P.04.08.1

Acta Cryst. (2005). A**61**, C226

Crystal Structures of *ox* and *sq* S64C Flavodoxin (*D. vulgaris*) Monomer and Dimer

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The crystal structures of the monomer and homodimer of S64C mutant from *D. vulgaris* in the oxidized and semiquinone states have been determined, at 1.80Å resolution with diffraction data at 100K, by molecular replacement using as starting model the wild type structure [1]. The interest for the new mutant S64C is due to the location of Cys64 in a key region for the interaction with the cofactor FMN and in a position favourable to the formation of homo- and heterodimers.

The structures of *ox* and *sq* S64C monomer crystallizes in the space group $P4_32_12$ and have the general fold of flavodoxin family. The pattern of hydrogen bonds between the protein and FMN is similar to that of the wild type. The main structural differences between the *ox* and *sq* monomer are in the loop-60, that is involved in a new hydrogen bond with the cofactor upon reduction [2]. The two forms of the dimeric S64C mutant crystallize in the space group $P4_12_12$ and the dimer link is due to Cys64, that makes the disulfide bridge with a simmetry related mate. These structural studies provide the seminal information towards a better understanding of the role of the protein moiety in tuning the redox potential and, therefore, the electron transfer.

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Keywords: flavodoxin, S64C mutant, disulfide bridge

P.04.08.2

Acta Cryst. (2005). A**61**, C226

Role of a Scaffold in the Inhibitory Process of a Serine Protease Inhibitor

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The inhibitory loop of a serine protease inhibitor has a characteristic conformation while the remaining part of the molecule, known as scaffold, has widely different folds in different families of inhibitors. To understand the exact contribution of the 'inhibitor scaffold' towards the inhibition process, a classic β-trefoil fold protein, Winged bean Chymotrypsin Inhibitor (WCI), has been chosen. Owing to the crucial strategic location, as seen in our previous crystallographic and molecular dynamics studies, a scaffolding residue Asn14 has been targeted for mutagenesis by residues of different shapes and charges and the ability of chymotrypsin inhibition by the resulting mutants has been measured. Crystal structures of the mutants were determined and it was observed that the degree of loop deformation is inversely proportional to the extent of chymotrypsin inhibition.

Similarly, through mutations in the WCI loop region, two chimeric proteins are attempted with loops of trypsin inhibitors like ETI and STI on the scaffold of WCI. A comparison of binding constants of these chimeric proteins with their respective wild type ones can be used to understand whether the scaffold of WCI is best suited for chymotrypsin inhibition or it can be used for trypsin inhibition as well. As a first step towards this approach, we found that the single mutation (Leu 65→Arg) at P1 converts WCI to a potent inhibitor of trypsin with a K_i value comparable to ETI and STI indicating that the role of the scaffold of WCI is comparable to that of ETI and STI. Structure of this mutant (L65R) at 2.15Å provides a clue to this altered inhibition.

Keywords: serine-protease inhibitor, mutational analysis, inhibitor design

P.04.08.3

Acta Cryst. (2005). A**61**, C226

Sructure Determination of sc*Pvu*II by Crystallographic and SAXS Methods

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*Pvu*II is the first restriction endonuclease which has been converted from its wild-type (wt) homodimeric form into a single chain (sc) protein by tandemly joining the two subunits through the peptide linker GlySerGlyGly. The DNA cleavage activity of the enzyme is thereby largely retained [1]. The single-chain enzyme provides a scaffold for the development of asymmetrically modified restriction endonucleases.

Crystals of sc*Pvu*II, obtained by the hanging drop vapor diffusion method, were measured at EMBL/DESY (X11 beamline). The crystals diffract to a resolution of 2.35Å and belong to space group $P4_2$ with the unit cell parameters a=b=102.0, c=100.3 Å and two molecules in the asymmetric unit. The crystal structure was determined by molecular replacement method using the AMORE program, using the DNA-binding subdomain (residues 36-157) of the wt*Pvu*II monomer [2] as search model. The crystal structure shows that sc*Pvu*II adopts a more compact conformation compared to the wt form. SAXS measurements at EMBL/DESY (X33 beamline) confirm this result.

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Keywords: restriction endonucleases, sc*Pvu*II, SAXS

P.04.08.4

Acta Cryst. (2005). A**61**, C226

Structure of NADH: Quinone Oxidoreductase from *Acidianus ambivalens*: Electron Entry Point of Aerobic Respiratory Chain

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NADH:quinone oxidoreductases (NDHs), constitute one of the electron entry points into membrane bound respiratory chains, oxidising NADH and reducing quinones. Type-II NDHs are functionally unable to translocate protons and are typically constituted by a single ~50 kDa subunit lacking iron-sulfur clusters and containing one flavin as redox centre [1]. NDH was isolated from the membrane fraction of *Acidianus ambivalens*, a thermoacidophilic archaeon capable of growing at 80º C and pH 2.0, as an enzyme of 47 kDa, containing a covalently bond flavin with a reduction potential of ~70 mV [2,3].

The membrane-bound NDH was crystallized using ammonium phosphate as precipitant at pH ~ 5. Crystals belong to the hexagonal space group ($P6_522$), with cell parameters a=b=178.76 Å and c=162.57 Å. We describe the first X-ray structure of a novel type-II NDH at 2.6 Å resolution, solved by MIRAS, which will contribute to a better understanding of the catalytic and electronic transfer mechanism.

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Keywords: membrane protein, respiratory chain, extremophile

P.04.09.1

Acta Cryst. (2005). A**61**, C226-C227

X-ray Structural Analysis of Carbonic Anhydrase from *Chlamydomonas reinhardtii*

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