	Experiment title: Structural characterisation of the restriction-modification controller protein C.AhdI in complex with its specific operator DNA.	Experiment number: MX-408
Beamline: BM16	Date of experiment: from: 01 November 2004 to: 02 November 2004	Date of report: 12/08/05
Shifts: 3	Local contact(s): Dr Gavin Fox	*Received at ESRF:*

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Report:

Background

Restriction-modification (R-M) systems are composed of a methyltransferase (MTase) and an endonuclease (ENase) that work in tandem to protect the host bacterium from bacteriophage infection (Wilson & Murry, 1991). The MTase recognises and methylates a specific DNA sequence and the ENase recognises the same sequence and cleaves within or close to this site. The ENase will only cleave DNA that is unmethylated at the specific site, thus host DNA is protected while non-host DNA is cleaved. Following DNA replication, expression of the ENase must be delayed until the host DNA is appropriately methylated. This is achieved at the transcriptional level through the action of the controller protein, or C-protein (Tao *et a.l*, 1991).

Following on from an extensive biophysical characterisation of the C-protein from *Aeromonas hydrophilia* (Streeter *et al.,* 2004), diffracting crystal were obtained of the native protein (McGeehan *et al*. 2004). We solved the structure of the first R-M C-protein, C.AhdI, using selenomethionine MAD which was refined to 1.69Å (McGeehan *et al*., 2005).

Experiment

Using DNA duplexes corresponding to the binding site of C.AhdI, putative co-crystals were obtained by vapour diffusion methods. Despite extensive trials with buffer, additives and

seeding methods, only small (<50µm), fragile crystals could be obtained. These were transported to the ESRF in drops and frozen on site at BM14 at 100K under various cryoconditions. A large number of crystals were screened and the best diffraction was obtained with a crystal grown with MPD at 20%.

A full dataset was collected for a putative C.AhdI complex with a 16mer duplex. The cell indexed in *P*1 with unit cell dimensions:
a=24.66, b=32.96, c=46.44, α=91.12°, β=82.62°, γ=86.70°, with a solvent content of 53.4%. The data extended to 1.96Å and was 94.1% complete. The data was processed and refined with an R-factor of 23.9% and Rfree of 30.0%.

Analysis of the electron density maps revealed that although the spacegroup and packing was different than our published native structure, the 16mer DNA duplex was not visible. The dataset has proved to be useful as important surface residues predicted to interact with DNA polymerase are similarly orientated in this structure despite the different crystal packing arrangement.

Since this data was collected we have undertaken extensive studies on the DNA binding properties of this protein. Substantial resources have been directed at screening different DNA duplex oligonucleotides for optimal binding vs. length. Results show that binding can be substantially increased by the addition of 1 or 2 bases as compared to the 16mer sequence used in our initial trials.

Recent studies using velocity analytical centrifugation have clearly demonstrated that two dimers of C.AhdI can bind to adjacent positions on the operator DNA. Again, using a series of truncated oligonucleotides, the shortest optimal binding sequence has been determined. Since no such structure is currently available in the PDB database, our efforts are currently focused on producing co-crystals with the single dimer-DNA and the larger tetramer-DNA complexes.

References

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