New structure of cold adapted esterase Est97 (RC97), derived from a metagenomic library
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By Hanna-Kirsti S. Leiros and Kenneth Johnson

Project number 1, Est97

Est97 has been proven to be an esterase, its DNA isolated from metagenome sample collected in the Arctic (Spitsbergen Islands) and the bacteria is probably belonging to the gamma-proteobacteria family.

Lipolytic enzymes include esterases and lipases which catalyze the hydrolysis and synthesis of short-chain (≤C10) and long-chain (≥C10) acylglycerols, respectively. These enzymes are widely used in detergents, textile, and dairy industries and synthesis of chiral pharmaceuticals (Rogalska et al., 1997, Bornscheuer, 2002, Houde et al., 2004, Zoumpanioti et al., 2010). Cold-adaptive enzymes have great potential in industry since they have high activity and can work at low and moderate temperatures which are beneficial economically (Cavicchioli et al., 2002, Smalås et al., 2000, Siddiqui & Cavicchioli, 2006). To search for new cold-adapted lipolytic enzymes, a metagenomic fosmid library was constructed by using environmental DNA from a Svalbard intertidal zone sediment and about 60,000 clones were screened on tributyrin plates at 12°C. In total, 139 positive clones were obtained. Based on subcloning or complete fosmid sequencing, the genes of putative lipolytic enzymes from positive clones were determined. Since lipolytic enzymes have numerous of applications, we are presently in dialogue with the enzyme-producing industry to elucidate the potential of our library in this respect.

The pure Est97 protein was crystallized using the in-house Phoenix RE robot with various screens. During optimization we have obtained diffracting protein crystals and one in-house X-ray data set was found to belong to space group P222. The unit cell axis are a=54.95, b=55.95 and c=67.75 Å, and one screw axis was found along b, thus the true space group is $P2_12_12$ or $P2_12_12_1$.

Results obtained

The native Est97 crystals diffracted as expected to much higher resolution at ID29, a native dataset to 1.6 Å of wild type Est97 was obtained, and in space group $P2_12_12$ with cell axis of a=55.86, b=69.87 and c= 54.06.

Then we tested crystals soaked with different heavy atoms (Hg, Sm, Cd, Ir, Pt, Pd, Zn), all data were collected in SAD mode and the peaks of the energy scans. None of the crystals were back soaked since the crystals made the crystallization robot and were small, thus we when slightly to the high energy side of the f`` peak for each heavy atom.

In total we collected two data sets for the native Est97 and 18 data sets for heavy soaked Est97 crystals.

At the beamline the anomalous signal of many of the data set was analysed and some zinc soaks seemed to be interesting. After returning home we solved the Est97 by the single wavelength

with anomalous dispersion (SAD) method with data collected with λ=1.282 Å on the high energy side of the Zn K-edge which have a theoretical peak at λ 1.2835 Å. The program suit HKL2MAP (Schneider and Pape 2004) was used in in SAD mode and 11 Zinc sites were identified and refined using ShelxD. Phase improvement was performed using ShelxE (Sheldrick 2008), where after ArpWarp (Cohen, Ben Jelloul et al. 2008) and Buccaneer (Cowtan 2008) were used for automated model building. The phases then we extended to 1.6 Å and a complete model only missing two loops adjacent to the active site was obtained.

The success for solving this structure was the use of the sample changed which allowed us to screen all the soaked crystal very efficiently. The available software and computer facilities were also very helpful. The additional data sets from the other heavy atoms were not tests to see if they could also be used to solve the Est97 structure.

Project No 2 was the Metallo-β-Lactamase (MBL) GIM-1
Metallo-β-lactamases (MBLs) are enzymes responsible for antibiotic resistance to virtually all β-lactam antibiotics in various Gram-negative bacteria, and the task of this project was to solve a high resolution crystal structure of GIM-1 from the opportunistic human pathogen *Pseudomonas aeruginosa*.

In total we collected three data sets on GIM-1 where the best was resolved to 1.6 Å in $P2_12_12$. This structure was by molecular replacement by use of the IMP-1 structure, and we found three molecules in the asymmetric unit. The analysis and structural refinement of this structure is in progress of a PhD student.

Project number 3
Protein DNA binding

We collected data on two protein DNA complexes namely *Deinococcus radiodurans* Proliferating Cell Nuclear Antigen (DrPCNA) bound to 10 nt oligo, Cy5 labelled in the 5' end (DrPCNA-DNA) which gives blue protein crystals.

DrPCNA is a 39.6 kDa protein with 362 residues that have a 29% sequence identity to *E. coli* PCNA. DrPCNA has been recombinantly expressed and purified with the aim to perform structural studies of PCNA alone and in complex with DNA and interacting DNA repair proteins from *D. radiodurans.*

We collected four data sets on DrPCNA-DNA from blue crystals grown in the presence of 2 different oligos with a blue dye, Cy5, attached at the 5' end of the oligonucleotide. The data were obtained at low resolution (about 3.5Å) and we solved the structures by molecular replacement using the 2.1Å resolution DrPCNA structure determined from the data collected on 27 Jul at beamline 14-4. The resulting electron density showed no evidence for ordered DNA. This was despite the evidence for DNA in the crystals as shown by single crystal UV spectroscopy. Future efforts will focus on obtaining crystals with differing oligonucleotide constructs in order to obtain a structure of the DrPCNA with ordered, bound DNA.

Four crystals of DNA bound to *D. radiodurans* Uracil DNA N-Glycosylase (DrUNG) were tested

with the aim was to obtain higher resolution data in order to better define the protein-DNA interactions in the complex. The result was a data set to 1.35Å useful for obtaining a high resolution structure to be published in in 2012.

Conclusion

The use of the PILATUS detection on ID29 was impressive in terms of reduced data collection times, very good statistics with very low R-merge values for most of the data. The scientific results were also satisfying.

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