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Structural Study of Human Prefoldin Involved in Protein Folding

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1 Introduction

Proper folding is a necessary process for proteins to express their functions. Prefoldin (PFD) is a molecular chaperone that helps protein folding in coupled with group II chaperonin [1]. Archaeal PFD is a hexamer consisting of two α subunits and four β subunits [2]. Crystal structure and biochemical analyses of archaeal PFD have suggested that hydrophobic patches on the coiled-coil tentacles are important for preventing aggregation of various target proteins [2-6]. On the other hand, eukaryotic PFD consists of six different subunits and is a specific chaperone of actin and tubulin [1]. To explore the unknown recognition mechanism of actin and tubulin by eukaryotic PFD, we approach with a crystallographic study of human PFD. Here we report the preliminary X-ray diffraction analysis of human PFD [7].

2 Experimental

Each subunit of human PFD was expressed in *E. coli* cells. The cells were mixed and lysed to form the heterohexamer of PFD. The PFD hexamer was purified by Co^{2+}-affinity, cation-exchange and gel-filtration chromatography. The crystals of human PFD were obtained by hanging-drop vapor-diffusion method using polyethylene glycol (PEG) 3350 as a main precipitant. K_2PtCl_4 derivative crystals were prepared by soaking in reservoir solutions containing 1 mM K_2PtCl_4 for 4 hours. Selenomethionine derivative crystals were prepared by expressing each subunit in minimal medium containing selenomethionine, and purifying and crystallizing as native human PFD. Prior to X-ray diffraction experiment, crystals were soaked in the reservoir solution containing 20% (*w*/*v*) PEG 4000 and flash-frozen in a nitrogen gas stream at 95 K. X-ray diffraction experiments were performed at beamline BL-1A and AR-NE3A in KEK. The data were processed and scaled with the HKL2000 package [8].

3 Results and Discussion

The crystals of human PFD appeared within 1 week with typical dimensions of 300 × 100 × 20 μm (Fig. 1). The best crystal diffracted X-rays up to 4.7 Å resolution. The data set was collected at beamline BL-1A with the detector Pilatus 2M-F (Dectris). The camera distance, wavelength, oscillation range and exposure time were 440 mm, 1.1000 Å, 1 degree and 0.5 sec, respectively. A total of 180 frames were obtained.

The crystals belong to the space group $P2_12_12$ with unit-cell parameters a = 123.2, b = 152.4, c = 105.9 Å. Assuming that two PFD hexamers are in the asymmetric unit, the Matthews coefficient [9] was calculated to be 2.5 $Å^3\ Da^{-1}$, corresponding to solvent content of 50.2%.

Since the molecular replacement (MR) method gave no clear solutions, the heavy atom derivatives were prepared for the single-wavelength anomalous dispersion (SAD) method. We have collected the data from the K_2PtCl_4 derivatives at 7.5 Å resolution and Se-Met derivatives at 8.8 Å resolution, respectively. Due to the poor quality of the data, no interpretable peaks were observed in the anomalous Patterson maps. Combination of the MR and SAD methods is in progress.

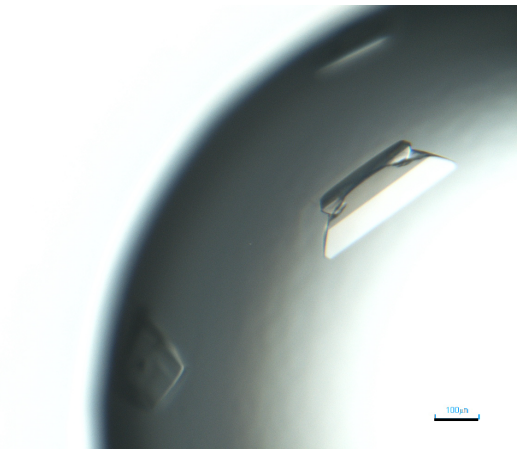

Fig. 1: Crystals of human PFD.
The black bar represents 100 μm.

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