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Report:

Ribonucleotide reductases (RNRs) catalyse the reduction of ribonucleotides to deoxyribonucleotides by a radical mechanism and are therefore crucial enzymes for our understanding of the evolution of life. They fall into three classes depending on primary sequence and radical generation method. However only one RNRs used in a given organism under given physiological conditions for reduction of all four nucleotides, and there is a complex allosteric regulation. We have good structural information for class I RNRs, which create a stable tyrosyl radical through the cleavage of dioxygen by a di-iron cluster. This radical is transferred to the active site along a hydrogen-bonded pathway extending over more than 35Å. The class III, anaerobically expressed RNRs have no obvious sequence homology to class I and create a stable glycyl radical using the cleavage of S-adenosylmethionine by an iron-sulphur containing "activase" protein. The enzyme is an $\alpha_2\beta_2$ homodimer, consisting of a 605 residue active site subunit (NrdD) and the 150 residue "activase" (NrdG). By virtue of its anaerobic origins, this RNR is more likely to resemble a putative common ancestor of modern-day RNRs; structural information on this system will thus be of great importance for our understanding of the functional relationships between different classes of RNR and also of the transition from RNA world to DNA world and the advent of oxygen.

We have determined and refined the structure of the class III anaerobic RNR from bacteriophage T4 using a native and two MAD data sets. Exposure of the complex NrdD/NrdG to oxygen results in cleavage of NrdD at Gly580 and inactivation; we thus chose to solve the structure of the complex NrdD(G580A)/NrdG. Crystals belong to space group $P4_32_12$ with cell dimensions **a=b=98.2Å, c=244.0Å**. There is one monomer in the asymmetric unit with 63% solvent. The crystals hardly diffract in the laboratory and all useful work has to be carried out at synchrotrons.

The native data were collected at the Swiss-Norwegian beamline in May 1997. The crystals diffracted to around **3.0Å** with 10 minute exposures or greater. However, a rapid test data set collected using wiggler beamline X25 of the NSLS, Brookhaven, using **only** 1 minute exposures, revealed that they clearly diffract beyond **2.8Å** given a bright enough source. The two derivatives were methyl mercury acetate (MMA) and potassium osmate, collected at BM14 in July 1997. For MMA, point of inflection and hard remote wavelengths were collected; for osmate we used the f'' peak and hard remote. These were used in conjunction with another hard remote wavelength from MMA collected at station Xl 1 of DESY. Despite the fact that: a) the anomalous signal was weak, b) the data had R_{merge}s around 8-9%, c) all five sites in the MMA and osmate derivatives were common, the MIRAS treatment with solvent flattening provided enough phase information to permit chain tracing at **3.5Å** resolution.

Refinement is still in progress and the current R_{model} is 27%, with R_{free}=36%. Despite the lack of sequence homology we observe a clear evolutionary relationship to the class I RNRs, with intriguing differences near the active site. However the small subunit NrdG is not at all clear in our current density, despite the fact that gels of redissolved crystals show it to be present; neither are the C-terminal 50 residues of NrdD. This is unfortunate since no structure is known for any homologue of NrdG and the obscure part of NrdD contains the glycyl radical site. To resolve these ambigutities and to characterise the actuive site better, we require better quality data, both at higher and lower resolution. We hope to obtain this, as well as high resolution data sets with substrates and allosteric effecters, during upcoming runs at the ESRF. We are also reconsidering our existing MAD datasets to ascertain if we can obtain better MIR phases from the data we have, as it appears that the disordered C-terminus may be affected by the solvent flattening process.