STRUCTURE OF FALS MUTANT CuZnSOD D125H: IMPLICATIONS BICARBONATE'S ROLE IN PEROXIDATIVE REACTIONS CATALYZED BY CuZnSOD

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Over 90 distinct single site mutations in the enzyme copper-zinc superoxide dismutase (CuZnSOD, SOD1) have been individually linked to the inherited (familial) form of the neurodegenerative disease amyotrophic lateral sclerosis (FALS, known also as Lou Gehrig's disease). We recently determined the X-ray structure of the FALS CuZnSOD mutant D125H to 1.4 Å resolution using synchrotron radiation and single-wavelength anomalous diffraction (SAD) phasing methods.

The nearly metal-free, 'as isolated' D125H crystallized from a solution containing 10 mM zinc sulfate. Surprisingly, zinc is unambiguously observed in both the copper- and the zinc-binding sites. The zinc in the zinc-binding site is coordinated in a fashion identical to that of the wild type protein. The zinc in the copper site is tetrahedrally coordinated to His46, His48, His120, and an oxygen atom of a sulfate anion. Another sulfate oxygen atom accepts hydrogen bonds from the epsilon and guanidinium nitrogens of Arg143, the residue primarily responsible for electrostatic guidance of the superoxide substrate into the active site channel. This region likely serves as a binding site for other anions such as bicarbonate that in turn participate in the radical inactivation of CuZnSOD, or in the oxidation of exogenous substrates as previously observed in multiple EPR studies performed on the native and mutant enzymes.

The D125H structure also reveals little electron density for the region from residues 127 to 140 that is important in maintaining the structural integrity of SOD1, in directing the substrate to the active site, and in sterically preventing large non-native substrates from gaining access to the catalytic copper ion.

Keywords: Cu Zn SUPEROXIDE DISMUTASE, METALLOPROTEIN, BICARBONATE

STRUCTURAL STUDIES OF PLASMINOGEN ACTIVATOR INHIBITOR TYPE 2

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Plasminogen activator inhibitor 2 (pai-2) is a member of the serpin superfamily. Pai-2 is an effective inhibitor of urokinase (upa) and an inhibitor of tissue-type plasminogen activator (tpa).

The mechanism of inhibition involves a profound change in conformation, which is called the stressed-to-relaxed transition (S-R). One of the aims of the present study is to define the structural features, which make this transition feasible. A modified form of PAI-2, the 66-98 loop deletion mutant was crystallised in the relaxed form in the orthorhombic space group $P2_12_12$ with unit cell parameters a = 91.75 Å, b = 103.71 Å, c = 41.13 Å. The relaxed form was obtained by annealing a synthetic RCL peptide into the protein.

The structure of PAI-2 in a complex with a peptide mimicking reactive center loop (RCL) has been determined at 1.6Å resolution. The structure shows the serpin in a relaxed state with the peptide inserted as an extra β-strand and allows the examination of the transition at an atomic level. The S-R transition in PAI-2 has been modeled as the relative motion between a quasirigid core domain and a smaller segment comprising helix hF and β strands s1A, s2A and s3A. A comparison of the Ramachandran plots of the stressed and relaxed state structures of PAI-2 reveals the hinge regions connecting these two domains.

Keywords: **SERPIN, PLASMINOGEN ACTIVATOR INHIBITOR TYPE 2, STRUCTURE**

CHARACTERIZATION OF TRUNCATED TUMOR-ASSOCIATED NADH OXIDASE (TTNOX)

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Bacterial, plant and animal cells possess novel surface proteins that exhibit both NADH oxidation (NOX) or hydroquinone and protein disulfide-thiol interchange. These enzymatic activities alternate to yield oscillating patterns with period lengths of approximately 24 minutes. The catalytic period of NOX proteins are temperature compensated and gravity responsive. We report the cloning, expression and characterization of truncated tumor-associated NADH oxidase (ttNOX), in which the membrane spanning region has been deleted.

The cDNA (originated from HeLa cells) was cloned into pET-34b and pET-14b (Novagen) vectors for *E. coli* expression. Optimized expression and purification protocols yielded greater than 300mg per liter of culture with greater than 95% purity. Circular dichroism data was collected from a 2.7 mg/ml solution in a 0.1mm cuvette with variable scanning using an Olis RSM CD spectrophotometer. The ellipticity values were scanned from 190 to 260 nm. The spectra recorded have characteristics for alpha proteins with band maxima at 216 nm and a possible shoulder at 212nm at 12°C and 25°C. Protein crystal screens are in progress and, to date, only small crystals have been observed.

The regular periodic oscillatory change in the ttNOX protein is indicative of a possible time-keeping functional role. A single protein possessing alternating catalytic activities, with a potential biological clock function, is unprecedented and structural determination is paramount to understanding this role.

Keywords: ENZYME, CD SPECTRA, PROTEIN EXPRESSION

THREE DIMENSIONAL STRUCTURE OF THE SUC1 MUTANT P90AP92A

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Cyclin-dependant Kinase Subunit (Cks) proteins are essential for mitosis, however their precise function has eluded researchers for two decades. Two conformations of Cks have been detected crystallographically, a compact monomer with the C-terminal fourth β-strand inserted into the core of the molecule between strands 2 and 3, and a strand-exchanged dimer in which the fourth β-strand is inserted into the core of the dimer partner in an equivalent position. There is an absolutely conserved "hinge" region, consisting of the motif PEP, N-terminal to the fourth β-strand. In the monomer this motif constitutes a β-turn while in the dimeric structure it is extended, allowing strand exchange. The mutant protein, $p13^{suc1P90AP92A}$, in which alanine residues replace both prolines of the turn, provides an opportunity to examine the role of prolines in this conformational plasticity. We have expressed and purified this mutant protein. 2mM $p13^{suc1P90AP92A}$ crystallised in 50mM Tris pH7.5, 30% PEG 1500K. Diffraction data were collected at room temperature on a MAR345 image plate using Cu Kα; radiation from a Rigaku RU200 rotating-anode generator source. The structure was solved to 2.7Å in space group P6(3); unit cell parameters were found to be a=b=75.1, c=34.9, α=β=90, γ=120. The average deviation of residue positions in the mutant "hinge" region from those of their wild type equivalents was 5.75Å, supporting the proposal that pyrrolidine side chains of proline residues in the PEP motif impose strain in the turn, providing a mechanism for strand extension leading to the formation of interdigitated dimers.

Keywords: **CKS-PROTEINS CELL-CYCLE P13SUC1**