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Structural Study of DNA Binding Protein pdcd5 from *Sulfolobus solfataricus*
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The Sso0352 (pdcd5) gene of the hyperthermophilic archaeon S*ulfolobus solfataricus* was predicted to encode a hypothetical DNA binding protein which is homologous to the apoptosis- related protein Tfar19 in human leukemia cell line. The full length of pdcd5 protein was expressed to a large scale in bacteria system successfully and its DNA-binding property was assessed by gel retardation experiments. With the ability to bind dsDNA in a sequence-general manner, the exact role of pdcd5 in the organism needs to be further investigated. The secondary structure was preliminarily analyzed to be an all helix structure by using circular dichroism and ^{1}H NMR spectrum. The pdcd5 crystals belong to the space group C2 with unit-cell dimensions of a = 79.7 b = 36.8 c = 36.99 Å, β= 94.45°. To understand the DNA binding feature, structure determination of pdcd5 protein and its complexes with DNAs are in progress.
Keywords: DNA binding protein, hyperthermophilic archaea, X-ray crystallography

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Serendipitous SAD Phasing
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We describe here the solution of the structure of a bisintercalator-DNA complex with the help of an anomalous scatterer fortuitously present in the structure.

The DNA bisintercalator Echinomycin is characteristic of a family of anticancer drugs that interfere with both replication and transcription. The crystal structures of Triostin A[1] and Echinomycin[1] in complex with (CGTACG)2 showed for the first time Hoogsteen base pairing in a double stranded DNA.

In our laboratory we grew crystals of the complex of Echinomycin with two other sequences: (GCGTACGC)2 and (ACGTACGT)2, which show also the canonical Hoogsteen base pairing outside the intercalation site. Over time, a different crystal form grew in the the same drops whose structure could not be solved by molecular replacement. A native high resolution dataset was collected at BL14.1 at BESSY on this crystal form and showed a weak but significant anomalous signal although no heavy atom was included in the crystallization conditions. SAD phasing showed one single heavy atom site from which an interpretable electron density map could be obtained. The heavy atom proved to be a mixture of metals of unknown origin.

The structure shows a mixture of the canonical Hoogsteen base pairing together with the Watson-Crick mode that was suggested by NMR data, showing great flexibility of the DNA duplex in accommodating the bisintercalation and that Hoogsteen base pairing is not a necessary condition for Echinomycin binding. It offers a detailed three-dimensional model for molecular dynamics simulations of binding for both Hoogsteen and Watson-Crick base pairing around the intercalation site.

[1]Ughetto G., Wang A.H.J., Quigley G.J., van der Marel G.A., van Boom J.H., Rich A., *Nucl. Acids Res*., 1985, **13**, 2305.
Keywords: SAD, bisintercalation, Hoogsteen/Watson-Crick base pairing

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Altered Structure and Function of a Beta-retroviral dUTPase
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dUTPase prevents uracil incorporation into DNA and contributes to dTTP biosynthesis. It is indispensable for keeping the appropriate high dTTP/dUTP ratio, essential for cell survival. Its vital character makes it a promising anticancer and antiviral drug target. The protein forms homotrimers with three identical active sites located between two monomers and closed by a flexible C-terminal arm reaching over from the distant third monomer[1].

The economic beta-retroviruses however encode for a shortened dUTPase with compromised catalytic efficiency[2]. Sequence alignments revealed that the gaps accumulate at the beginning of the C-terminal segment, suggesting serious difficulties in reaching the targeted active site. To explain how the enzyme preserves its catalytic ability for dUTP hydrolysis, we combined structural analysis with enzyme kinetics, mutational techniques and modeling approach. Crystal structures of wild type and a truncated mutant dUTPase have been determined in unliganded form, and substrate analogue and product complexes. Structure-based molecular modeling predicted the catalytically relevant conformation of the C-terminal arm. Results propose localization of the C-terminal catalytic residues to the active site formed by the same subunit, and offer insights into the role of the C-terminal arm in catalysis.

[1] Barabas O., et al., *J. Biol. Chem*., 2004, **279**, 42907. [2] Barabas O., et al., *J. Biol. Chem*., 2003, **278**, 38803.
Keywords: DNA repair enzymology, enzyme mechanism, evolution

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Y225F/A for Met-tRNA Synthetase Reveals Importance of Hydrophobic Circumstance
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In *Thermus thermophilus* Met-tRNA synthetase, CP1 domain laps the entrance region of the hydrophobic pocket, on which Met binds. In order to investigate a possible role of this lapping in creation of hydrophobic circumstance around the enzymatic active center, *K*m values of substrates and *k*cat value for aminoacylation reaction for tRNA were comparatively measured for mutants Y225F and Y225A of Tyr225, which locates inside the ribbon portion of CP1 domain but out of the hydrophobic pocket for Met-binding. The crystal structures of these mutants determined were also closely compared with that of the wild type protein. Observed *K*m values for Met and tRNA are 27 and 4.7ìM for Y225F, 1300 and 11 ìM for Y225A and 30 and 4.5 ìM for wild type protein, respectively. On the other hand, observed *k*cat are 0.59 sec^{-1} for Y225F, 1.4 sec^{-1} for Y225A and 17 sec^{-1} for wild type, respectively. Remarked effect on the binding affinity for Met induced by Y225A may be due to the decreased hydrophobicity caused by mutation from Tyr to Ala, whereas little effect observed in Y225F reflects comparable hydrophobicity between Phe and Tyr. The decreased hydrophobicity may affect the conversion process of hydrated form of Met into un-hydrated form thereof, which process is a precedent step to Met loading into the hydrophobic pocket of the enzymatic center. In contrast, the considerably decreased kcat appears to suggest that the presence of the phenolic hydroxyl group of Tyr may play some determinant role in the coming-up step of the 3' terminal CCA of tRNA to the carbonyl group of Met.
Keywords: aminoacyl-tRNA synthetases, tRNA, mutations