BL-20B/2018G547

Improvement of Hen Egg White Lysozyme Crystal Quality by Control of Dehydration Process

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Equal-thickness fringes that are attributed to the Pendellösung effect were clearly observed in the region of a tapered tetragonal hen egg white (HEW) lysozyme crystal with a wedgelike edge, which indicates that the misorientation between subgrains was on the order of 10^{-4}°. This is attributed to control of the dehydration process for crystal growth from solution, which plays an important role. Both the local quality and whole homogeneity of the crystals were improved by regulating the dehydration process. Oscillatory contrast was observed in X-ray topographs from regions of the same thickness in the crystals. However, the area of such a contrast was quite small, which indicates the presence of defects, even in crystals for which equal-thickness fringes could be observed. We also discuss why the area of oscillatory contrast was small for tetragonal HEW lysozyme crystals with misorientations between subgrains on the order of 10^{-4}°.

1 Introduction

The dehydration process plays a dominant role during crystal growth from solution when atoms or molecules are incorporated into the steps on the crystal surface [1]. The dehydration process determines the crystal growth rate [1, 2], which indicates the importance of the hydration structure for crystal growth from solution. However, the relationship between the dehydration process and the mechanism of crystal growth is not yet fully understood, because it is difficult to directly observe the hydration structure.

We have recently revealed that the crystal growth rate is increased and the crystal quality is improved when the concentration of an added salt, such as NaCl, is higher in protein solutions [3], which is attributed to the faster dynamics of water around protein molecules [4, 5]. This indicates that the dehydration process could have an effect not only on the crystal growth rate but also on the crystal quality. More recently, we have also clarified that perfect crystals can be grown even for protein crystals, by using glucose isomerase crystals [6, 7]. However, it has not been understood why the grown glucose isomerase crystals become perfect, although research concerning the quality of macromolecular crystals grown from solution has been actively performed using proteins [8]. In our previous works [6, 7], glucose isomerase crystals were grown under the faster dynamics of water around protein molecules. Therefore, it is quite important to elucidate whether the faster dynamics of water is closely related to the growth of crystals with perfect quality.

In this report, we demonstrate that equal-thickness fringes, which are attributed to the Pendellösung effect, can be observed in the region of a tapered tetragonal hen egg white (HEW) lysozyme crystal with a wedgelike edge when the dynamics of water around the protein molecules are controlled by the concentration of an added salt. This suggests that the misorientation between subgrains in the crystal is quite small: i.e., the crystal quality is almost perfect. The results also indicate that the whole homogeneity of the grown crystals is improved by regulating the dynamics of water around protein molecules. This could be a universal phenomenon for the crystal growth of macromolecules from solution, which is characterized by the dehydration process.

2 Experiment

The HEW lysozyme used in this work was purchased from Wako Pure Chemical Industries, Ltd.. Dialysis was employed to remove NaCl from the HEW lysozyme solutions prior to crystal growth. The growth of tetragonal HEW lysozyme crystals was conducted using a cross-linked seed crystal, which has been described in detail elsewhere [9]. Tetragonal HEW lysozyme crystals were also grown under NaCl concentrations of 0.34, 0.68, 0.86, and 1.20 mol/L. The crystals were grown using a growth cell (25 × 25 × 2 mm) with a growth time of 1 week.

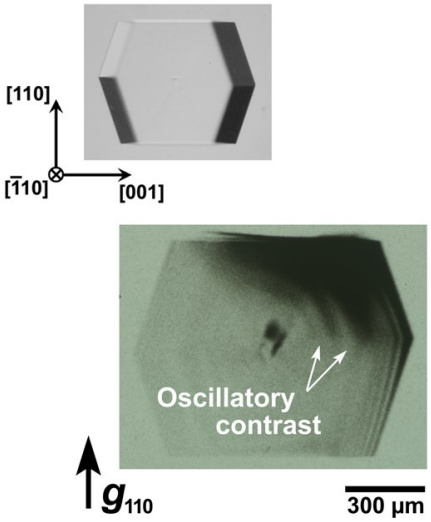

Figure 1: Synchrotron monochromatic-beam X-ray topograph of a HEW lysozyme crystal obtained using the 110 reflection for a crystal grown with a NaCl concentration of 1.20 mol/L.

Monochromatic-beam X-ray topography was conducted at room temperature using the BL8S2 beamline at the Aichi Synchrotron Radiation Center (AichiSR) of Japan. A two-crystal monochromator consisting of a Si(111) crystal was located 16 m from the source and was used to select an X-ray wavelength of λ = 1.0 Å. Topographs were obtained using the 110 reflection and were recorded on nuclear plates (Ilford) with an exposure time of approximately 3 min.

XRD rocking-curve measurements were performed at room temperature using the BL20B beamline at the Photon Factory, part of the High Energy Accelerator Research Organization (KEK) of Japan. The detailed setup for the XRD rocking-curve measurements is described elsewhere [9].

3 Results and Discussion

Figure 1 shows a synchrotron monochromatic-beam X-ray topograph of a HEW lysozyme crystal obtained using the 110 reflection. The crystal was grown with a NaCl concentration of 1.20 mol/L, by which the dynamics of water around protein molecules are considered to become much faster [4, 5]. The contrast in the center part in the topograph is attributed to strain in the seed crystal, although no contrast due to dislocations is observed. As shown in Figure 1, clear straight fringes are observed in the region of a tapered tetragonal HEW lysozyme crystal with a wedgelike edge. Thus, the observed fringes are not Moiré interference but equal-thickness fringes, which are attributed to the Pendellösung effect. This indicates that the quality of the grown crystal is almost perfect: i.e., the misorientation between subgrains is only about 10^{-4}°.

Table 1 shows the misorientation between subgrains in crystals grown with various concentrations of NaCl. As shown in Table 1, higher NaCl concentrations result in smaller misorientations. It was recently reported that an increase in the salt concentration of an aqueous solution results in faster dynamics of water around protein molecules [4, 5]. Thus, the quality of a crystal composed of macromolecules is closely related to the dynamics of water around the macromolecules: i.e., the dehydration process for crystal growth from solution.

Table 1 Misorientation between Subgrains for Tetragonal HEW Lysozyme Crystals Grown with Various Concentrations of NaCl

NaCl concentration mol/L (w/v %)	supersaturation σ	misorientation deg
0.34 M (2.0 w/v%)	2.34	0.00143
0.68 M (4.0 w/v%)	2.39	0.00103
0.86 M (5.0 w/v%)	2.35	0.00069
1.20 M (7.0 w/v%)	2.32	0.00041

We have recently observed clear oscillatory profiles in rocking curves for glucose isomerase crystals with subgrain misorientations of about 10^{-4}° [7]. However, rocking curves for tetragonal HEW lysozyme crystals with subgrain misorientations of the order of 10^{-4}° do not have oscillatory profiles. This suggests that the quality of the HEW lysozyme crystal is not perfect.

Let us discuss why the area of the oscillatory contrast is small for the tetragonal HEW lysozyme crystal grown with 1.20 mol/L NaCl. Strain is clearly observed in the crystal using the 001 family of reflections, although no strain is detected using the 110 family of reflections. This suggests that stress is applied in the direction of the c axis. Therefore, the indistinct oscillatory contrast could be mainly caused by stress in the c axis direction. However, the origin of the strain is not yet fully understood. There are water-filled channels along the [001] direction in tetragonal HEW lysozyme crystals. The diffusion coefficient of this water is less than that for bulk water molecules, which suggests that the water molecules form a structure in the channels. It has also been reported that the mobility of water molecules decreases when the NaCl concentration is higher [10]. Therefore, the stress along the c axis direction for tetragonal HEW lysozyme crystals grown with 1.20 mol/L NaCl could be attributed to the structure of the hydrogen bond network of water molecules in the channels.

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