Crystallography

10A/2008G667

X-ray diffuse scattering from protein crystals caused by the lattice defects.

Kenji KAWAMURA[1], Takeshi YOKOYAMA[2], Katsuhiro KUSAKA[2], Yuuki OHNISHI[2], Ichiro TANAKA*[2], Nobuo NIIMURA[2]
[1]Graduate School of Science and Engineering
[2]College of Engineering, Ibaraki Univ. Hitachi, 316-8511, Japan

Introduction

Protein crystals with high quality are essential for high resolution X-ray protein crystallography. The disorder of the molecular orientation in the crystal gives rise the decrease of the intensities of the higher order Bragg reflections. A scattering phenomenon caused by the disorder is observed as a diffuse scattering on the foot of the Bragg diffraction profile. The quantitative analysis of the diffuse scattering provides the information of the molecular orientational disorder in protein crystals which is the cause of the crystalline quality. Currently, it has been developed to estimate the quality of protein crystals by measuring over-all B-factors by Wilson plot.[1]

The B-factor consists of static and dynamic components. However, these components can not be distinguished precisely from only the B factors obtained. We would like to obtain the essential character in protein crystalline quality by observing and analyzing the diffuse scattering from protein crystals.[2]

Materials and Method

The measurements of the X-ray diffuse scattering from protein crystals, cubic insulin (a=80Å) and trypsin-BPTI complex (a=76Å, b=85Å and c=123 Å: orthorhombic) have been performed by a 4-circle diffractometer installed at BL10A in Photon Factory in KEK. The used X-ray wavelength was 1.54 Å. These crystal volumes were 0.5 x 0.5 x 0.25mm^3 and 0.5 x 0.5 x 0.5mm^3, respectively. Observed rocking curves were deconvoluted into three components, such as the main term of the Bragg reflection, the diffuse scattering and the background and they are fitted by Gaussian, Lorentzian and linear line, respectively.

The maximum resolutions and over-all B-factors of each crystal were measured by X-ray diffractomater in Ibaraki Univ(DIP2000).

Result and discussion

The profile fitting of the (080) Bragg reflection of insulin crystal is shown in Fig.1 (a) and the obtained parameters are summarized in Table 1. The rocking curves of (800), (080) and (008) reflections are shown in Fig.1 (b). These FWHMs are 0.006°, 0.0045° and 0.0085°, respectively. Since these rocking curves were asymmetry, they cannot be fitted by symmetrical functions. The quality of this crystal was separately observed, and it was found to be not a so good crystal because the maximum resolution was 2.0 Å and over all B factor was obtained as 21Å^2. We found that the quality of the crystal became worse after the measurement of the rocking curve as follows:

1) The integrated intensity of the (080) Bragg reflection decreases in 70%.
2) The resolution and the over all B-factor became worse from 1.9Å to 2.1Å and 25Å^2 to 28Å^2, respectively.

The X-ray radiation damage might be observed quantitatively. It is very interesting to measure the relation between the change of the crystal quality and the X-ray dose.

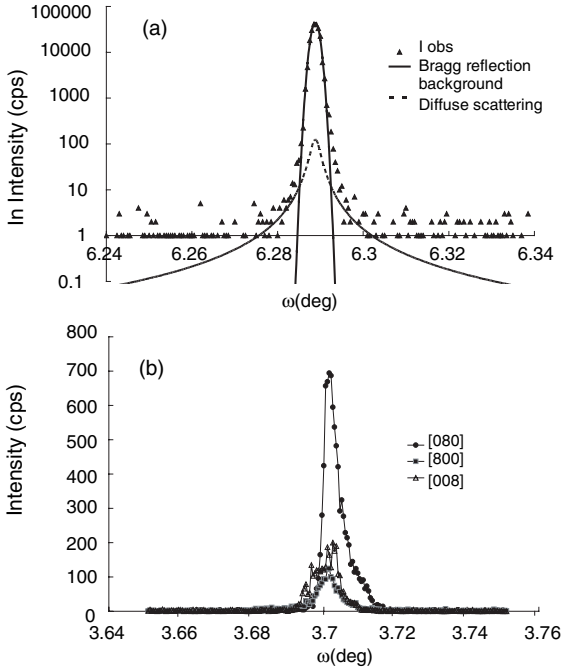

Fig1:(a) The result of profile fitting for rocking curve from insulin crystal. (b) The measurement of rocking curve for Trypsin-BPTI (orthorhombic) crystal.

Table 1: The obtained parameters by profile fitting.

	$I_{integrate}$	I_{peak} (cps)	FWHM(deg)
Bragg reflection	198679.7	43996.9	0.0021
Diffuses scattering	919.8	125.814	0.0024

References

[1]S.Arai, T.Chatake, N.Suzuki, H.Mizuno and N.Niimura: Acta Cryst. D60, 1032-1039 (2004)
[2] K.Kawamura *et al*: PF Activity Report, 2007 #25 PartB (2008) 176
* i.tanaka@mx.ibaraki.ac.jp