Determination of crystal structures of DNA repair proteins from *Deinococcus radiodurans* in complex with DNA

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Project number 1 Proliferating Cell Nuclear Antigen (PCNA)

DNA polymerase III β-subunit, or Proliferating Cell Nuclear Antigen (PCNA) is a ring-shaped protein that encircles DNA. PCNA acts as a processivity factor, or sliding clamp, for a wide variety of proteins that act on DNA, including DNA polymerases, DNA ligase, endonucleases and glycosylases (reviewed in (Warbrick, 2000). PCNA sliding clamps are loaded on DNA in ATP-dependent reactions by clamp loaders. Clamp loaders are multisubunit complexes where ATP hydrolysis is coupled to conformational changes that enable the clamp loader to open the sliding clamp and place it on DNA (Indiani & O'Donnell, 2006). Once loaded, the sliding clamp allows the binding of the other polymerase III subunits. The current knowledge of the complex and dynamic interactions between the subunits and the sliding clamp is reviewed by (Lopez de Saro, 2009), but much remains to be discovered.

Earlier we obtained a low resolution data set (2.8Å) from a crystal of the PCNA from the extreme radiation resistant bacterium *Deinococcus radiodurans (***Dr***)*. We solved and partially rebuilt and refined the structure. On this trip, we collected data to much higher resolution (2.1Å) on crystals of DrPCNA grown in the presence of a short oligonucleotide with a blue dye, Cy5, linked at the 5' end. Using this new data, we solved the structure by molecular replacement using the unfinished low resolution structure of DrPCNA. The improved resolution allowed for autobuilding by Buccaneer Pipeline (Cowtan, K., 2006 and Cowtan, K., 2008 of the entire protein chain, but the resulting electron density maps after refinement with Refmac5(G.N. Murshudov, A.A.Vagin & E.J. Dodson,1997) showed no evidence for ordered, bound DNA in the structure. We did on a subsequent visit show by single crystal UV spectroscopy that there was DNA in the blue crystals.

Project number 2 Screening Zn and Br 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 (Dec. 2009, ID29), several data sets from crystals of native sDNAse and from crystals soaked in various heavy atom solutions were taken. The best native crystal diffracted to 1.9Å. The heavy atom data sets showed very little anomalous signal except for that from a crystal soaked in 200mM zinc sulfate and 500mM sodium bromide. The goal for this trip was to take a zinc or bromide SAD data set order to solve the structure. This

was accomplished with the very first data set taken from a sDNAse crystal soaked in 250mM zinc sulfate. A absorption scan was used to plan the data collection with the peak data set being taken a few eV(at 1.2982Å) and a high energy remote taken a few hundred eV (at 1.256Å) above peak that as estimated from the absorption scan.

The resulting data were processed with XDS and scaled with XSCALE (Kabsch, W. 2010) and showed a useful anomalous signal to about 2.7Å (27% anomalous correlation). Zinc sites were located using the HKL2MAP interface(Pape, T. & Schneider, T.R., 2004) to ShelxC,D and E programs(Sheldrick, G.M., 2002, 2003 and Schneider T.R. & G.M. Sheldrick, 2002). Phases were improved using SOLVE(Terwilliger, T.C. and J. Berendzen, 1999) and RESOLVE(Terwilliger, T.C., 2000) in the calculate mode and the structure was built automatically by the new Buccaneer Pipeline(Cowtan, K., 2006 and Cowtan, K., 2008) program as implemented in the CCP4 package(CCP4 no. 4, 1994). Analysis of the resulting structure is ongoing and is expected that the paper will be published this year.

Project number 3 Screening small molecule inhibitors of Protein Kinase A.

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 two are redundant. The complex structures of the catalytic domain of protein kinase A in complex with the inhibitors ARC-1408 and ARC-1028 could be solved at a resolution of 2.3 Å and 2.2 Å respectively. In both cases electron density defines the desired complex, revealing the position and binding mode of the ligand. Both structures will be deposited in the PDB and published.

Conclusion

It was fortunate that we were allowed to use beamline 14-4 instead of the assigned 14-1 because this allowed us to solve the shrimp DNAse structure. We were also able to take very useful data for the *D. radiodurans* PCNA project. The data for the Protein Kinase A/inhibitors complexes were also very informative. The collected data will result in a publication for each of these three projects.

The beamline worked well with all the beamline components functioning flawlessly. The computer system failed at about midnight because of a very odd event in that the system ran out of inodes with which to identify new files. The beamline continued to work but no new files could be saved. Nevertheless it was quite a successful trip with useful data collected for three publications.