Time-Resolved X-ray Diffraction with Rotating Nanocrystal

Y. C. Sasaki(4114)*[1)4)], H. Noji(4288)[2)], S. Adachi(325)[3)], Y. Suzuki(1177)[4)], And N. Yagi(1129)[4)]

1) PRESTO, JST 2) CREST, JST 3) RIKEN 4) JASRI, Spring-8

1. Introduction

In order to understand how protein molecules operate in bio-systems such as cells, various x-ray techniques have been developed to give the averaged structural information of protein molecules. Recently, the behaviors of single bio-molecules have been directly visualized in real time under an optical microscope. This optical technology for imaging single molecules will be extended to observe the conformational changes of functional protein molecules in cells.

In this experiment, we try to observe the behaviors of single protein molecules by using a new x-ray technique called X-ray Diffraction with Rotating Nanocrystal (XDRN). XDRN monitors x-ray diffraction spots from a nanocrystal, which is labeled with the individual single protein in bio-systems such as membranes (Fig. 1.). Time-resolved XDRN can analyze the behavior of individual bio-molecules in real time and space. In time-resolved XDRN, we utilize both three-dimensional crystals (e.g., Au, Fe_2O_3, Al_2O_3) and multi-layer clusters (e.g., Si/Mo layers).

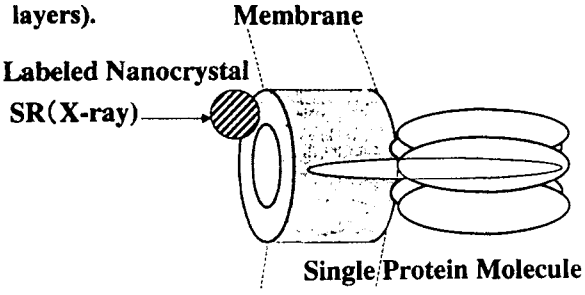

Fig. 1. Single molecular detection by X-ray diffraction with nanocrystal.

2. Experiments

We used a beamline BL44B2 (Structural Biology II, Spring-8) in the white X-ray mode to record x-ray diffraction spots from nanocrystals. Diffraction spots were monitored with an X-ray image intensifier (Hamamatsu Photonics, V5445P) and a CCD camera (Hamamatsu Photonics, C4880-82). The average exposure time was within 36 msec. The observed molecules were labeled with nanocrystals using chemical reaction or chelating.

3. Results and Discussion

3.1. Dynamics of affinity gel with multi-layer clusters

Figure 2 shows a Laue pattern made from multi-layer clusters (Si/Mo layers, average diameter = 50 nm). These diffraction spots were stationary at 237K. However, we observed the spots moving in all directions as the temperature of the gel solution increased.

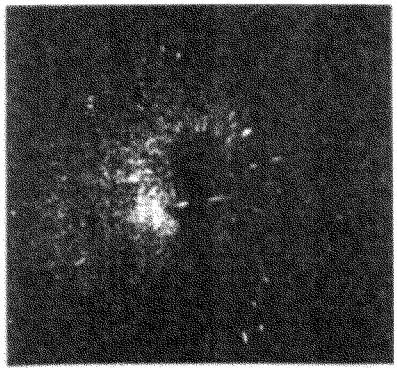

Fig. 2. Laue spots from multi-layer cluster (Si/Mo layer).

3.2. Rotation of portion of ATP synthase with colloidal gold

The diffraction spots (average diameter = 20 nm) were stationary when there were no ATP molecules. On the other hand, movements of the Laue spots with the labeled colloidal gold were detected in the presence of ATP molecules (200nM).