PS-03.04.24 CRYSTAL STRUCTURE OF Xenopus laevis COPPER-ZINC SUPEROXIDE DISMUTASE B. Djinovic Carugo1, A. Coda1, C. Collery2, M. T. Cripps3, F. Polticelli1, A. Desideri1, G. Rustico1, M. Solognac1-4, D. di Girolamo4, G. Zanotti5, S. Tartamella6, E. Erba, Centro Ricerche, Nerviano, Italy, 4 Dip. di Biologia, Universita' di Roma "Tor Vergata", Italy, 5 Centro Biotechnologie Avanzate, Universita' di Genova, Italy, 6 Dip. di Genetica, Universita' di Pavia, Italy.

Cu/Zn superoxide dismutases (SODs) are highly stable enzymes, characterized by an extremely rapid reaction rate, due to the use of active site residues that form the active site. The crystallographic structure of human, bovine, spinach and yeast SODs 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 modelling.

XSODA has been expressed in E. coli inserting the whole coding sequence in plasmid pEAK32-2 under control of the trc promoter. The purified recombinant enzyme is identical to natural XSODA as judged by 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 mm3, in 6 weeks, at 28°C.

APRT and XANTHINE SUBSTITUTED ARABINONUCLEIC ACIDS FROM ALCALIGENES DENTRIFICANS. By Richard L. Kingston, Kerry A. Blackwell, Bryan F. Anderson1 and Edward N. Baker*, Department of Chemistry and Biochemistry, Macquarie University, Palmerston North, New Zealand.

The structures of both apo-arizin and Cd(II)-substituted arizin from Alcaligenes denticribium have been determined at 1.8 Å resolution. Crystals of apo-arizin were obtained by slow diffusion of solutions of the apo-protein, while the Cd(II) derivative was obtained by soaking apo-arizin crystals in 10 mM CaCl2. In both cases, Crystals were grown at 4°C. Data were measured on a CAD4 diffractometer, were merged with high-resolution synchrotron data obtained with a Weissenberg camera equipped with image plate detectors. One asymmetric unit contained 24 water molecules, 4 apo-arizin molecules and 2 partial occupancy (0.13 and 0.15) copper atoms. Cd(II)/apo-arizin = 0.168 for all data (23,332 reflections) in the range 10.0 - 1.8 Å, for a model comprising 1954 protein atoms, 247 water molecules, 4 apo-arizin, 2 partial occupancy (0.13 and 0.15) copper atoms and 2 water molecules.

Removal of copper, from arizin causes virtually no structural change. There is a slight inward movement of the lid sidechains such that the radius of the metal binding cavity shrinks from 1.3 in reduced arizin to 1.2 Å for oxidized arizin 1.1 Å. 1.2 Å for arizin. This is consistent with the observation that the geometry of the metal site is determined primarily by the constraints of the protein structure. There is no significant change in the outward movement of the ligand over 1.17 Å seen for apo-arizin from Pseudomonas aeruginosa. Cadmium binding again causes no significant change in the protein structure, the only movement involves the carboxyl oxygen ligand, which moves towards the metal to give a Ca-O distance of 2.7 Å, compared with a Ca-O distance of 3.1 Å in oxidized, Cu(II)-arizin (Baker, J. Mol. Biol., 1988, 223, 1071-1090). This demonstrates a limited flexibility in the metal site in response to the different coordination preferences of Cd(II).

PS-03.04.25 THE RIC NUCLEIC ACID MONOMER REDUCTASE. Per Nordlund, Anders Åberg, Ulla Uhlin & Hans Eklund, Department of Molecular Biology, Swedish University of Agricultural Sciences, Biomedical Center, S-751 29 Uppsala, Sweden.

Proteins containing non-nucleic acid iron centers perform several functions which can also be made by iron-containing proteins. Hemerythrin (a hemerythrin reversibly bind oxygen, i.e. perform the same function as hemoglobin, myoglobin), myoglobin and an extracellular protein which will bind to the phage repressor can be performed by the non-nucleic acid monomeric ferric iron as well as the single-stranded DNA binding enzyme P450. Tyrrosin radical containing proteins also exist in two groups: ribonucleotide reductase (RNR) and ribosomal H synthesis 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 to 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 helices. The R2 dimer has two equivalent heme iron centers which are well buried in the subunits. Each monomer contains contains two ferric ions and monooxygen and oxygen dioxane were also found.

The R2 protein without iron, apoR2, is a precursor of active R2 and forms 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 RValue 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 ions and their environment.

The removal of iron results in a clustering of four carbohydrate side chains in the interior of the subunit. The distance between Asp34, Glu115, Glu204 and Glu215 is 1.5 Å, suggesting that some of them are uncharged. When the case of protein R2 the energy cost of the clustering of the carbohydrate side chains in the interior of the protein must have been accounted for by other means. Hydrogen bond between the side chains in the vicinity of the carbohydrate residues partly reduces the effect of the charges. The most important, the folds state of the subunit is stabilized by extensive van der Waals interactions and hydrogen bonds between the uncharged 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 two manganese clusters in which the two manganese ions occupy the natural iron sites and are bridged by carboxylate ions from glutamates 115 and 238. Mn-R2 could provide a model for the active ferrous 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 manganese monooxygenases (MMA) with the sequences of the iron coordinating four helix bundle in R2. The model confirms that the four helix bundle of R2 should be present in both proteins and that the iron coordination in R2 is similar in MMA and R2. With two histidine ligands and two carboxylate ligands in both cases. The terminal carboxylate ligands appear to have less importance than the other ligands and may be Asp or Glu. In apoR2 only His 241 is hydrogen-bonded to an Asp residue but in MMA both histidine ligands are probably bound by Asp residues. This may allow high
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Transient oxidation states of the binuclear iron center in MMO. The
reduces the propionic oxygen binding site in MMO are significantly
smaller in MMO than in MO due to binding of both molecular oxygen
and methane at this site. The binding site is lined by residues Cys151,
Thr213, Ile217 and Lys219.

This binding site in MO can be involved in other reactions than
producing the radical species of the protein as shown by the mutant of
Mmo208 to Tyr. This Tyr is transformed into a catalytic in the oxygen
reduction and a blue protein with ferric heme interaction is created. The
structure of this mutant has been determined at 2.8 Å resolution. The
coordination geometry is changed significantly and the dopes 2O3 and
Glu208 become tetrahedral.

**PS-03.04.26 THE ROLE OF THE METAL AND IMPORTANT
ACTIVE SITE RESIDUES IN ENZYMATIC CATALYSIS OF
ZINC PROTEINASES.** By H. Feinberg, H. M. Greenblatt, O.
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Several series of crystallographic studies, combined with enzyme
kinetics, surface interactions and electrostatic potential analysis,
have been performed in order to determine the yet unknown general
mode of catalysis of zinc proteinases. For the detailed structural
analysis we chose the one-zinc carboxy-exo-proteinase bovine
carboxyamidase A (CPA) and the two-zinc amono-exo-proteinase
Streptomyces griseus amidoamidase (SGAP). In attempts to fully
understand the role of the zinc ions in these and other zinc
proteinases, and in zinc enzymes in general, several metal
derivatives have been prepared and their kinetic behavior studied.
These metal-substituted enzymes show very interesting pattern of
activities towards various peptidic and ester substrates. The catalytic
activity is shown to change dramatically with the particular metal
substituted and with the specific substrate chosen. Although some
 crystallographic studies of metal substituted zinc proteinase
derivatives have been carried out in the past, no satisfactory
 explanation has been given to the large kinetic deviations observed.
 Moreover, the specific role of the active site zinc ion(s) in both the
 binding of the substrate and the actual enzymatic hydrolysis has
 never been fully clarified for this family of metallo proteinases.
 We have prepared apo-CPA (zinc removed) in which no trace of
 zinc remains, and have used the resulting enzyme for the
 preparation of a series of metallo-CPA derivatives. These
derivatives were crystallized and their crystal structures were
determined at high resolution. In addition to the detailed structures
of the metallo-derivatives themselves, we have also analyzed the
structures of their complexes with a number of inhibitors and
reaction coordinate analogues.

The purpose of these studies was to clarify the role of the active site
metal in enzymatic catalysis and especially to ascertain whether the
observed changes in the activity of metallo-derivatives of CPA (or
zinc proteinases in genera) are due to differences in the way in
which the metal is bound to the enzyme, due to local changes in the
conformation of the active site, or whether different metals affect
the binding of substrates in various ways.

Similar studies were also carried out on the apo-enzyme, in order to
determine the effect of the zinc ion on the so-called "Michaelis-
Menten" complex, formed before the reaction takes place. We will
present the high resolution and refined structures of native-, apo-
and metallo-enzymes described above, both alone and in complex
with various effectors, and discuss the relevance of these data to the
role of zinc in biological catalytic activity. Results will be presented
of electrostatic potential calculations which are based on the
structure determined. These results indicate that in addition to the
roles of substrate binding and transition state charge stabilization,
the active site metal plays also an important role in long range
attraction of the substrate to the active site. Surface interactions in
the structures of the enzyme-effectors complexes provides will be
considered for the future rational design of inhibitors and second
generation analogues.

Prozymic crystallographic data will be presented for the calcium
activated amurcarapinidase SGAP. In addition to its importance for
the understanding of zinc proteinases this enzyme represents a new
class of low molecular mass amurcarapinidases of various potential
biotechnological applications.

**PS-03.04.27**

**Magnetostuctural behaviour of the superexchange coupled Cu-dimers**

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Some biomimetic molecules are formed by exchange coupled
Cu-dimers in the active site. The kind of diamagnetic ligands
between the paramagnetic copper can vary.

The contribution is summarising the related magnetostuctural
results even over extended bridges (\( \sim 1 \)), in general different
ligands, e.g. hydrogen bridges.

Theoretical calculations of the exchange coupling parameters are
presented for long distance bridges, oximate groups and hemochromogen
model compounds.

Some new crystal structures on Cu-oligomers are presented and
discussed.

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