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Crystallization Technique of High-Quality Protein Crystals Controlling Surface Free Energy

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The relationship between protein crystal quality and growth kinetics was assessed by measuring the normal growth rates vs supersaturation of the (110) face of tetragonal hen egg white lysozyme crystals at three precipitant concentrations, with NaCl as the precipitant. Assuming a two-dimensional birth and spreading nucleation mechanism, an increase in the surface free energy of the step edges was realized with increasing NaCl concentration, as established by decreases in the full width at half-maximum values of X-ray diffraction rocking curves obtained from crystals. These results demonstrate that controlling the surface free energy of the step edges is an important aspect of obtaining high-quality protein crystals.

1 Introduction

The three-dimensional (3D) structures of protein molecules are closely related to their functions in living tissue, so determining these structures is an important aspect of drug design and controlled drug delivery [1]. Since protein structures can be elucidated using high-brilliance synchrotron radiation, many researchers have attempted to develop high-brilliance sources and/or to improve the sensitivity of detectors [2]. The goal in such cases is to obtain accurate 3D structures of protein molecules with a resolution of less than 1.5 Å, equivalent to the length of a covalent carbon-carbon bond. However, this level of resolution has only been achieved for 9% of all protein molecules registered with the Protein Data Bank (PDB; http://www.rcsb.org/pdb/), even with the use of high brilliance synchrotron radiation facilities, such as SPring-8. This lack of high resolution structures is a direct result of the difficulty associated with growing high-quality protein crystals which have high diffraction efficiency (diffractivity). Therefore, in order to obtain high-quality specimens for analysis, it is important to understand the relationship between the growth kinetics of protein crystals and the resulting crystal quality.

Previously, we have succeeded in the improvement in the crystal quality of protein crystals by applying an external electric field at 1 MHz [3-5]. It has been revealed that this is attributed to a decrease in the entropy of the solid under application of an external electric field at 1 MHz, resulting in the increase in the surface free energy of the step edges [6].

Generally, salt precipitants such as NaCl and $(NH_4)_2SO_4$ are added when protein crystals are grown using the batch and hanging drop techniques. The addition of these salts to protein solutions adjusts the anisotropic Coulomb interactions in a way that favors crystallization. That is, certain intermolecular orientations can be attractive by adding the salts, even if the repulsion (long-range electrostatic interactions) dominates the azimuthally average potential [7-9]. It has also been reported that the long-range electrostatic interactions in protein crystals in a low ionic environment are significant, based on analyses of macrobonding and electrostatic energy transfer [10]. These earlier reports suggest that the intermolecular interactions in protein crystals can be modified by varying the concentrations of the precipitant salt. That is, the enthalpy of the solid can also be controlled by changing the concentrations of the precipitant salt, leading to the change in the surface free energy of the step edges. Such changes in the surface free energy of the step edges would, in turn, be expected to change the crystal quality, similar to improvements seen in previous research involving the application of an external electric field [6]. Herein, we report that the surface free energy of the step edges increases with increasing concentrations of the precipitant salts, based on assessing the normal growth rates R of tetragonal hen egg white (HEW) lysozyme crystals. Moreover, we demonstrate that tuning the surface free energy in this manner improves the protein crystal quality, as verified by X-ray diffration (XRD) rocking-curve measurements.

2 Experiment

The HEW lysozyme employed in this study was purchased from Wako Pure Chemical Industries, Ltd. Because commercial HEW lysozyme typically contains a high concentration of NaCl, dialysis was employed to

remove NaCl from the HEW lysozyme solutions prior to experimental trials. Tetragonal HEW lysozyme crystals grown from seed crystals were used in this work. The growth solutions contained 50 mg/mL HEW lysozyme together with 0.34, 0.50, or 0.68 M NaCl in a 100 mM sodium acetate buffer at pH 4.5. The supersaturation value of each HEW lysozyme solution σ (=ln(C/C_{eq}), where C is the concentration of the solution and C_{eq} is the solubility), was changed by varying the temperature.

XRD rocking-curve measurements were conducted at room temperature using the BL20B beamline at the Photon Factory, part of the KEK, Japan. The monochromatic synchrotron beam with 1.2 Å was almost parallel to the $[\bar{1}10]$ crystallographic direction of the crystal in the growth cell. Rocking-curve profiles for the 12 12 0 reflection were acquired using a high-spatial-resolution, two-dimensional (2D), digital CCD camera (Photonic Science X-RAY FDI 1.00:1, effective pixel size 6.45×6.45 μm^2). The beam spot size at the region being analyzed was 258 μm (40 pixels). The associated full width at half-maximums (FWHMs) were evaluated using a Gaussian function.

3 Results and Discussion

Fig. 1 shows typical data summarizing the dependence of the normal growth rates R on $1/(\sigma T^2)$ at all three NaCl concentrations. Here, the normal growth rates R are related to the surface free energy of the step edges as follows:

$$\ln\left(\frac{R}{\sigma^{1/6}\left(1-e^{-\sigma}\right)^{2/3}}\right) = A - \frac{\pi\Omega\alpha^2 h}{6k_B^2}\frac{1}{\sigma T^2}, \qquad (1)$$

where α is the surface free energy of the step edge, h is the step height, Ω is the crystal volume per molecule, k_B is the Boltzmann constant, T is the absolute temperature, respectively. The surface free energy of the step edges α for the (110) face can be estimated using Eq. (1), assuming that the step height of the (110) face is 5.6 nm [11]. On the basis of the straight line fit to the data in Fig. 1, the α values for NaCl concentrations of 0.34, 0.50, and 0.68 M were estimated to be 0.92, 1.25, and 1.74 mJ/m^2, respectively, for the (110) face. These data show that the

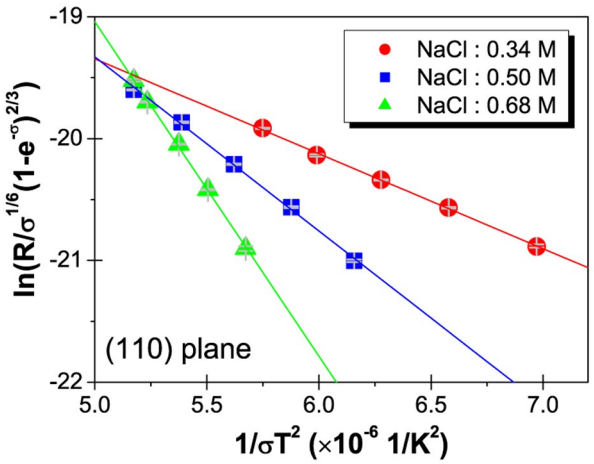

Fig. 1 Typical plots of the growth rates R of the (110) face at each NaCl concentration as functions of $1/(\sigma T^2)$.

Table 1 Comparison of the FWHM Values for the 12 12 0 Reflections Obtained from Tetragonal HEW Lysozyme Crystals Grown Using the Same Degree of Supersaturation†

NaCl concentration	supersaturation (temperature)	R (μm/s)	FWHM (SD)
0.34 M (2.0 w/v%)	2.04 (10 ˚C)	5.37±0.03	0.0031˚ (0.0003˚)
0.50 M (3.0 w/v%)	1.95 (21 ˚C)	4.28±0.03	0.0022˚ (0.0003˚)

†The standard deviations (SD) are also provided.

α values for both faces clearly increased as the NaCl concentration was raised. These results confirm that the intermolecular interactions in protein crystals can be modified by changing a precipitant salt concentration.

Next, we assessed the effect of the surface free energy of the step edge on the quality of the tetragonal HEW lysozyme crystals by comparing the quality of crystals grown with the same normal growth rate. Table 1 summarizes the growth parameters at two NaCl concentrations, from which it is evident that the supersaturation and normal growth rates were almost equivalent in each trial, while the FWHM values for the 12 12 0 reflection were completely different. The FWHM was clearly reduced at the higher NaCl concentration, corresponding to an increased surface free energy of the step edge. Thus, it is concluded that high-quality protein crystal can be grown with increasing the NaCl concentration. That is, these results confirm that the protein crystal quality can be greatly affected by changes in the surface free energy of the step edge and that this factor therefore plays an important role in the growth of high-quality protein crystals.

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