Biological Science

10A/2005G161

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

Takuya ISHIKAWA[1], Daichi YAGI[1], Toshikatsu ICHIGE[1], Yuusuke SAWA[1], Kenji KAWAMURA[1], Yuuki OHNISHI[2], Ichiro TANAKA[3], Izumi YOSHIZAKI[4], Nobuo NIIMURA[1]
[1]Graduate School of Science and Engeneering, and [3]College of Engeneering, Ibaraki Univ. Hitachi, 316-8511, Japan, [2]Kaken Co. Ltd., Mito, Japan and [4]JAXA, Tsukuba, Japan

Introduction

Recent high resolution (better than 1Å) X-ray protein crystallographic analysis requires high quality protein crystals. High quality crystals in turn produce a high-resolution diffraction data set. In protein crystallography the quality of the crystal is usually defined as follows: an often-used alternative criterion is whether the crystal produces a high resolution Bragg reflection data set or not. One possible explanation of the quality of protein crystals is whether the protein molecules are orientationally ordered within the unit cell. In that case, the overall B-factor obtained by the Wilson plot technique [1] becomes a good measure of the quality of the protein crystals. As a matter of the fact, we have already made such a proposal and we have proved that the overall B-factors obtained from Wilson plots correlate much better with the quality of the crystals experimentally [1]. In other words, the quality of the protein crystals is discussed by the degree of the disorder of the protein molecules in the unit cell.

Generally in X-ray diffraction phenomena it is well known that the disorder of molecules in unit cells provides diffuse scattering under a Bragg reflection profile. In the past such a diffuse scattering from a protein crystal has been often observed and this phenomenon has been discussed from the view point of molecular dynamics. We do not deny that one part of the diffuse scattering is the contribution of molecular dynamics, but we think that the main part is one of the disorder of molecules, incompleteness of crystals. The diffraction pattern on the imaging plate observed at 77K shows very distinctive diffuse scattering under Bragg reflections, which do not depend on temperature. We tried to measure the accurate profile of diffuse scattering in order to analyze the relation between the crystal quality and the molecular disorder.

Sample Preparation

Porcine insulin was purchased from Wako Pure Chemicals Industries, Ltd. Insulin readily dissolves in pure water with the addition of ammonium hydroxide. Crystals of cubic insulin were grown by a dialysis method at several crystallization condition such as at different protein and precipitant concentrations. (The precipitant is Na_2HPO_4.)

Experiment

We have carried out the preliminary measurement of the accurate profile of diffuse scattering in order to analyze the relation between the crystal quality and the molecular disorder.

We have used the 4 circle diffractometer at BL10A in PF of KEK. The wavelength is 1.54 Å Figure 1 shows the rocking curve profile of the Bragg reflection of cubic insulin. The FWHM is 6/1000 deg. A diffuse scattering is seen under the Bragg reflection from the logarithmic display of the profile as shown in Fig.2. We will continue the measurement of the diffuse scattering from several protein crystals as a function of crystallization condition and crystal quality determined by B factors in order to discuss the nature of the quality of protein crystals.

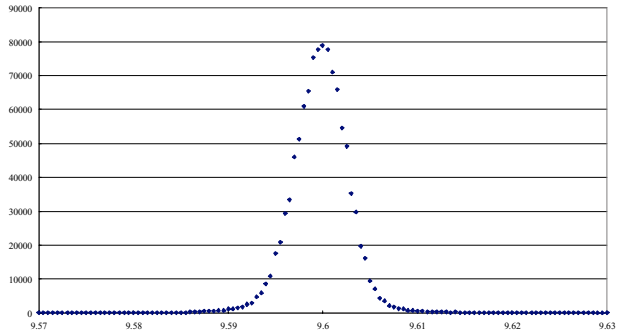

Fig.1 Rocking curve profile of the Bragg reflection.

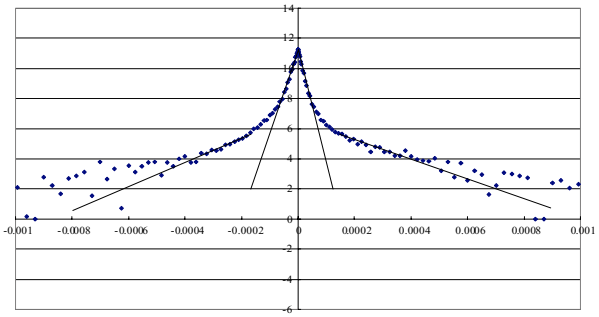

Fig.2 Ln I vs. Q^2 display of Fig.1.

Reference

[1] S.Arai, T.Chatake, N.Suzuki, H.Mizuno,N.Niimura, Acta Cryst. D60, 1032-1039 (2004)

niimura@mx.ibaraki.ac.jp