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Keywords: proteases, crystallization, X-ray structure

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Do the Strong Magnetic Fields Modify the 3D Structure of Proteins?
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In this work we explore the feasibility of a new method to increase the crystal quality of biological macromolecules for X-ray crystallography. The method consist of growing protein crystals in capillary tubes containing a gelled protein/precipitant solution under the presence of a strong magnetic field of 10 Tesla. The strong magnetic field applied to the crystal growth cell was the conventional NMR magnet normally used in Chemistry laboratories.

From our preliminary results by solving the 3D structure of these analyzed protein crystals and controls, it was observed that crystals grown under the presence of a strong magnetic field in gel/capillary tubes improved substantially their electron density maps where electron density was not observed in all controls.

This promising methodology will help most of biocrystallographers to increase the crystal quality, a typical problem in most of the research laboratories for structural biology. Because of the existence of two different gels (double protective-chamber), this methodology seems to be also an available way to transport safely crystals to the synchrotron facilities without using the classical heavy laboratory Dewars for data collection.
Keywords: biomacromolecule X-ray, crystal growth from solution, crystal perfection

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Crystal Structure of the NADP-dependent 3-hydroxyisobutyrate Dehydrogenase
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3-Hydroxyisobutyrate, a central metabolite in the valine catabolic pathway, is reversibly oxidized to methylmalonate semialdehyde by a specific NAD/NADP-dependent dehydrogenase. To gain insight into the function of this enzyme at atomic level, we have determined the first crystal structures of 3-hydroxyisobutyrate dehydrogenase from *Thermus thermophilus* HB8: holo enzyme, 3-hydroxyisobutyrate complex, and sulfate ion complex. The crystal structures reveal a unique tetramer consisting of four identical protomers. The protomer folds into two distinct domains with open/closed interdomain conformations. The cofactor NADP(H) and the substrate 3-hydroxyisobutyrate are bound at the cleft between the two domains of the closed protomer. The observed tetramer structure might be important for the catalytic function through forming the active site involving two adjacent subunits. A kinetics study confirms that this enzyme has strict substrate specificity for 3-hydroxyisobutyrate and serine, but it cannot distinguish the chirality of the substrates. This enzyme prefers the physiological cofactor NADP rather than NAD. We propose a reaction mechanism based on the structures of cofactor/substrate bound at the cleft; Lys^{165} is the probable catalytic residue of the enzyme.

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Keywords: 3-hydroxyisobutyrate, 3-hydroxyisobutyrate dehydrogenase, MAD

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Visualization of the Forward and Reverse Reactions Catalyzed by Nitrite Reductase
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The process of denitrification involves the sequential reduction of nitrate (NO_3^-) and nitrite (NO_2^-) to dinitrogen (N_2). The nitrite reductase from *A. faecalis* (NiR) is a green 110 kDa homotrimer with each monomer containing one type I and one type II copper (Cu) sites. The type I is the site of electron transfer from pseudoazurin. Electrons are then donated internally to the type II Cu site, where NO_2^- is reduced to NO. Crystals of NiR are orthorhombic with a trimer in the asymmetric unit.

To visualize the product bound at the active site, ascorbate *reduced* crystals of NiR were exposed to a NO saturated solution and frozen in liquid N_2 in the absence of oxygen. Data were collected at SSRL to 1.3 Å resolution. After refinement at full occupancy, the average B factor for NO is 29 $Å^2$, similar to that observed for water bound to the resting state of the enzyme. The N and O atoms of NO are equidistant from the Cu, thus the Cu-nitrosyl of NiR is characterized with side-on coordination of a diatomic molecule [1].

To examine the ability of the enzyme to catalyze the reverse reaction, *oxidized* crystals of NiR were exposed to a saturated NO solution. Refinement of the structure to 1.4 Å revealed nitrite bound to the copper via its oxygens, indicating completion of the reverse reaction in crystal. Spectroscopic studies [2] further support the conclusion that Cu-NiR can catalyze the reverse reaction.

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Keywords: structures of metalloproteins, copper complexes, nitric oxide

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The Structural Basis for Ser/Thr Protein Phosphatase Inhibition
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Serine/Threonine Protein Phosphatases are important in many cellular processes including glycogen metabolism and immunosuppression. Many marine prokaryotic organisms produce structurally diverse phosphatase inhibitors that can be toxic. The surface of Ser/Thr Phosphatases contain an inhibitor-binding loop which is important in inhibitor activity. How this loop determines inhibitor-specificity is unknown. We have solved the structures of Protein Phosphatase-1 (PP1) bound to four marine natural product inhibitors: okadaic acid, motuporin, clavosine and microcystin-LA(2H)[1]. These inhibitors bind in a similar manner to the phosphatase, exhibiting analogous interactions and showing no structural rearrangement of the inhibitor-binding loop. A structure solved using a mutant PP1, where the inhibitor-binding loop from calcineurin has been substituted for the native loop, reveals repositioning of only specific amino acid side-chains. These results indicate that inhibitor specificity in Ser/Thr Phosphatases is most likely due to specific interactions within the inhibitor-binding loop and not structural rearrangements. There are observable differences in the binding of inhibitors to PP1 and the PP1-calcineurin hybrid, information that may be utilized in the design of new immunosuppressant calcineurin inhibitors.

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Keywords: protein phosphatase, marine natural products, enzyme inhibitor design

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Active Site Structure of Actinorhodin Polyketide (*act* III) Reductase

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Polyketides produced by bacteria and other organisms include antibiotics, anticancer and antifungal compounds. In Type II polyketide synthesis, a minimal system of three enzymes are sufficient to form a polyketide of the requisite chain length, one of which is acyl carrier protein (ACP) which mediates transport of pathway intermediates between the proteins. Addition of ketoreductase (KR) to this system results in the correctly cyclised and reduced product. We have determined the 2.5 Å crystal structure of the tetrameric polyketide Type II ketoreductase, a member of the SDR family, with its cofactor $NADP^+$. Of two subunits in the crystallographic asymmetric unit, one is 'closed' around the active site. Formate is observed in the other 'open' subunit, indicating possible locations for substrate binding. A model for the binding of ACP has been constructed, based on observed non-crystallographic contacts. Based on these observations, we hypothesize that approach and binding of ACP triggers a conformational change from the closed to the open, active, form of the enzyme that allows the polyketide chain to enter the active site and be reduced. The model suggests a mechanism for ACP recognition which is applicable more generally to a range of protein families with NAD(P) cofactors which rely on ACP to provide the substrate. Substrate analogue soaks are being carried out to elucidate the binding of the substrate in the active site.

Keywords: ketoreductase, polyketide synthesis, ACP

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The MutT Crystal Retains the Ability to Hydrolyze 8-oxo-dGTP

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Escherichia coli MutT specifically hydrolyzes a potently mutagenic and DNA replicative substrate, 8-oxo-dGTP to 8-oxo-dGMT and pyrophosphate in the presence of Mg^{2+} (Mn^{2+}) so as to prevent misincorporation of 8-oxoguanine (8-oxoG) opposite adenine and A:T to C:G transversion by the resulting A:8-oxoG mispair. Recently we have determined the crystal structures of MutT in the presence and in the absent of the reaction product 8-oxo-dGMP. The structures reveal that MutT specifically recognizes 8-oxo-dGMP through a wealth of hydrogen bonds to the protein and waters in the binding pocket with the large ligand-induced conformational change. The catalytic mechanism of MutT still remains unclear.

In this paper we report the crystal structure of MutT in complex with its substrate, 8-oxo-dGTP at 1.8 Å resolution. The structure confirms the substrate induced conformational change and sodium ions bound to the triphosphate moiety and residues in the MutT (Nudix) motif. In order to elucidate the reaction mechanism of MutT, the MutT-8-oxo-dGTP crystals were soaked in $MnCl_2$ under a variety of conditions and freeze-trapped. The crystal soaked in 2mM $MnCl_2$ for 2 days showed that a manganese ion occupied one sodium ion site in the two ones. When the crystals were soaked in 20mM $MnCl_2$ for 4 hours, the substrate was perfectly hydrolyzed. The trial of the catching the intermediate state of catalysis is in progress. These results would provide insights into the hydrolysis mechanism.

Keywords: hydrolysis, reaction snapshot, DNA repair

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Crystal Structure of Recombinant Human Cyclophilin J and its Complex

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Cyclophilins (CyPs) are a large class of highly conserved ubiquitous peptidyl-prolyl cis-trans isomerase. CyPs have also been identified to be a specific receptor for the immunosuppressive drug cyclosporin A (CsA). CyPJ is a novel member of the CyP family, and human CyPJ (hCyPJ) is the protein encoded by a cyclophilin-like gene from human fetal brain. The three-dimensional structure of recombinant hCyPJ has been determined by molecular replacement using the cyclophilin A (hCyPA) structure as the search model and has been refined at 2.6 Å resolution. The hCyPJ molecule contains four helices and one β-barrel composed of eight antiparallel β-strands. The overall secondary and tertiary structures of hCyPJ are similar to those of hCyPA, but hCyPJ contains an additional disulfide bridge and four segments with conformations that are strikingly different from those of hCyPA. His43 and Gln52 of hCyPJ are expected to be the active sites based on sequence alignment with hCyPA. The hCyPJ structure shows a conserved water molecule close to His43 and Gln52, which appears to support the solvent-assisted mechanism. The crystal structure of hCyPJ in complex with CsA has been determined by molecular replacement and the refined structure will be presented. The crystallization of the complexes of hCyPJ with various ligands is in progress.

Keywords: cyclophilin J, mechanism, complex

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Structural Studies of an Antibiotic Resistance Factor

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The number of multidrug resistant microorganisms has increased in clinical settings and the danger of new pathogenic bacterial strains spreading has placed emphasis on understanding the biochemical basis of resistance. One class of unique last resort antimicrobials is the streptogramins which consist of naturally occurring macrocyclic lactone ring compounds. This family is comprised of Group A and Group B compounds which independently are bacteriostatic against Gram-positive bacteria acting to decrease protein production. However, in combination they exhibit synergistic bactericidal effects due to the permanent inhibition of peptide bond formation in the 50S ribosomal peptidyl transferase centre of prokaryotes.

Resistance to the Group B streptogramins can be conferred enzymatically by cleaving the ring structure of these small peptide drugs. The enzyme responsible is found both on chromosomal DNA and on bacterial plasmids allowing for potential quick dissemination of resistance. Recently, selenomethionine crystals were obtained for this enzyme by hanging drop diffusions method. Diffraction data were collected at the NSLS (Upton, NY) to 1.6Å resolution. Difficulties in solving the structure were encountered as a result of heavy atoms falling close to special positions and extensive internal symmetry of the protein structure. Neither MAD nor molecular replacement on their own were sufficient in obtaining phasing information thus the two techniques were employed in combination to solve the structure. Here we present the high resolution three dimensional structure of streptogramin B linearizing enzyme using X-ray crystallographic techniques and place it in a biological context.

Keywords: antibiotic resistance, enzyme, crystal structure