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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