Structure-function studies of a highly active, highly selective and thermolabile nuclease from shrimp

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By Hanna-Kirsti S. Leiros, Kenneth A. Johnson, Alexander Pflug and Ulli Rothweiler

Project number 1 Screening heavy atom derivatives of the shrimp DNAse

Shrimp deoxyribonuclease (sDNAse) is an endonuclease cleaving phosphodiester linkages in DNA to yield oligonucleotides with 5'-phosphate and 3'-hydroxyl termini. DNase from shrimp has a very high specific activity, estimated to be 30 times higher than bovine DNase I, which is widely used in modern biotechnology. In addition, shrimp DNase is heat labile, and it has a particularly strong preference for the hydrolysis of double-stranded DNA (dsDNA). In the presence of magnesium as the only divalent cation, single-stranded DNA (ssDNA) is hydrolyzed at a rate of 1-5% of that of dsDNA. Shrimp DNase can therefore be used to specifically degrade dsDNA, leaving ssDNA essentially intact. The higher catalytic efficiency, together with the reduced thermal stability, has made the enzyme an important component in kits used in molecular biology.

On a previous trip to the ESRF (27 Jun. 2011, ID14-4), the structure of sDNAse was solved by collecting SAD data from a crystal soaked in 250mM zinc sulfate. On this trip, data sets from 4 sDNAse crystals soaked in 250mM magnesium sulfate were collected with the aim to obtain the catalytically active conformation of magnesium in the active site of sDNASe. The highest resolution obtained was 2.1Å; the resulting electron density maps clearly showed the presence of octahedrally coordinated magnesium atom. This data and the phasing data(ESRF, Jun 2011) and the native data (ESRF, Dec 2009) will lead to a publication this year (2012).

Project number 2, Protein DNA binding in *Deinococcus radiodurans*

Data were collected on data a protein DNA complex of *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 three data sets from two different crystals with the best resolution being 2.1Å. However, as on the previous data collection trip (Jun 2011, ID29,) the resulting electron density maps showed no ordered, bound DNA. Future plans are to screen different oligonucleotide constructs to obtain crystals of DrPCNA with bound and ordered DNA as we know that DNA is present in the crystals from previously taken (ESRF, Jun 2011) single crystal UV spectra.

Project number 3, the Cold-adapted esterase Est97

Starting with DNA isolated from metagenome sample collected in the Arctic (Spitsbergen Islands), a novel cold adapted esterase Est97 was cloned, expressed and purified, and native protein crystals have been obtained. The native structure was solved in June 2011 also at ID29, with data collected under experiment MX-1311.

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). 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.

On this trip we collected data on Est97 crystal soaked with the substrate tributyrate in order to obtain a complex structure. Different times and substrate concentrations were used, 8 crystals were tested and 8 data sets collected to 1.55-1.8 Å resolution. Unfortunately no complex structure was obtained, but in one of the unsuccessful soaks, many additional loop residues were defined. This loop was disordered in the native Est97 structure and it role in the structure is under investigation and the result will be published.

Project number 4, GIM-1

Antibiotic resistant bacteria is a growing problem throughout the world (Walsh, 2010), metallo-β-lactamases (MBLs) are enzymes responsible for resistance to virtually all β-lactam antibiotics in various Gram-negative bacteria. GIM-1 (German imipenemase) from the opportunistic human pathogen *Pseudomonas aeruginosa* represents a new class of MBLs (Castanheira et al., 2004), and the native structure was solved in June 2011 at ID29, under experiment MX-1311 to 1.6 Å in $P2_12_12$.

On this trip the native GIM-1 crystals were soaked with three commercially available inhibitors. The aim was to obtain details from the active site and adjacent binding sites. In total 8 crystals were tested and 5 complete data sets were obtained. Unfortunately no complex structure with an inhibitor was obtained, but the protein had crystalized in one new space group with an apo-active site, thus missing two active site zinc ions. The implication of this result is currently under investigation.

Project number 5, Protein Kinase A with small molecule inhibitors

A different approach for design of active site-directed kinase activity regulators is the bisubstrate inhibitor, whereby a fragment of ATP or an ATP-competitive inhibitor is combined with a non-phosphorylatable substrate peptide analogue into a single molecule, enabling higher target selectivity and affinity than the solely ATP-competitive starting compound. This bisubstrate inhibitor strategy has recently produced adenosine analogue-oligoarginine conjugates (ARCs) that are highly potent inhibitors of basophilic protein kinases, with significant potential for further derivatization, diversification and application in a wide variety of kinase assays (Lavogina et al. ChemMedChem. 2010 Jan;5(1):23-34; Pflug et al. J Mol Biol. 2010 Oct 15;403(1):66-77).

The aim of the project is to co-crystallize the catalytic domain of protein kinase A (PKAc) in complex with ARC inhibitors in order to study the atomic details of their interaction and guide the further development and optimization of the compounds. On this trip, three full datasets were collected, of which one represents a novel complex of the catalytic domain of protein kinase A and the inhibitor ARC-1411. The structure features a resolution of 1.95 Å and depicts a highly flexible binding mode for the molecule ARC-1411. Thus, this data adds vital information to the understanding of the compound's mode of action allowing for further optimization of the protein kinase inhibition by ARCs. The structure will be deposited and published including data collected previously(ESRF, Jun 2011).

Project number 6, Cyclin-dependent kinase 2 with small molecule inhibitors

The research project aimed to facilitate structure based anti-cancer drug design by studying the structural determinants of protein kinase inhibitor selectivity profiles. This is done in detail for key protein kinases as model systems, and in a structural proteomics fashion for a suitable panel of crystallizable protein kinases. This would facilitate the determination of the crystal structures of protein kinase-inhibitors in order to study the interactions and the dependence on point mutations, domain architecture, phosphorylation state, and intermolecular domain-domain interactions.

Several hits for inhibitors for kinases were found in in vitro screening with new unique inhibitor scaffolds and new fragments based on these finds were synthesized in an attempt to improve the kinase inhibition. The *in vitro* data show that the inhibitors hit the different kinases with variable inhibition properties. CDK2 was used as a model kinase because it is easily crystallized. On this trip, data sets

were collected from 8 crystals of CDK2 that were soaked with 4 different inhibitors. The crystals diffracted from 1.75Å for the best crystals to 2.9 Å for the worst crystal. Unfortunately, the resulting electron density maps showed that none of the CDK2 structures had an inhibitor molecule bound in the active site.

Conclusion

The beam line worked flawlessly with excellent assistance from the beamline scientist. The new Pilates detector made the data collection especially efficient with data being collected from 6 different projects in only 1 shift of beam time.