

## Observation of lysozyme crystals by X-ray topography and diffraction-enhanced X-ray imaging

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### Introduction

Crystal defects, especially dislocations, in hen egg-white lysozyme crystals that have multiple polymorphism, i.e. tetragonal, orthorhombic, monoclinic forms, and so on, were investigated by means of synchrotron monochromatic-beam X-ray topography. The observed topographic images of dislocations were much clear and beautiful, compared with those for any protein crystals have been reported so far. It was demonstrated that millimetre-size crystals larger than extinction lengths for X-ray topographic reflections are required to obtain clear images, i.e. direct images, for protein crystals. In addition, to characterize defects in protein crystals, diffraction-enhanced X-ray imaging[1] was carried out.

### Experiment

Orthorhombic HEW lysozyme crystals were grown by a liquid-liquid interfacial precipitation method. A HEW lysozyme solution containing 53 mg/ml HEW lysozyme and the 3.5 % NaCl at pH 4.7 was prepared. The bottle containing the interface of the HEW lysozyme solution and the Fluorinert liquid was placed in a thermostatic bath and maintained at 40 °C. After approximately two weeks, large crystals up to a size of 20 mm were grown at the interface. The crystals were orthorhombic with space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>, lattice constants of a=56.4 Å, b=73.7 Å, c=30.4 Å, and four molecules per unit cell.

X-ray topography was carried out with synchrotron radiation in BL15B1 and BL14B at the PF. The monochromatic-beam of 1.2 Å was selected by adjusting the monochromator. In addition, diffraction-enhanced X-ray imaging for protein crystals was carried out in BL14B.

The synchrotron radiation was strongly scattered in the test tube or glass bottle in which HEW lysozyme crystals were grown. The crystal in the test tube or glass bottle was gently transferred into a thin container, e.g. a short straw, which is transparent for the synchrotron radiation. To avoid the evaporation of water contained in the crystal, it was surrounded in the growth solution and both sides of the straw were sealed with parafilms. The sealed straw was mounted on the goniometer. A habit crystallographic face of the crystal was adjusted to be almost normal to the incident beam.

For X-ray topography, X-ray flat panel sensor was employed to find interest reflections. This sensor with high sensitivity in the low energy range is very useful for monochromatic-beam X-ray topography for protein

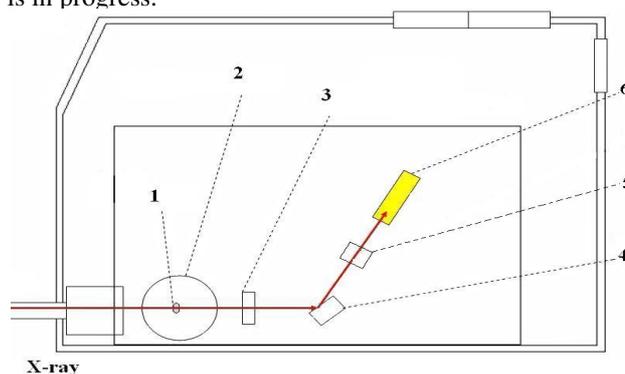
crystals. X-ray films or nuclear plates was set after finding the reflections.

For diffraction-enhanced X-ray imaging method, a rocking curve is measured with a scintillation counter. The images of the high-angle and low-angle sides of the rocking curve observed with CCD camera.

### Results and Discussion

For X-ray topography, weak beam technique was found to be useful for obtaining more clear images. Not only straight dislocations but also other types (curve and loop ones) of dislocations were clearly resolved on the topographs. It was found that various types of dislocations have been observed in common inorganic crystals and organic crystals of small molecules can be also introduced even into protein crystals. The shape and configuration of dislocations strongly depended on the crystal form in the polymorphism. These results suggest that the growth mechanisms in the crystal forms are different from each other. From such clear topographic images, it is concluded that X-ray topography is real available for the characterization of dislocations for protein crystals.

For diffraction-enhanced X-ray imaging, the images of the refraction contrasts was taken on CCD. The analysis is in progress.



X-ray

Fig.1 A schematic figure of setting sample for monochromatic X-ray topography and diffraction-enhanced X-ray imaging at BL14B.

1. Sample
2. Goniometer
3. Film or Flat panel sensor
4. Si crystal
5. Scintillation counter
6. CCD camera

### References

- [1]. D.C. Chapman, W. Thomlinson, R.E. Johnston, D. Washburn, E. Pisano, N. Gmur, Z. Zhang, R. Menk, F. Arfel, D. Sayers, Phys. Med. Biol. 42 (1997) 2015.

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