BL-20B/ 2021G037

Control of strain in subgrains of protein crystals by the introduction of grown-in dislocations

Haruhiko KOIZUMI[1,*], Satoshi UDA[2], Ryo SUZUKI[3] and Masaru TACHIBANA[3]
[1] Graduate School of Integrated Sciences for Life, Hiroshima University,
1-4-4 Kagamiyama, Higashi-Hiroshima, 739-8528, Japan
[2] New Industry Creation Hatchery Center, Tohoku University,
6-6-10 Aramaki Aza Aoba, Aoba-ku, Sendai, 980-8579, Japan
[3] Graduate School of Nanobioscience, Yokohama City University,
22-2 Seto, Kanazawa-ku, Yokohama 236-0027, Japan

It is important to reveal the exact cause of poor diffractivity in protein crystals in order to determine the accurate structure of protein molecules. It is shown that there is a large amount of local strain in subgrains of glucose isomerase crystals even though the overall crystal quality is rather high, as shown by clear equal-thickness fringes in X-ray topography. Thus, a large stress is exerted on the subgrains of protein crystals, which could significantly lower the resistance of the crystals to radiation damage. It is also demonstrated that this local strain can be reduced through the introduction of dislocations in the crystal. This suggests that the introduction of dislocations in protein crystals can be effective in enhancing the crystal quality of subgrains of protein crystals. By exploiting this effect, the radiation damage in subgrains could be decreased, leading to the collection of X-ray diffraction data sets with high diffractivity.

1 Introduction

The three-dimensional (3D) structures of protein molecules are intimately related to the proteins found in living organisms. Therefore, it is important to determine the 3D structures of protein molecules from the points of view of both fundamentals and applications in medical science. The determination of the 3D structures of protein molecules has typically been carried out at synchrotron-radiation facilities with high brilliance. A structure determined using data at a resolution of better than 1.5 Å, corresponding to the length of a covalent carbon–carbon bond, is necessary to achieve structure-guided drug design and controlled drug delivery. This means that the collection of many high-order reflections from protein crystals, i.e. high diffraction efficiency (diffractivity), is desirable to obtain accurate 3D structures of protein molecules. As such, many researchers have concentrated on developing high-brilliance sources and/or improving the sensitivity of the detector systems [1]. However, protein molecules useful in structural analysis (<1.5 Å resolution) for structure-guided drug design and controlled drug delivery represent only 9% of all protein molecules deposited in the Protein Data Bank, even when using synchrotron-radiation facilities with high brilliance such as SPring-8. This suggests the importance of the growth of high-quality protein crystals that allow the collection of many high-order reflections. In fact, it has been reported that the improvement of crystal quality leads to an increase in the integrated intensities of diffraction spots [2]. Therefore, it is important to enhance the crystal quality of protein crystals.

In this report, we observed a large amount of local strain in the subgrains of the present glucose isomerase crystals, even though the overall quality of the crystal as a whole is near-perfect as verified by microscopic XRD rocking-curve measurements. We believe that this points to a inherent crystal imperfection in protein crystals that prevents the achievement of high diffractivity, which is associated with radiation damage due to synchrotron radiation. Moreover, we demonstrate control over the local strain in subgrains of glucose isomerase crystals by introducing dislocations.

2 Experiment

Glucose isomerase was purchased from Hampton Research and was used without further purification. The crystallization conditions were the same as those used previously [3, 4]. Glucose isomerase crystals were grown from seed crystals that were either cross-linked using a glutaraldehyde solution or left non-cross-linked. The crystals grown under these conditions were approximately 2.5 mm in size. The crystals were orthorhombic, with space group *I*222 and unit-cell parameters a = 93.88, b = 99.64, c = 102.90 Å, and contained two molecules per unit cell [5].

Microscopic XRD rocking-curve measurements were performed at room temperature using synchrotron radiation on the BL20B beamline at the Photon Factory, High Energy Accelerator Research Organization (KEK), Japan. A monochromatic beam with a wavelength of 1.2 Å was selected. Instrument parameter details are provided in a previous report [4]. The glucose isomerase crystals were sealed in an acrylic cell as reported previously [3, 4]. A high spatial resolution, two-dimensional digital CCD camera (Photonic Science X-RAY FDI 1.00:1, 6.45 × 6.45 μm effective pixels) was employed to measure the microscopic XRD rocking curves for the 011 family of reflections obtained from glucose isomerase crystals. XRD rocking curves were reconstructed from reflected intensities outside the crystals using various beam-spot sizes ranging from 64.5 μm (ten pixels) to 1096.5 μm (170 pixels).

3 Results and Discussion

Fig. 1 shows a digital X-ray topograph of a glucose isomerase crystal taken using the 011 reflection. As seen in Fig. 1, no contrast attributable to the seed crystal or dislocations was observed because the seed crystal was not cross-linked using glutaraldehyde solution. XRD rocking curves for each beam-spot size were reconstructed from the region within the white circle. Fig. 2 shows the estimated dependence of local strain and misorientation on beam-spot size in dislocation-free glucose isomerase crystals. The filled and open symbols represent the data for type A_1 and type A_2 crystals, respectively. As shown in Fig. 2, the local strain increases with decreasing beam-spot size for beam-spot sizes below 451.5 μm (70 pixels), leveling off at around 28.2 με. This means that a large amount of local strain is accumulated in subgrains, because the beam-spot size (64.5 μm) is smaller than the previously estimated subgrain size of tetragonal henn-egg-white (HEW) lysozyme crystals (200–600 μm) [6].

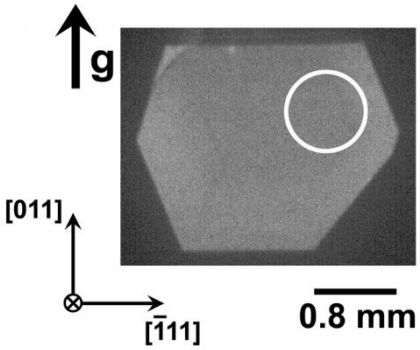

Fig. 1: Digital X-ray topograph of a glucose isomerase crystal taken using the 011 reflection. The white circle is the region from which the XRD rocking-curves for each beam-spot size were reconstructed.

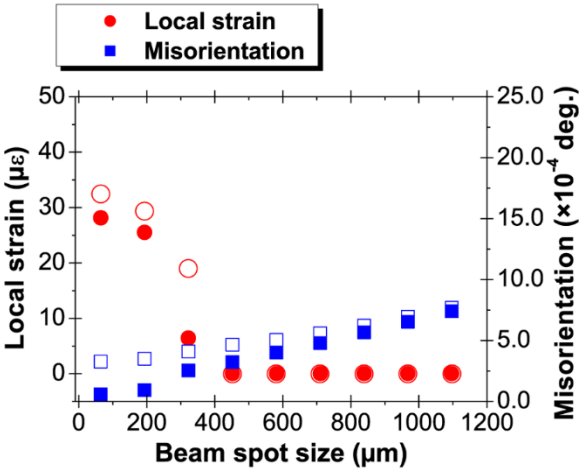

Fig. 2: The dependence of local strain and misorientation on beam-spot size estimated using a glucose isomerase crystal grown from a non-cross-linked seed crystal. The filled and open symbols represent data obtained from type A_1 and A_2 crystals, respectively.

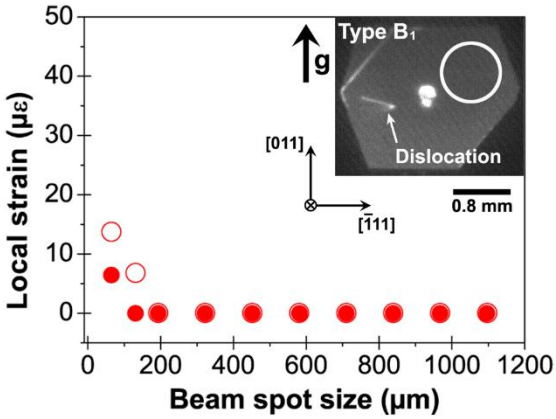

Fig. 3: Dependence of the local strain on beam-spot size estimated using a glucose isomerase crystal with a few dislocations. The filled and open symbols represent data obtained from type B_1 and type B_2 crystals, respectively. The analyzed region enclosed by the white circle does not contain any dislocations.

Next, we demonstrate that the local strain in subgrains can potentially be controlled by introducing grown-in dislocations in the crystal. However, no dislocations were included in the analyzed region in the present case. Fig. 3 shows the dependence of local strain on beam-spot size for glucose isomerase crystals containing a few dislocations. The filled and open symbols represent data obtained from type B_1 and type B_2 crystals, respectively. As clearly seen in the digital X-ray topograph in Fig. 3, dislocations were generated in the crystal. In the case of glucose isomerase crystals with a few dislocations (types B_1 and B_2), it is observed that the local strain in the subgrains decreases as a whole relative to that for dislocation-free crystals (type A_1 and type A_2), and the beam-spot size at which the local strain in the subgrains becomes detectable also decreases. This means that dislocations have a significant effect on the local strain in subgrains, even if the analyzed region, which is free of dislocations, is far removed from the grown-in dislocations. Thus, the influence of the strain field of each dislocation could extend over a distance of the order of a millimetre.

References

[1] N.E. Chayen *et al.*, *Macromolecular Crystallization and Crystal Perfection.* (2010).
[2] E.H. Snell *et al.*, *Acta Cryst.* **D51**, 1099 (1995).
[3] H. Koizumi *et al.*, *Cryst. Growth Des.* **14**, 5111 (2014).
[4] H. Koizumi *et al.*, *Cryst. Growth Des.* **16**, 4905 (2016).
[5] H. Carrell *et al.*, *PNAS* **86**, 4440 (1989).
[6] H. Koizumi *et al.*, *Cryst. Growth Des.* **14**, 5662 (2014).

* h-koizumi@hiroshima-u.ac.jp