Report on MX-1667 experiment.

Structural studies of bacterial Tn7 transposase complex

Tn7 is a bacterial DNA cut-and-paste transposon (1,2,3) that is involved in DNA rearrangements which play an important role in genome evolution. It encodes five proteins TnsA (nuclease), TnsB (transposase from DDE/RNase H fold family), TnsC (ATPase), TnsD and TnsE (DNA-binding proteins). TnsABC complex is constitutes the core transposition machinery. Although Tn7-like transposons were found in chromosomes of many bacterial species and Tn7 has been characterized biochemically, the available structural information is limited and the exact mechanism of Tn7 action, in particular the role of TnsC ATPase and target immunity, is poorly understood. Our aim is to determine the architecture of the TnsABC complex and elucidate the mechanism of nucleic acid substrate binding and processing. We have obtained three types of crystals: those of TnsABC (two crystal forms), TnsB with DNA substrate and TnsAC of full length proteins. These crystals diffracted poorly at a small synchrotron (maximum resolution we can obtain is ~8 Å) and we are not able to collect good quality data. Crystals that we obtained are regular but relatively small. Therefore, we wanted to use a microfocus setup at Diamond for data collection. We tested 35 crystals for TnsABC complex and 5 crystals for TnsB-DNA. Unfortunately, we could not improve the resolution for any of the two types of crystals and the maximum resolution varied between 18 and 8 Å. We also prepared crystals soaked with heavy metals (tantalum bromide, tri-sodium phosphotungstate, hexa-sodium metatungstate, deca-ammonium paratungstate) for phasing experiments, however, the soaked crystals did not diffract X-rays. We recently obtained a new type of TnsABC crystals, so our immediate goal is to test whether they will have better diffraction properties.

The other project that we worked on during our last experiment at ESRF was Ydc2 protein that is a Holliday junction resolvase. HJ is an intermediate during the process of homologous recombination. The proper resolution of such structures is of key importance for an organism because their accumulation is mutagenic. HJ resolving enzymes are structure-specific endonucleases that are present in all branches of life. They share biochemical properties but their structures differ. Best characterized is the bacterial RuvC (4), much less is known about resolvases from other organisms. The resolvase identified in fungi is called Ydc2. This enzyme has been characterized biochemically (5) and the structure of the apo protein is known (6) which confirms a distant evolutionary relationship to the bacterial RuvC family, but reveals structural features which are unique to the eukaryotic enzymes. We tested 42 crystals of Ydc2 in complex with HJ both of native crystals and soaked with heavy metals (tantalum bromide, tri-sodium phosphotungstate, hexa-sodium metatungstate, deca-ammonium paratungstate). We were able to collect good quality data sets to 3.8 A for native crystals (space group P222, unit cell 142.3, 148.7, 157.7, 90, 90, 90) but soaked crystals diffracted to ~7.5 Å at best. We tried to solve structure by molecular replacement using known structure as a model but this approach failed. We obtained crystals of selenomethionine derivative and we are planning to test them in the future.

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