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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.

[1] Artali R., Bombieri G., Cavazzini D., Meneghetti F., Gilardi G., Rossi G.L., Sadeghi S.J., *Acta Cryst. D*, 2002, **58**, 1787. [2] Watt W., Tulinski A., Swenson R.P., Watenpaugh K.D., *J. Mol. Biol*., 1991, **218**, 195.

Keywords: flavodoxin, S64C mutant, disulfide bridge

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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

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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.

[1] Simoncsits A., et al., *J. Mol. Biol*., 2001, **309**, 89-97. [2] Athanasiadis A., et al., *Stuct. Bio.*, 1994, **1**, 469-475.

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

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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.

[1] Yagi T., et al., in *Respiration in Archaea and Bacteria*, 1st Ed., Kluwer Publishing, Germany, 2004, 15. [2] Gomes C. M., Bandeiras T. M., Teixeira M., *J. Bioenerg. Biomembr*., 2001, **33**, 1. [3] Bandeiras T. M., et al., *FEBS Letters*, 2002, **531**, 273.

Keywords: membrane protein, respiratory chain, extremophile

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

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Carbonic anhydrase (CA) is a zinc-containing enzyme that catalyzes the reversible hydration of CO_2. Based on the amino acid sequences, CAs are classified into three evolutionarily distinct families, designated α-, β- and γ-CAs. CA from *Chlamydomonas reinhardtii* belong to the α type enzyme and, homologous to CA from animal and eubacteria source. To reveal the structure, we have been engaged on X-ray study of *Chlamydomonas* CA. The enzyme was extracted from Cultured *Chlamydomonas* cells and highly purified by column chromatography [1]. The enzyme is composed of two subunits, small and large. Crystals were obtained in a solution containing ammonium sulfate. X-ray diffraction data were collected at 100K with synchrotron radiation at NW12 of PF-AR (Tsukuba). Diffraction patterns were processed with the program HKL2000. The crystal diffracted to a maximum resolution of 2.4Å. The unit-cell dimensions were a=b=134.6 and c=120.0Å with a space group of $P6_1$ or $P6_5$. Initial phases were derived by the molecular replacement method using the atomic coordinates of CA from *Neisseria gonorrhoeae*, which has a sequence identity of 37 % with the present CA.

[1] Yang S-Y., Tsuzuki M., Miyachi S., *Plant Cell Physiol*.,1985, **26**, 25-34.

Keywords: carbonic anhydrase, *Chlamydomonas reinhardtii*, X-ray crystal structure analysis

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Crystallographic Study of Fructokinase from *Sulfolobus tokodaii* strain7

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Fructokinase (FK) (ATP:D-fructose-6-phosphotransferase, EC 2.7.1.4) catalyses the phosphorylation of fructose-6-phosphate with ATP. This enzyme is essential for removing fructose to prevent the reverse reaction, in glycolytic pathway. The crystal structures of FK were reported only for prokaryote, *Bacillus subtilis* and *Salmonella enterica*. To reveal the tertiary structure of FK from an Archaea *Sulfolobus tokodaii* strain7, we have performed X-ray analyses of the protein. *Sulfolobus* FK produced in *E. coli* was purified by column chromatography, and crystallized. X-Ray diffraction data taken at 100K show that there are two forms with $P2_12_12_1$ and $P6_122$. Their initial crystal structures were derived by molecular replacement with 2-keto-3-deoxygluconate kinase from *Thermus thermophilus*, and refined to 2.8Å and 1.85Å resolutions, respectively. The two tertiary structures are similar to each other, but quite different from that of *Bacillus* FK with a little bit high sequence identity (29%). The present structures are rather similar to *Salmonella* FK though the sequence identity is low at 27%. An interesting feature is that although the function is different from *Thermus* 2-keto-3-deoxygluconate kinase, but the two tertiary structures are similar at higher sequence identity (35%).

Keywords: fructokinase, *Sulfolobus tokodaii* strain7, X-ray crystal structure analysis

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Crystal Structure of Translation Initiation Factor IF2beta-IF2gamma Complex

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IF2 is a heterotrimeric GTPase composed of alpha, beta, and gamma subunit. It has been identified in both of eukaryotes and archaea. The gamma subunit is core subunit which binds Met-$tRNA_i^{Met}$ and GTP. The beta subunit was reported to interact with eIF5 that stimulates GTP hydrolysis, and also interact with Met-$tRNA_i^{Met}$ and mRNA [1,2], while alpha subunit functions as a regulatory element of IF2.

We have determined the structures of aIF2beta-aIF2gamma and aIF2beta-aIF2gamma-GDP complex

The aIF2beta subunit was located at G-domain of aIF2gamma and was close to GTP-binding site. The detail of the complex structure will be discussed and the model of ternary complex IF2-GDP-Met-$tRNA_i^{Met}$ will be given in this presentation.

[1] Das S., Maiti T., Das K., Maitra U., *J. Biol. Chem.*, 1997, **272**, 31712. [2] Laurino J. P., Thompson G. M., Pacheco E., Castilho B. A., *Mol. Cell. Biol.*, 1999, **19**, 173.

Keywords: translation, translation initiation factor, IF2

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Fusidic Acid Resistance and Sensitivity in Ribosomal Elongation Factor G

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Elongation factor G (EF-G) catalyzes translocation in protein synthesis on the ribosome. EF-G is inhibited by Fusidic acid (FA), a commonly used antibiotic. Structural information on FA binding to EF-G has not yet been available.

We present three crystal structures of two mutant EF-G factors; G16V, highly FA sensitive, and T84A, highly FA resistant. The crystal structures provide a first insight into the conformational changes induced by GTP binding and how this affects FA binding. These structural conformations show the general importance of the interface of domain G, III and V as a key component of the FA binding site and the specific role of Phe90 as a gatekeeper and conformational regulator. We provide an explanation on how EF-G is able to discriminate between GDP and GTP.

Keywords: protein synthesis, translation factors, antibiotic resistance

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The *thrS* Messenger Path on the Ribosome

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Initiation of translation generally determines the efficiency of protein synthesis, and is the key step for the control of gene expression when fast adaptation is required. Binding of the messenger to the eubacterial ribosome involves several mRNA elements, which affect the kinetics of the initiation complex formation. These elements may include binding sites for translational regulatory proteins. *E. coli* threonyl-tRNA synthetase (ThrRS) plays such a role, as it inhibits its own synthesis by binding to *thrS* mRNA in a region located upstream from the ribosome binding site.

Here we show the path of *thrS* mRNA on the ribosome in the presence of the initiator $tRNA^{Met}$ at 5.5 Å of resolution determined by X-ray crystallography. Our data show the first visualization of a translational regulatory domain of mRNA and explain how a repressor protein can interfere with initiation of ribosome translation.

Keywords: ribosome structure and function, tRNA synthetases, regulation

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Productive and Non-productive Binding of Polyketides to the Ribosome Large Subunit

Rita Berisio[a], Maya Amit[b], David Baram[b], Joerg Harms[c], Anat Bashan[b], Harly Hansen[c], Frank Schluenzen[c], Ada Yonath[b], [a]*IBB,*