03-Crystallography of Biological Macromolecules

PS–03.04.23 HIGH RESOLUTION STRUCTURE ANALYSES OF APO-AZURIN AND CADMIUM-SUBSTITUTED AZURIN FROM *ALCALIGENES DENITRIFICANS*. By Richard L. Kingston, Kerry A. Blackwell, Bryan F. Anderson* and Edward N. Baker*, Department of Chemistry and Biochemistry, Massey University, Palmerston North, New Zealand.

The structures of both apo-azurin and Cd(II)-substituted azurin from *Alcaligenes denitrificans* have been determined at 1.8Å resolution. Crystals of apo-azurin were obtained by vapour diffusion of solutions of the apo-protein, while the Cd(II) derivative was obtained by soaking apo-azurin crystals in 10mM $CdCl_2$ In both cases medium-resolution data, collected with a CAD4 diffractometer, were merged with high-resolution synchrotron data obtained with a Weissenberg camera equipped with imaging plates. Refinement was by restrained least squares (program PROLSQ). Final refinement statistics were: *Apo-azurin* R=0.160 for all data (22,687 reflections) in the range 10.0 - 1.8Å, for a model comprising 1954 protein atoms, 247 water molecules, 4 SO_4^{2-} ions and 2 partial-occupancy (0.13 and 0.15) copper atoms. *Cd(II) - azurin* R= 0.168 for all data (23,332 reflections) in the range 10.0 - 1.8Å, for a model comprising 1954 protein atoms, 239 water molecules, 4 SO_4^{2-} ions and two cadmium atoms.

Removal of copper, from azurin causes virtually no structural change. There is a slight inwards movement of the ligand sidechains such that the radius of the metal binding cavity shrinks from 1.31Å in reduced azurin and 1.24Å in oxidised azurin to 1.16Å in apo-azurin. This is consistent with the view that the geometry of the metal site is determined primarily by the constraints of the protein structure. There is no trace of the outward movement of the ligand His 117 seen for apo-azurin from *Pseudomonas aeruginosa.* Cadmium binding again causes no significant change in the protein structure; the only movement involves the carbonyl oxygen ligand, which moves towards the metal to give a Cd-O distance of 2.76Å, compared with a Cu-O distance of 3.13Å in oxidised, Cu((II)-azurin (Baker, *J. Mol. Biol.*, 1988, **203**, 1071-1095). This demonstrates a limited flexibility in the metal site in response to the different coordination preferences of Cd(II).

PS–03.04.24 CRYSTAL STRUCTURE OF *Xenopus laevis* COPPER-ZINC SUPEROXIDE DISMUTASE B. K.Djinovic Carugo[1], A.Coda[1], C.Collyer[2], M.T.Carri'[3], F.Polticelli[3], A.Desideri[3], G.Rotilio[3], M.Bolognesi[1,4]. [1]Dip. di Genetica e Microbiologia, Univ.di Pavia, Italy. [2]Farmitalia Carlo Erba, Centro Ricerche, Nerviano, Italy. [3]Dip. di Biologia, Universita' di Roma "Tor Vergata", Italy. [4]Centro Biotecnologie Avanzate, Univ.di Genova, Italy.

Cu,Zn superoxide dismutases (SOD's) are higly stable enzymes, characterized by an extremely rapid reaction rate, due to electrostatic guidance of substrate to the active site. The crystallographic structure of human, bovine, spinach and yeast SOD's have indicated that the core of the enzyme is essentially a flattened eight-stranded, Greek key, β-barrel. Three-dimensional structures of the two SOD variants from *Xenopus laevis* (XSODA and XSODB) have been proposed based on computer graphics homology modeling.

XSODB has been expressed in *E.coli* inserting the whole coding sequence in plasmid pKK233-2 under control of the *trc* promoter. The purified recombinant enzyme is identical to natural XSODB as judged from electrophoretic mobility and by the identity of the absorption and EPR spectra, and of the N-terminal amino acid sequence. The recombinant protein was purified to homogeneity and crystallized by vapour diffusion techniques, using polyethylene glycol as precipitating agent (18% w/v), at pH 6.0, in phosphate buffer. The crystals grow as flattened prisms, of about 0.003 mm^3, in about 6 weeks, at 28° C.

X-ray diffraction data, to a limiting spacing of 3.0 Å were collected on an image plate area detector system (from MarResearch, Germany), installed on a Rigaku RU200 rotating anode generator. The collected intensities were reduced to 6,238 structure factors using the MOSFILM program suite; the R-merge factor for the 51,860 observed intensities, in the 15.0-3.0 Å resolution range (99.1% completeness) was 8.2%.

Solution of the three-dimensional structure was achieved by molecular replacement methods, using the program suite AMORE. The search model employed was that of Cu,Co bovine SOD dimer, previously refined in our laboratory. The molecular replacement search produced unambiguously unique peaks for both the rotational and translational functions. After rigid body refinement of the search dimer in XSODB unit cell, the crystallographic R-factor, calculated in the 15.0-3.0 Å resolution range, was 0.41. Inspection of the corresponding electron density shows characteristic features of XSODB amino acid sequence.

Furhter data collection and refinement of the three-dimensional structure is in progress together with the design and purification of novel variants obtained by site-directed mutagenesis.

PS–03.04.25 THE IRON CENTER IN RIBONUCLEOTIDE REDUCTASE Pär Nordlund, Anders Åberg, Ulla Uhlin & Hans Eklund, Department of Molecular Biology, Swedish University of Agricultural Sciences, Biomedical Center, S-751 24 Uppsala, Sweden

Proteins containing binuclear non-heme iron centers perform several functions which can also be made by heme containing proteins. Hemerythrin (myohemerythrin) reversibly binds oxygen, i.e. perform the same function as hemoglobin (myoglobin), monooxygenase chemistry can be performed by the non-heme methane monooxygenase as well as the heme-protein cytochrome P450. Tyrosyl radical containing proteins also exist in both groups - ribonucleotide reductase protein R2 and prostaglandin H synthetase respectively.

The crystal structure of the free radical protein R2 of ribonucleotide reductase has been determined by multiple isomorphous replacement and two-fold molecular averaging. The structure has been refined at 2.2 Å resolution to R=0.175. The subunit structure of the R2 protein has a fold where the basic motif is a bundle of eight long helices.

The R2 dimer has two equivalent binuclear iron centers. The iron centers are well buried in the subunits. Each iron center contains two ferric ions which are coordinated by Asp84, Glu115, His118, Glu204, Glu238 and His241. The coordination is octahedral for one of the ferric ions an distorted octahedral for the other. The tyrosine harboring the stable free radical is buried in the protein with its η-oxygen 5.1 Å away from the closest iron ion.

The R2 protein without iron, apoR2, is a precursor of active R2 and folds into a stable protein which is transformed into active R2 by ferrous ions and molecular oxygen. Diffraction data on apoR2 crystals was collected to 2.5 Å and the structure has been refined to a crystallographic R-value of 18.7 %. A comparison with the iron containing protein shows no large global differences. Differences found are local and mainly restricted to the former metal sites and their environment.

The removal of iron results in a clustering of four carboxylate side chains in the interior of the subunit. The distances between Asp84, Glu115, Glu204 and Glu238 are short, suggesting that some of them are uncharged. In the case of protein R2 the energy cost of the clustering of carboxylate side chains in the interior of the protein must have been accounted for by other means. Hydrogen bonding to polar side chains in the vicinity of the carboxyl residues partly reduces the effect of the charges. Most importantly, the folded state of the subunit is stabilized by extensive van der Waals interactions and hydrogen bonds between the unusually long helices of R2.

ApoR2 has a very strong affinity for four stable $Mn2^+$ ions. The manganese containing form of R2, named Mn-R2, has been studied by X-ray crystallography. It contains binuclear manganese clusters in which the two manganese ions occupy the natural iron sites and are only bridged by carboxylates from glutamates 115 and 238. Mn-R2 could provide a model for the active diferrous form of protein R2.

Guided by the three-dimensional structure of the R2 protein of E. coli ribonucleotide reductase, we have aligned the sequences of two different methane monooxygenases (MMO) with the sequences of the iron coordinating four helix bundle in R2. The model confirms that the central four helix bundle of R2 should be present also in MMO. The iron coordination is similar in MMO and R2 with two histidine ligands and four carboxyl acid ligands in both cases. The terminal carboxyl ligands appear to have less restrictions than the other ligands and may be Asp or Glu. In R2 only His 241 is hydrogen bonded by an Asp residue but in MMO both histidine ligand are probably bound by Asp residues. This may allow high

