ESRF	**Experiment title:** Data collection on ribonucleotide reductase. R2 subunit, acylphosphatase and protein tyrosine phosphatase	**Experiment number:** LS 118
Beamline: D2AM	**Date of experiment:** from: 22-7-95 to: 23-7-95	**Date of report:** 1 o-2-97
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Report:

Data collection at D2AM was rapid: we could collect 3 data sets in 16 hours. Much time was lost at the beginning of the shift due to problems with the cryo-cooling system and lengthy alignment of the beamstop. We collected data on the following crystals:

1) Ribonucleotide reductase protein R2

RNR R2 is a dimer containing one non-haem diiron centre per monomer. We were interested in a high resolution structure of RNR R2 in the resting, Fe(III)-Fe(III) form, for which the structure is already known at 2.2A resolution [l], in order to compare it with the high resolution structure of the Fe(II)-Fe(II) form which we have determined at :170°C [2]. R2 crystals are in space group $P2_12_12_1$ with cell dimensions a=75A, b=85A, c=105A.

Our crystal, cooled to –170°C, diffracted to beyond 1.7Å but data collection was limited to this resolution by the available swing angle of the CCD detector. Data collection was rapid, with exposure times of 2s. We collected a high resolution data set to 1.7Å and a low resolution set to 2.6Å, both with ϕ ranges of 0.3" and 360 images in each set. The merged data were 82% complete to 1.7A, with R_{merge} = 6.2%. Between 1.8–1.7Å the data were 53% complete with R_{merge} = 20.0%. The lower completeness is presumably due to detector geometry. The overall multiplicity was 2.3, but in the intermediate resolution bins where the two data sets overlapped it was around 4.5.

Despite the apparently excellent data quality, we chose not to use the data because the only processing program available at the time was XDS, whose algorithm automatically sets to zero weak intensities which are negative due to errors in counting statistics. In our case, this led to unacceptable loss of useful data at high resolution and we abandoned the data set. We were unable to use Denzo as the data collection philosophy at D2AM used XDS's local scaling algorithm to compensate for detector non-uniformity. However we are currently reassessing the data in view of the new possibility to process converted images with Denzo.

2) Acylphosphatase potassium osmate derivative

Acylphosphatase is a low molecular weight phosphomonohydrolase catalysing with high specificity the hydrolysis of the carboxyl-phosphate bond present in acylphosphates. It is one of the smallest enzymes known, with only 98 residues. Crystals are in space group C2 with unit cell a = 63.2, b = 35.3, c = 45.2 A, β = 104.1" and diffract to 1.8Å on a laboratory source. We were interested in solving the structure by MIR, so we collected data from a crystal soaked in potassium osmate.

This data set was unfortunately not used as, before we had processed it, high quality MIR maps were obtained using three other derivatives. These included one derivative which we had previously believed to be of no use, but which could be solved in retrospect with the aid of the other two. The structure has since been published [3].

3) Low molecular weight protein tyrosine phosphatase in complex with a peptide

We also collected a dataset from a low molecular weight protein tyrosine phosphatase (ImPTP), mutant S12C. This mutant does not have any catalytic activity but does bind substrate molecules with the same affinity as wild type. The protein was complexed with a peptide containing a phosphorylated Tyr. The spacegroup was $P2_1$, with cell dimensions a = 60, b = 45, c = 137 Å, β = 106°. The crystal diffracted rather weakly. We were able to collect 120° of data to around 2.8Å on 0.3° rotation images with an exposure time of 7.5s, before our beam allocation ran out. However due to the above-mentioned problems with XDS and the fact that this was weak, incomplete data we decided not to process it. We will process this data using Denzo in the near future.

References

[l] Nordlund P., Sjoberg B.M. & Eklund H. (1990). The three-dimensional structure of the free radical protein of ribonucleotide reductase. Nature 345, 593-598.

[2] Logan D.T., Su X.D., Aberg A., Regnstrom K., Hajdu J., Eklund H. & Nordlund P. (1996). Structure of reduced protein R2 of ribonucleotide reductase: the structural basis for oxygen activation at a dinuclear iron site. Structure 4, 1053-1064.

[3] Thunnissen M.M.G.M., Taddei N., Liguri G., Ramponi G. & Nordlund P. (1997) Crystal structure of common type acylphosphatase from bovine testis. *Structure 5, 69-79.*