Report on MX-998 experiment

Our main project and the topic of the proposal were crystallographic studies of human RNase H2. We also collected data from bacterial Holliday junction resolvase RuvC.

Human RNase H2

RNases H are divided into two main classes called type 1 and 2. The preferred substrate of type 1 enzymes is an RNA/DNA hybrid with one strand containing a minimum of four consecutive ribonucleotides, while type 2 enzymes require only one ribonucleotide for cleavage to occur. Eukaryotic type 2 RNases H are composed of three subunits [1]. They have been first identified in S. cerevisae and are called RNase H2A (catalytic subunit), RNase H2B and RNase H2C (auxiliary subunits) The exact role of auxiliary subunits in the activity of RNase H2 is not known. It has been shown that mutations in any of the three subunits of human enzyme result in Aicardi-Goutières syndrome (AGS), an autosomal recessive genetic disorder with symptoms similar to in utero viral infection that severely affects the nervous system [2]. How RNase H2 deficiency leads to AGS is not clear.

We obtained crystals of human RNase H2 A/B/C complex in the form of thin needles. They belong to P1 spacegroup and the initial X-ray diffraction experiments produced patterns to only 7 Å resolution. In order to improve the diffraction we performed a dehydration procedure by gradually increasing the precipitant concentration. This improved the resolution to around 4,5 Å at a conventional synchrotron beamline. By using the microfocus beamline (23-2) we were able to collect a 3,1 Å dataset. Application of helical data collection was also essential to overcome the radiation damage problem and allowed us to collect a complete dataset. Since our efforts to solve the structure with Molecular Replacement method and available structures of archaeal RNases H2 failed, we produced selenomethionine-labeled protein and grew its crystals. We collected a 3,5 Å SAD dataset from these crystals at 14-4 beamline. The crystal belong to P1 spacegroup, but the dimensions of the unit cell are different from the native crystal. So far, our attempts to solve the structure using these data have failed. It is possible that too many methionines present in the strcture are disordered to obtain phase information (see below).

While we were working on the SeMet crystals, a structure of mouse RNase H2 complex was published [3]. The two proteins are closely related with around 80% identity of catalytic subunits. We solved the structure of human protein based on the 3.1 Å native dataset and using the mouse protein as a search model in Molecular Replacement. The asymmetric unit contains six A/B/C complexes of human RNase H2. The refinement and analysis of the structure is now underway and it is very tedious given the large number of residues in the asymmetric unit. The preliminary analysis of the structure shows that human RNase H2 adopts a structure very similar to the mouse enzyme with large parts of subunits B and C disordered. The catalytic subunit A adopts a structure very similar to other known monomeric RNases H2. It contains two domains - the catalytic and the C-terminal one. The key element of the catalytic domain is the central β-sheet flanked by α-helices. The active site is composed of carboxylates located in the C-termini of the first strand and fourth strands of the central β-sheet. The B and C subunits adopt a structure of the highly intertwined dimer. At its center there are two mixed β-sheets and both B and C subunits contribute to the formation of each sheet. The interactions with the catalytic

subunit are mainly mediated by protein C and the B/C binding site is away from the nucleic acid substrate interface. This suggests that the role of the auxiliary subunits is not to influence the catalytic activity of the enzyme but to bring along other interacting proteins. We are now in the process of finishing the refinement of the structure and designing biochemical experiments to complement it.

RuvC

Genetic recombination mediates the replacement and rearrangement of DNA sequences. It is used to rearrange the genes between chromosomes and consequently generate genetic diversity and promote evolution. It also serves to protect genetic information when used to repair the DNA damage such as double-strand breaks [4]. The obligatory intermediate in this process is a four-way DNA strcture called Holliday junction. In bacteria these junctions are resolved by a protein called RuvC. It is a 19 kDa dimeric endonuclease that requires divalent metal ions for activity. It resolves Holliday junctions by cleaving symmetrically two strands of the same polarity. Substrate binding does not depend on the DNA sequence, but the cleavage is specific and occurs only at (A/T)TT↓(G/C) cognate sequence [5, 6]. Apo protein structure of RuvC has been reported [7] and it revealed a dimeric structure with the two active sites within 30 Å from each other. The fold of RuvC is very similar to that of RNases H and other members of retroviral integrase superfamily.

We have solved the structure of bacterial RuvC in apo form and in complex with a synthetic Holliday junction, but in the complex the DNA was bound non-catalytically. In crystallization trials we also obtained another crystal forms that produced only very weak diffraction at synchrotron sources. We hoped that this crystal form might contain a catalytic complex of RuvC, so we performed a very careful optimization of the cryoprotection conditions and devised a crystal dehydration protocol. This treatment already improved the diffraction but in order to obtain usable data we used microfocus beamline 23-2. Even though the crystals grow in the form of thick rods (up to 0.2 mm in width) the combination of a small and very bright beam of 23-2 with the helical data collection to circumvent the radiation damage problem produced much better data than other beamlines. After testing of a large number of crystals we found one from which we collected a 3,8 Å data set. The structure was solved by molecular replacement and it corresponds to a catalytic RuvC-Holliday junction complex. Even at the relatively low resolution at which the structure was solved, the electron density for the substrate is very clear. The protein is mostly traced as a poly-alanine model.

The structure reveals that the DNA in the complex is in an unfolded 2-fold symmetrical conformation. The DNA conformation can be described as a square planar conformation in which, upon binding to the protein two arms containing the cleavage site are pulled downwards from the plane and two other arms pushed upwards. The junction has the same two-fold symmetry as the protein dimer. The base pairing at the Holliday exchange point appears to be maintained and the scissile phosphate is located 1 nt from the branch point towards the 3'-end of the cleaved strand. Substrate binding, DNA conformation and the site of cleavage are very different in RuvC compared to to published complex structures of two phage resolvases Endo I and Endo VII [8, 9]. Our structure is also the first one for a substrate complex of a resolvase which is specific for four-way junctions.

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