided drug design and controlled drug delivery, because the crystal quality governs the distinctness of the 3D structure of protein molecules obtained from X-ray structure analysis. The distinctness of a 3D protein structure is defined by the resolution, and a resolution of less than 1.5 Å is desirable to achieve structure-guided drug design and controlled drug delivery, which enables each carbon atom on a protein molecule to be distinguished. However, it is quite difficult to obtain high quality single crystals of proteins with a resolution of less than 1.5 Å. Therefore, the establishment of a crystallization technique that can obtain high quality single crystals of proteins is required.

Recently, we have successfully achieved both an increase and decrease in the nucleation rate of HEWL crystals by changing the frequency of an applied electrostatic field [1, 2, 3, 4] by focusing on the electrostatic term added to the chemical potentials of the liquid and solid phases. Furthermore, we have revealed that this crystallization technique is also adaptable to porcine insulin [5]. If crystal quality could also be improved by application of an external AC electric field, then this crystallization technique could be an excellent tool, because the nucleation rate can also be controlled. In this paper, we report on improvement of the crystal quality of HEWL crystals by application of the external AC electric field, as verified by X-ray diffraction rocking-curve measurements.

2 Experiment

HEWL was purchased from Wako Pure Chemical Industries, Ltd., and was used without further purification. Solutions of 57 mg/mL HEWL 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 2.9 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.

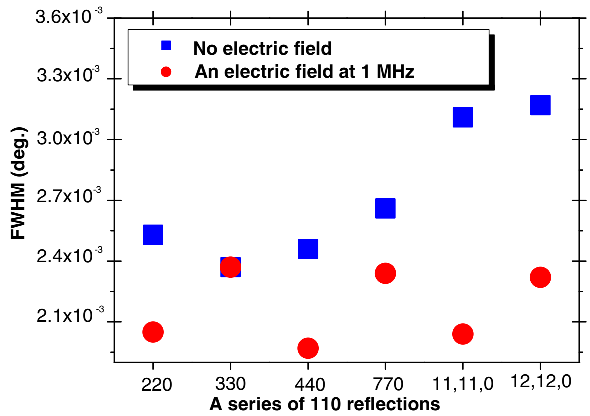

Fig. 1: Dependence of FWHM on a series of 110 reflections for HEWL crystals grown with and without application of an external electric field.

X-ray diffraction rocking-curve measurements were conducted in BL15B1 at the Photon Factory (PF) of the High Energy Accelerator Research Organization (KEK). A two-crystal monochromator consisting of a Si(111) crystal was located 24 m from the source and was used to select an X-ray wavelength of λ = 1.2 Å. Samples were measured with a high-resolution step by mounting on a precision goniometer (minimum angular step width: 5.3×10^{-5} °). Under such a condition, the reflected images of entire crystals for a series of 110 reflections were detected using a high spatial resolution, two-dimensional digital CCD camera (effective pixel: 6.45 μm × 6.45 μm). X-ray rocking-curve profiles for a series of 110 reflections were reconstructed from the reflected intensities using the same region of the crystal with a beam spot size of 212.85 μm (33 pixels). Therefore, the beam divergence was 1.7×10^{-5} rad (0.9×10^{-3} °). The full width at half maximum (FWHM) of each rocking-curve profile measured for samples prepared with and without an external electric field was evaluated using a Gaussian function.

3 Results and Discussion

Figure 1 shows the dependence of the FWHMs on a series of 110 reflections from crystals prepared with and without an external electric field. The FWHMs obtained from crystals prepared without an external electric field increased for diffraction peaks with higher-order than the 440 reflection, as shown in Fig. 1. In particular, the FWHMs were larger for the 11,11,0 and 12,12,0 higher-order reflections. This is due to the sensitivity of the higher-order reflection to strain in the crystals; therefore, the FWHMs of the higher-order reflections are suitable for assessment of the crystal quality. In contrast, for crystal growth with an external electric field at 1 MHz, the FWHMs were almost constant for all order diffraction peaks, as shown in Fig. 1. Furthermore, the FWHMs of crystals grown with an external electric field were smaller than those without, except for the 330 reflection. It should be noted that the FWHMs for the 11,11,0 and 12,12,0 reflections of crystals grown with an external electric field were significantly smaller than those without. This indicates that the crystal quality of HEWL crystals was significantly improved by application of an external AC electric field.

With respect to improvement of the crystal quality, Taleb *et al*. have suggested that the crystal quality of HEWL crystals was slightly improved by application of an external DC electric field [6], and they reported a 17% improvement of the crystal quality for crystals grown under application of an external DC electric field, although the diffraction vector of the measured X-ray rocking-curve profiles was not described in Ref. [6]. The present enhancement of 26% for the crystal quality was observed for the 440 reflection by application an external AC electric field, and an enhancement of 34% was achieved for the 11,11,0 higher-order reflection. Therefore, the results suggest that application of an external AC electric field can be employed to improve the quality of protein crystals.

The higher-order reflection cannot often be observed for X-ray structure analysis of protein molecules, which results in a lowering of the resolution for the structure of protein molecules. This phenomenon is attributed to deterioration in the crystal quality of the protein crystals. However, we have observed that the FWHM for the higher-order reflection was improved when an external AC electric field was applied during crystal growth, which suggests that it may be possible to distinctly observe the higher-order reflections of crystals grown under application of an external AC electric field. Thus, enhancement of the resolution for the structural analysis of protein molecules could be expected by application of an external AC electric field during crystal growth.

In summary, we have observed that the FWHMs of X-ray rocking-curve profiles for crystals grown with an external electric field were smaller than those without. The FWHMs for the higher-order reflections were significantly smaller for those crystals grown with an external electric field than those grown without. These results indicate that the crystal quality of HEWL crystals could be improved by application of an external AC electric field during growth. This is a novel crystallization technique for proteins, which can be employed to not only enhance the nucleation rate [1, 2, 3, 4, 5], but also improve the crystal quality.

Acknowledgement

X-ray diffraction rocking-curve measurements were performed at the Photon Factory (PF) under the auspices of the Photon Factory Program Advisory Committee of KEK (Proposal No. 2010G605, 2011G073) and at SPring-8 with approval of the Priority Program for Disaster-Affected Quantum Beam Facilities (Proposal No. 2011A1947).

References

[1] H. Koizumi, K. Fujiwara and S. Uda, Cryst. Growth Des. **9** (2009) 2420.

[2] H. Koizumi, K. Fujiwara and S. Uda, Cryst. Growth Des. **10** (2010) 2591.

[3] H. Koizumi, S. Uda, K. Fujiwara and J. Nozawa, J. Crystal Growth **312** (2010) 3503.

[4] H. Koizumi, S. Uda, K. Fujiwara and J. Nozawa, Langmuir **27** (2011) 8333.

[5] H. Koizumi, S. Uda, K. Fujiwara and J. Nozawa, J. Crystal Growth **352** (2012) 155.

[6] M. Taleb, C. Didierjean, C. Jelsch, J. Mangeot, B. Capelle and A. Aubry, J. Crystal Growth **200** (1999) 575.

* h_koizumi@imr.tohoku.ac.jp