Time-resolved X-ray Diffraction with Rotating Nanocrystal

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1. Introduction

Most X-ray experiments are based on the averaged observations of many molecules, so the behavior of each molecule cannot be determined. Nevertheless, in the wavelength region of visible light, recent advances in single molecular detection using optical imaging and spectroscopy have made it possible to investigate individual molecular properties at ambient conditions [1-5]. This optical technology for imaging single molecules will be extended to observe the conformational changes of single functional protein molecule in cells. Here we demonstrated the direct observation of the rotating motion of an individual nanocrystal in the gel network and the protein molecules using time-resolved Laue Diffraction. One of the goals in this experiment is to observe the behaviors of single protein molecules by using a new X-ray technique, called Laue Diffraction with Rotating Nanocrystal (LDRN). LDRN monitors X-ray diffraction spots from the nanocrystal, which is labeled with the individual single protein molecules in bio-systems such as membranes and cells (Fig. 1.). We can determine the orientation of the labeled nanocrystal because of the high orientational sensitivity of X-ray diffraction. The orientation of the crystal can be determined to an accuracy of the order of 1 mradian.

In other words, we can detect the displacement of 10/1000 nm with the labeled nanocrystal by assuming the distance (=10 nm) from the axis of a rotor spinning. In this experiments, the rotation of the labeled nanocrystal is assigned to the movement of each local chain in the gel network or single protein molecules when the nanocrystal is tightly coupled to the observed individual chain. Thus, time-resolved LDRN can analyze the behavior of individual bio-molecules in real time and space.

2. Experiments

We used the white X-ray mode (Laue mode) of a beamline BL44B2 (RIKEN Structural Biology II, SPring-8, Japan) to record Laue diffraction spots from the labeled nanocrystals in thin glass capillaries. Photon flux at the sample position was estimated at 10^{15} photon/sec/mm^2 in the energy range of 7-30 kV. The X-ray beam focal size was 0.2 mm (horizontal) × 0.2 mm (vertical). A diffraction spot was monitored with the X-ray image intensifier (Hamamatsu Photonics, V5445P) and the CCD camera (Hamamatsu Photonics, C4880-82) with 656 × 494 pixels. The averaged exposure time was 36 msec. The detector's effective size was 160mm in diameter with 310mm sample-to-detector distance. We could detect the diffraction angle 2θ between zero and 0.25 radian. In time-resolved LDRN, we utilize both three-dimensional crystals (*e.g.*, Au, Fe_2O_3, Al_2O_3) and multi-layer clusters (*e.g.*, Si/Mo layers). The observed molecules were labeled with nanocrystals using chemical reaction or chelating [6-8].

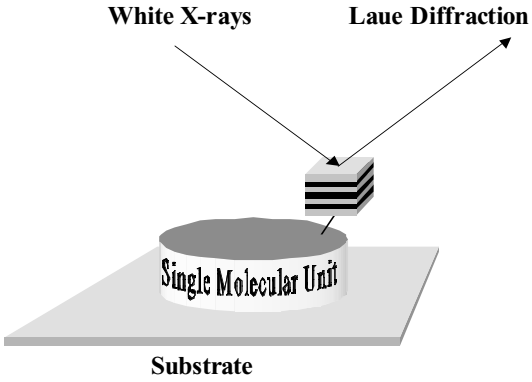

Fig. 1. Single molecular detection system by X-ray diffraction with nanocrystal.

3. Results and Discussion

3.1 Dynamics of Affinity Gel with Multi-layer Clusters

We use the cluster of a two-dimensional crystal, we call the multilayer (Silicon/Molybdenum) cluster, because many nanopaticles are not perfectly crystallized. Thus, we can detect the single diffracted spot from a single two-dimensional crystal with Laue method, and the number of the diffracted spots enables us to determine the number of the observed nanocrystals. Mulilayers used in X-ray optics are periodic structures made from alternating layers of light and heavy elements, *e.g.*, silicon and molybdenum. If we consider multilayers as artificial crystals, both their lattice constants and the length of the stacking period can be chosen to fit experimental needs. In this experiment, we chose both lattice constants and the stacking period (2d= 8 nm, 20 pairs) for the Si/Mo multilayer. The total thickness of the Si/Mo multilayer is 80 nm.

The nano-clusters for the artificial crystals in this study were fabricated by a sequential process using silicon substrate, polymer layer, silicon dioxide (SiO_2) bead, and microelectronic processing techniques including reactive ion etching. Silicon dioxide beads were used as a model protective coating array because the rate of the reactive ion etching with SiO_2 is much lower than that of the Si/Mo multilayer. Polymer film was used to separate the Si/Mo multilayer from the silicon substrate with ethanol. We confirmed that the diameters of the Si/Mo clusters (20-30 clusters/μm^2) on the Si substrate were approximately 80 nm by using the scanning electron microscope (SEM).

In this work, LDRN monitors the motion of individual nanocrystals to detect the dynamical movements of the polymer local chain in the beaded Agarose gel containing ultrapure water. To fix the Si/Mo clusters in the gel, we used the chemical coupling between the carboxyl groups (COOH) in the local chain of the gel and the amino groups (NH_2), which were placed on the surface of the Si/Mo clusters with silane coupling reagent. The Agarose gel, which was labeled with the Si/Mo clusters, was mounted in thin glass capillaries (typically 1.0-mm inner diameter) at room temperature (20 ^oC). We controlled the temperature of the sample capillary with a cryostat using liquid nitrogen and dry air. The temperature of the samples was fixed at 0 ^oC, -20 ^oC, and - 40 ^oC to decrease Brownian motion of beaded gel. Figure 2 shows a Laue pattern made from multi-layer clusters (Si/Mo layers, average diameter = 80 nm). These diffraction spots were stationary at - 40 ^oC. However, we observed the spots moving in all directions as the temperature of the gel solution increased.

3.2 Rotation of Portion of ATP Synthase with Colloidal Gold

The observed diffraction spots (average diameter of clusters = 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).

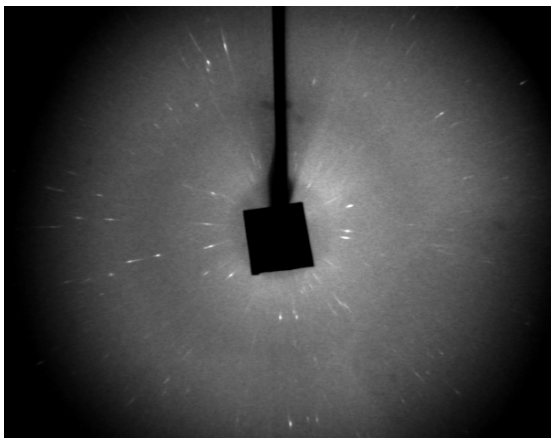

Fig. 2. Laue spots from multi-layer clusters (Si/Mo layer).

4. Summary

We have observed the rotating motion of an individual nanocrystal in the gel network and the ATP synthase using time-resolved Laue Diffraction for the first time. LDRN can be utilized for detecting single molecular units with X-rays when the nanocrystal is tightly coupled to the observed individual molecular units. In addition, LDRN is possible to monitor an unprecedented observation of single molecular units at the surface of the living cells as well as in inner or deeper cells because of the high transmittance of X-rays.

In this experiment, two-dimensional motion was monitored because we utilized Laue spots from the two-dimensional periodic crystals. We might use LDRN to obtain information about the three-dimensional motion of the single molecular units with the guidance of plural diffraction spots from the individual three-dimensional nanocrystal which is tightly coupled to the single molecular units.

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