Crystal structure of AML1-RNA aptamer complex

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Introduction

RNA aptamers are short single-stranded nucleic acids with high affinity to target molecules, and can be produced by a method called SELEX (systematic evolution of ligands by exponential enrichment). The concept is based on the ability of short sequences to fold, in the presence of a target, into unique three-dimensional structures, which allow the aptamer to bind target molecules with high specificity. The first aptamer-based therapeutic, Pegaptanib (Macugen), which targets vascular endothelial growth factor, was approved by the FDA in 2004 for the treatment of age related macular degeneration (AMD). Thus, the applications of the RNA aptamers are expected to be potential candidates for RNA therapeutic agents.

Despite such useful properties of the RNA aptamers, their molecular mechanisms are poorly understood. Although RNA aptamers have been examined for their three-dimensional structures by X-ray crystallography or NMR spectroscopy, only a few high resolution structures of RNA aptamers in complex with target proteins have been reported so far [1].

We previously identified two RNA aptamers containing 2'-fluoro pyrimidine nucleotides, which binds to the AML1 protein involved in leukemia. Interestingly, the primary structures of these RNA aptamers are totally different, suggesting that these aptamers interact in the distinct binding modes.

To clarify these unique molecular mechanisms of the RNA aptamers, we crystallized the AML1-RNA aptamer complex, and performed X-ray diffraction measurement of the crystals of the complex in KEK-PF, JAPAN.

Preparation of AML1 crystals

Preparation of RNA aptamer and protein

AML1 was overexpressed in E. coli, and purified by affinity and size-exclusion chromatography. The final concentration of the AML1 protein was 5 mg ml⁻¹ and it was stored at 193 K. The chemically synthesized two RNA aptamers containing 2'-fluoropyrimidines were purchased from GeneDesign Inc. (Japan). These aptamers were purified by denaturing PAGE and extensive desalting.

Data collection of RNA aptamer complex crystals

The aptamers were mixed with the AML1 protein in a molar ratio of 1:1.2 for crystallization. Crystals were obtained by vapor diffusion method using ammonium sulfate or polyethylene glycol (PEG) as precipitant.

The crystals were soaked briefly in Paratone-N oil and then frozen by rapidly submerging them in liquid nitrogen. X-ray diffraction measurements were performed at the beamlines BL5A and BL17A.

Synchrotron radiation experiments

The X-ray diffraction data were collected from a single crystal, on a CCD camera at beamlines BL5A and BL17A (λ=1.00 Å), at a maximum resolution of 3.5 Å.

Since the crystal structure of DNA-AML1 complex has been determined; we first tried to solve our RNA aptamer-AML1 by molecular replacement method using the coordinates of DNA-AML1 complex as a probe. However, there were no solutions obtained. We then prepare the modified RNA aptamers including iodine to determine the structure using multi- or single-wavelength anomalous dispersion. Although we crystallized the modified RNA aptamer-protein complex, the modified RNA aptamer-AML1 complex suffered X-ray damage during data collection even at cryogenic temperatures. At present, we are merging the different data sets from different crystals to collect the accurate diffraction data and determine the structure of the complex.

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Reference


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