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Improvement of protein crystal quality by forced flow solution

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Introduction

The influence of solution flow on protein crystal growth has been investigated. Convection affects growth kinetics, enhances impurity incorporation, morphological instability, inclusion formation, and nucleation [1]. Hence, it has been widely recognized that high-quality crystals can be hardly obtained when solution flow exists.

However, Adachi *et al*. demonstrated a new method that decelerates the nucleation rate by stirring the solution [2]. The gentle stirring accelerated the growth of protein crystals, prevented additional nucleation, and allowed large protein crystals to be obtained. This result suggested that carefully controlled flow may improve the crystal quality. However, the quality of crystals in terms of X-ray diffraction was not discussed. To reveal whether solution flow benefits the protein crystal quality or not is very important for protein structure determination. Therefore, the current study was carried out to examine the effect of solution flow on the protein crystal quality. We intended to estimate the crystal quality with respect to several properties measurable by X-ray synchrotron diffraction.

Experiment and Results

Materials and Methods

Hen egg-white lysozyme (HEWL) used as the model protein in this study was purchased from Seikagaku Kogyo Corporation and was further purified to 99.99% purity. Solutions were prepared in 50 mM NaOAc buffer, pH 4.5 and mixed with appropriate volumes of NaCl to obtain the desired final lysozyme (15 mg/ml) and NaCl (25 mg/ml) concentrations.

The crystallization solution was circulated through a growth chamber by a peristaltic pump. The chamber was placed in a temperature controlled incubator at 10 °C. The circulation loop consisted of silicon tubes and was kept at 24 °C to avoid additional nucleation. The solution was undersaturated at this temperature. The average flow velocities (0 to 1000 μm/s) were controlled by volumetric flow velocities from the 4 mm^2 cross-sectional area of the growth chamber.

The crystal size was carefully controlled to be the same to compare the crystal quality without compensation. We collected all data at room temperature at the BL-6A of the Photon Factory (PF), Tsukuba, Japan. Nearly complete diffraction data sets were collected using an ADSC Quantum 4R CCD detector by the oscillation method with a wavelength of 0.978 Å. Eight samples were analyzed in total. The X-ray diffraction data were auto-indexed and integrated using the program *DPS/MOSFLM/CCP4* [3] and then merged and scaled with *SCALA/CCP4* [4].

Results

The crystal quality of flow-crystals quiescent-crystals were evaluated by the X-ray data statistics. Over the entire resolution range, the $\langle I \rangle / \langle \sigma(I) \rangle$ values of the flow-crystals were higher than those of quiescent-crystals although the crystals were of identical size. Also, a clear improvement of the maximum resolution limit and R_{merge} was found in flow-crystals.

A possible improvement mechanism is as follows. (1) Step bunching occurred by solution flow. (2) The growth rate decelerated owing to the increase of steps. (3) Slow growth yielded better quality. Step bunching is actually observed in flow conditions [5,6,7]. Details are reported elsewhere [8].

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