15B1/2007G150

Digital topographic system for characterizing imperfection in protein crystals

Yu Mukobayashi, Natsuko Kitajima, Yu Yamamoto, Naoki Ohya, Mayumi Koishi, Kenichi Kojima, Masaru Tachibana*
International Graduate School of Arts and Science, Yokohama City Univ., 22-2 Seto, Yokohama, Kanagawa 236-0027, Japan

Introduction

X-ray topography and rocking curve measurements have been widely used for the evaluation of protein crystal perfection. However, these measurements have been carried out separately [1]. For the comprehensive understanding of perfection in protein crystals, it is desired that X-ray topography and rocking curve can be simultaneously measured for the same crystal. In this report, we report the system which could simultaneously carry out X-ray topography and rocking curve measurement with a high resolution CCD camera for characterizing perfection in hen egg-white lysozyme (HEWL) crystals.

Experiment

Tetragonal HEW lysozyme crystals were grown by a liquid-liquid interfacial precipitation method. A HEW lysozyme solution containing 30 mg/ml HEW lysozyme and the 6.7 % $NiCl_2$ at pH 4.3 was prepared. The bottle containing the interface of the HEW lysozyme solution and the Fluorinert liquid was kept at 23 °C in a clean room. After approximately one month, large crystals up to a size of 5 mm were grown at the interface. The crystals were tetragonal with space group $P4_32_12$, lattice constants of a=79.1 Å, b=79.1 Å, c=37.9 Å, and eight molecules per unit cell.

X-ray topography and rocking curve measurement was carried out with synchrotron radiation in BL15B1 at the PF. The monochromatic-beam of 1.2 Å was selected by adjusting the monochromator.

The crystal in the 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 and rocking curve measurement, X-ray flat panel sensor was employed to find target reflections. This sensor with high sensitivity in the low energy range is very useful for monochromatic-beam X-ray topography for protein crystals. After finding the target reflections, X-ray films or nuclear plates were set and X-ray topographs were provided. In addition, CCD camera was mechanically positioned based on this information. Then we obtained X-ray diffraction intensity data from the whole crystal depending on the diffraction angle, i.e. rocking curve.

Results and Discussion

We obtained much clear images of dislocations by monochromatic X-ray topography. Not only straight dislocations but also other types (curve, loop and pair) of dislocations were clearly observed on the topographs. In addition, we identified the Burgers vectors of various types of dislocations in tetragonal HEW lysozyme crystals. For rocking curve measurement, we could evaluate the perfection at any local positions in the crystal. Such clear topographic images and rocking curve measurement will lead to a more detailed understanding of crystal growth and defects in protein crystals.

The analysis of the correlation between X-ray topography and rocking curve is in progress.

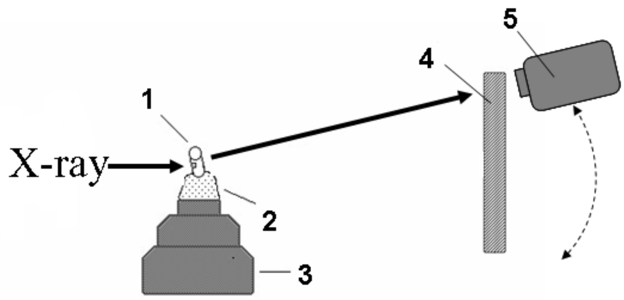

Fig.1 A schematic figure of experimental apparatus for monochromatic X-ray topography and rocking curve measurement at BL15B1.
1.Sample 2.Clay 3.Goniometer head
4.Film, Flat panel sensor 5.CCD camera

References

[1]. I.Yoshizaki et al., J.Cryst. Growth 290, 185 (2006)

*tachiban@yokohama-cu.ac.jp