Master project proposal in structural biology

Crystallisation and structure determination of protein-DNA complexes of Endonuclease III from the extreme radiation and desiccation resistant bacterium *Deinococcus radiodurans*

Introduction and aim of study

Deinococcus radiodurans (DEIRA) is a gram-positive bacterium, which exhibits extreme resistance to ionising radiation and desiccation. DEIRA tolerates doses ranging from 5,000 to 30,000 grays whereas most other organisms cannot survive doses above 50 grays. Such a massive radiation dose is estimated to induce several hundred double-strand breaks, thousands of single-strand gaps and about one thousand sites of DNA base damage per chromosome.

Endonuclease III (EndoIII) is a DNA glycosylase which belongs to the Base Excision Repair (BER) pathway and removes oxidation damaged bases from DNA. The enzyme is bi-functional and catalyse the removal of thymine glycol (Tg) from DNA and generates a nick in the DNA on the 3'side of the damaged base (AP-lyase activity) in order to prepare the DNA for further processing by AP-endoncuclease and repair by DNA polymerase and ligase.

DEIRA possesses three genes encoding Endonuclease III (DR_2438 [1], DR_0289 [2] and DR_0928 [3]). We already determined the crystal structures of EndoIII1 and 3, and generated a homology model of EndoIII2 based on the crystal structure of EndoIII from *E.coli* (43% sequence identity) since this protein does not want to crystallise in our hands. The structures consist of two α-helical domains with an active site cleft between them, and with a [4Fe-4S] cluster in one of the domains. We are currently working on determining the substrate specificity of the proteins, and studies of the involvement of the [4Fe-4S] clusters for catalysis are also ongoing.

So far it seems that the substrate specificity of EndoIII1 and 3 is different from EndoIII2. In order to explain the differences in substrate specificity we would now like to determine the crystal structures of EndoIII1 and 3 in complex with DNA, and this is the main goal of this project.

Approach and expected outcome

The genes encoding the proteins are already cloned and protocols for expression and purification are also established, thus the focus will be on crystallisation and structure determination. Crystallisation of protein-DNA complexes are known to be challenging. However, we will use a chemical which will cross-link the protein with the DNA and thus increase the probability of obtaining crystals.

Methods

1. Expression of recombinant proteins in *E.coli*.
2. Protein purification.
3. Preparation of DNA for co-crystallisation (annealing and purification).
4. Crystallisation (use of nano-litre crystallisation robot and optimisation by manually setting up hanging drops/vapour diffusion methods).
5. X-ray crystallography (use of *in-house* X-ray generator and diffractometer, training on data collection and processing (PROTEUM, iMosflm, CCP4 package, etc.), model building (Coot) and refinement (PHENIX)).

Supervisors

Elin Moe: Protein expression and purification, DNA preparation and crystallisation.
Pedro Matias: Crystallisation, data collection and processing, structure determination, model building, refinement and structural analysis.