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Recipe for SR Laue topography of protein crystals

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The characterization of crystal defects in protein crystals is very important for their technical applications. Laue topography using synchrotron radiation (SR) is one of the most powerful methods to characterize crystal defects because a large number of topographs with different reflections can be obtained by one short exposure. However, protein crystals that include a large amount of water are very fragile. In addition, they suffer radiation damages even in the short exposure. These mean that it is difficult to take SR Laue topographs of protein crystals in the same way as other inorganic crystals. In this paper, we report the recipe for SR Laue topography of protein crystals such as hen egg-white (HEW) lysozyme crystals.

HEW lysozyme crystals were grown at 23°C by the solubility-gradient method [1]. Tetragonal HEW lysozyme crystals (a=b=7.91 nm, c=3.79 nm) of millimeter-size were obtained in the test tube after about two weeks.

The HEW protein crystals with the test tube of 1.0 mm thickness can provide SR Laue topographs with the intense X-ray background due to the test tube. Consequently, the image contrast of crystal defects on the topographs is much poor. Therefore, for the SR Laue topography, the crystal grown in the test tube must be transferred to the thin container that is transparent for white beam. The crystal was carefully skimmed from the solution with fine tip of a soft wood. As shown in Fig.1, the crystal was set inside a short straw of about 0.2 mm thickness, and both sides were sealed with parafilms. The use of the straw was effective for the reduction of X-ray background on the topographs. As the other way, the skimmed crystal was mounted on spider cobwebs crossing a metal ring and sealed with a piece of wetted filter paper. This way for holding the sample is also effective for fragile crystals [2,3].

The high power of the SR white beam gives rise to the radiation damage even in one short exposure. Most of radiation damages are due to the heating of the crystals by irradiating long wavelengths. To avoid the heating, metal plates are usually used as a filter. However, the metal filters were not effective for protein crystals. We considered that the radiation damages for protein crystals are due to the long wavelengths absorbed by the water in the crystals. To avoid the irradiation of the long wavelengths, the water was used as a filter. As a result, the radiation damage was drastically reduced [2,3]. The water filter is very effective for the SR Laue topography of protein crystals. Such recipes should accelerate the study on crystal defects in protein crystals.

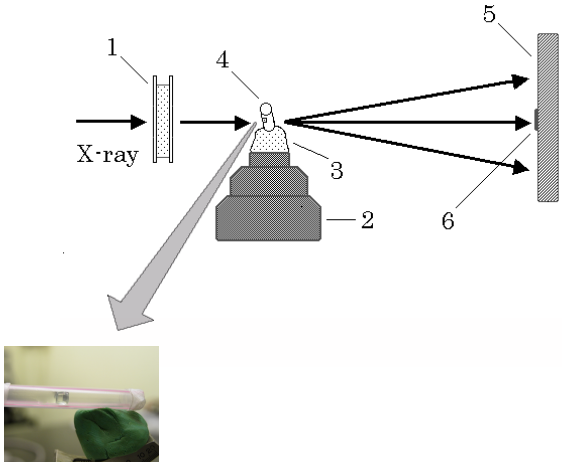

Fig.1 A schematic figure of setting sample for Laue topography using synchrotron radiation.
1.Watre filter 2.Goniometer head 3.Clay 4.Sample 5.File 6.Beam stopper

References

[1] M. Tachibana *et al*., *J. Crystal Growth* **198-199**, 661 (1999).
[2] K. Izumi *et al*., *J. Crystal Growth*, **206**, 155 (1999).
[3] M. Tachibana and K. Kojima, *Current Topics in Crystal Growth Research* (in press).

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