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Control of Subgrain Formation in Protein Crystals by the Application of an External 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, Katahira. Aoba-ku, Sendai 980-8577, Japan
[2]Graduate School of Nanobioscience, Yokohama City University, 22-2 Seto, Kanazawa-ku, Yokohama, 236-0027, Japan
[3]Department of Education, Yokohama Soei University, 1 Miho-tyou, Midori-ku, Yokohama, 226-0015, Japan

We show that improvements in the quality of tetragonal hen egg white (HEW) lysozyme crystals, as assessed by a decrease in the misorientation between subgrains, can be achieved by imposing a 1 MHz electric field during crystal growth. Analysis of the full width at half-maximum (FWHM) of X-ray diffraction (XRD) rocking curves indicated that misorientation between subgrains and local strain in the crystals contributed to the broadening of the experimentally determined rocking curves for the tetragonal HEW lysozyme crystals. In particular, the data suggested that imperfections in tetragonal HEW lysozyme crystals are predominantly caused by misorientation between subgrains.

1 Introduction

The structural analysis of protein molecules using X-ray diffraction (XRD) analysis is an active research field primarily aimed at achieving structure-guided drug design and controlled drug delivery. To obtain accurate three-dimensional structures of molecules by XRD analysis, it is important to detect many diffraction spots, particularly high-order reflections using high-quality single crystals. However, it is difficult to grow high-quality single crystals of proteins, which is an impediment to the accumulation of high-order reflections for protein crystals. Therefore, the establishment of a crystallization technique to obtain high-quality single crystals of proteins is required.

We have previously demonstrated that the nucleation rate [1, 2] and nucleated phases [3] of proteins can be regulated under application of an external electric field, by focusing on the electrostatic field added to the chemical potentials of the liquid and solid phases. When an electrostatic field is applied to a protein solution, the difference in the magnitude of the electrical permittivities between the liquid and solid phases is an important factor when discussing the effect of an electrostatic field on the nucleation rate. The electrostatic energy is added to the chemical potential in both liquid and solid phases, and we can select for which phase the effect is larger, by changing the frequency of the external electric field, because the conservation of dielectric flux holds at the interface between the liquid and solid [1, 2]. Such a thermodynamic effect is added not only to the chemical potential but also to the entropy. Therefore, we have recently succeeded in the improvement of the local crystal quality for tetragonal HEW lysozyme crystals under application of an external electric field at 1 MHz [4, 5]. Notwithstanding these prior results, our understanding of the improvement of crystal quality in response to the application of a 1 MHz field remains incomplete.

The measured FWHM of XRD rocking curves may be increased by various effects, such as lattice tilting, lattice strain, and particle size, so the broadened profiles can be resolved into different components depending on the lattice misorientation, the local strain, and the particle size [6, 7]. In this article, we report the origins of the broadening contributions to the rocking curves obtained for tetragonal HEW lysozyme crystals grown both with and without the application of an external electric field. This approach would be extended to a less perfect protein crystal in the future.

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 trials were conducted at 21±0.2°C using the batch method. The details of the electrode arrangements by which the electrostatic field was applied to the protein solution have been described in ref [4]. We have previously found that the strong electric field (10^4 V/cm) generated in the electric double layer (EDL) at the inner surface of a drop of protein solution is important with regard to controlling the nucleation rate of the proteins [8-10], so HEW lysozyme crystals grown on the sides of the electrodes were used to obtain XRD rocking curves. The distance between the electrodes was 12 mm, and the solution volume was 2.7 mL. An external electric field of 400 V/cm was applied at 1 MHz, and crystals were grown over the course of 9 days, both with and without the application of this external electric field.

XRD rocking-curve measurements were conducted at room temperature on beamline BL15B1 at the Photon Factory (PF) of the High Energy Accelerator Research

Organization (KEK) in Japan. Details of the experimental procedures are described in ref [4]. During these measurements, the reflected images of entire crystals for the 110 family of reflections were detected using a high spatial resolution, twodimensional, digital CCD camera (effective pixels 6.45×6.45 μm). XRD rocking curves for the 110 family of reflections were reconstructed from the reflected intensities over a region corresponding to a beam spot size of 896.55 μm (139 pixels). Based on the above procedure, the instrumental resolution function (IRF') [11], which takes into account the dimensions of the sample and the horizontal beam divergence (0.178 mrad), can be calculated to be 1.91×10^{-3} degrees. All the XRD rocking curves were observed to contain only single peaks. The FWHM of each rocking-curve profile measured for samples prepared with and without the external electric field was evaluated using a Gaussian function.

3 Results and Discussion

In this analysis, the experimentally determined FWHM for the protein crystals was separated into the effects of local strain, $<\varepsilon>$, and misorientation between subgrains, $|\theta-\varphi|$. Therefore, the sum of the broadening contributions for the protein crystals are expressed as follows:

$$\begin{aligned}\beta_{adj}^2 &\approx \beta_\alpha^2 + \beta_\varepsilon^2 \\ &= K_\alpha + K_\varepsilon \tan^2\theta, \qquad (1)\\ \beta_\alpha^2 &= 2\pi \ln|\theta-\varphi|^2 = K_\alpha, \\ \beta_\varepsilon^2 &= 8\ln 2 <\varepsilon>^2 = K_\varepsilon \tan^2\theta,\end{aligned}$$

where β_{adj} is the FWHM adjusted to account for the intrinsic FWHM, which is defined as $\beta^2_{adj} = \beta^2_M + \beta^2_0 + \beta^2_{ins}$ (β_M is the measured FWHM, β_0 is the intrinsic FWHM of the rocking curve for a perfect crystal and β_{ins} is the broadening contribution due to IRF'), β_α represent the line broadening due to lattice tilting and β_ε represent the line broadening due to local strain. Figure 1 shows the relationship between β^2_{adj} and $\tan^2\theta$ for the tetragonal HEW lysozyme crystals prepared with and without an external electric field. From a straight line fit to the data in Fig. 1, $|\theta-\varphi|$ and $<\varepsilon>$ were estimated to be 0.0031° and 157 με for tetragonal HEW lysozyme crystals prepared without an external electric field, respectively. From these data, the individual broadening contributions of β_α and β_ε can be calculated to be 0.0065° and 0.0026°, suggesting that imperfections in the protein crystals are primarily the result of misorientation between subgrains [12].

In contrast, from a straight line fit to the data in Fig. 1, $|\theta-\varphi|$ and $<\varepsilon>$ were estimated to be 0.0019° and 107 με for tetragonal HEW lysozyme crystals prepared with an external electric field, respectively. These results suggest that misorientation between subgrains and the local strain are both reduced as the result of applying an external electric field at 1 MHz. However, the predominant broadening contribution in the case of the crystals prepared with an external electric field at 1 MHz was not the local strain, β_ε (= 0.0019° for the 12 12 0 reflection) but rather the misorientation between subgrains, β_α (= 0.0040°). That is, the improvement of the crystal quality under a 1 MHz applied field is achieved by a decrease in the misorientation between subgrains in the crystal [12]. This crystallization technique can be expected to enhance the resolution of protein molecule structure analysis by X-ray diffraction.

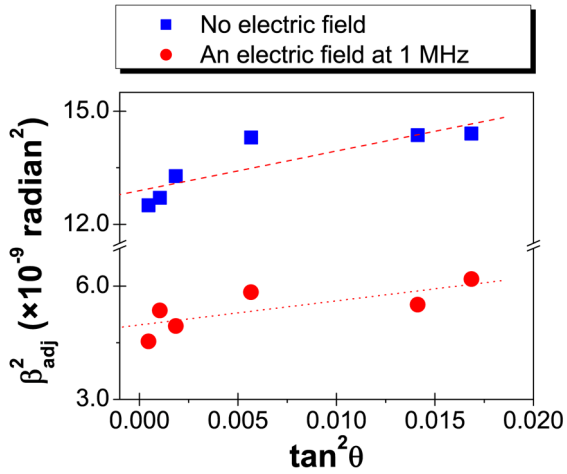

Fig. 1: Relationship between β^2_{adj} and $\tan^2\theta$ for tetragonal HEW lysozyme crystals prepared with and without an external electric field.

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* h_koizumi@imr.tohoku.ac.jp