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STRUCTURE OF THE RTP/DNA COMPLEX AND ITS ROLE IN POLAR FORK ARREST

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The arrest of replication forks and the coordinated termination of DNA replication in *Bacillus subtilis* is dependent upon the binding of the replication terminator protein (RTP) to its cognate DNA binding site (Ter site). The cooperative binding of two RTP dimers to the Ter site is able to arrest the helicase catalyzed unwinding of duplex DNA in a polar manner. A complex between a 15N-labelled mutant form of RTP and a symmetrical form of a DNA binding site was formed using NMR spectroscopy. By titrating the DNA NMR was used to assess the stoichiometry of complex formation, with the sample containing the most homogenous solution of complex used in crystallization trials. Diffraction experiments were conducted at 100 K, with the initial resolution limit not exceeding 6 angstroms. A technique known as annealing, in which a barrier is placed in front of the cryo-stream allowing the crystal to reach ambient temperature before recryo-cooling, was used and increased the resolution limit to beyond 2.5 Å.

The 2.5 Å resolution structure reveals a novel DNA interaction by a dimeric winged-helix domain protein that differs from the predicted models. As expected the recognition helices of RTP are in contact with the major grooves of the DNA. However, the wings and N-termini of RTP do not significantly contact the DNA as forecast. This structure provides insight into the molecular basis of polar replication fork arrest and has been used to model the cooperative binding in the functional terminator.

Keywords: DNA REPLICATION REPLICATION FORK ARREST WINGED HELIX

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STRUCTURAL ANALYSIS OF TBP-INTERACTING PROTEIN (Tk-TIP26) FROM HYPERTHERMOPHILIC ARCHAEON *THERMOCOCCUS KODAKARAENSIS* STRAIN KOD1

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TBP-interacting protein from *Thermococcus kodakaraensis* (Tk-TIP26) has been found as the protein that binds to TATA-binding protein (TBP). The binding of Tk-TIP26 disables TBP to interact with TATA-box and transcription become unable to start. From this fact, Tk-TIP26 has been thought to be one of the factors that negatively regulate transcription machinery, and the determination of its three-dimentional structure will aid to understand the regulation mechanism in archaeal transcription. We have succeeded in the cultivation, purification, and crystallization of Tk-TIP26. We have also succeeded to prepare selenomethionine(SeMet) substituted crystals to solve phase problems using multiwavelength anomalous dispersion (MAD) method. SeMet Tk-TIP26 was crystallized in the tetragonal space group $P4_32_12$ with cell dimensions of a = b = 74.34 Å, and c = 86.38 Å , providing one molecule is locating in the asymmetric unit. MAD data collection proceeded at SPring-8 (beamline BL40B2) and the crystal diffracted up to 3.0 Å resolution at three wavelengths. The collected data was processed with MOSFLM, SCALA, and TRUNCATE (CCP4 Program package). The Bijvoet difference Patterson function showed strong peaks on the Harker planes. After the phase calculation, two Se sites were determined in the asymmetric unit. The initial solvent-flattened electron-density map was of excellent quality. The present model with more than 90 % of all residues reveals the whole structure of Tk-TIP26. The model re-building and the refinement are in progress.

Keywords: TBP-INTERACTING PROTEIN TATA-BOX MAD

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STRUCTURE OF PYROCOCCUS ABYSSI Sm PROTEIN IN COMPLEX WITH RNA, SUGGESTIONS FOR EUKARYOTIC Sm PROTEINS

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Sequence comparisons as well as biochemical and structural data indicate that archaeal Sm proteins are the ancestral precursors of eukaryotic Sm proteins present in the core domains of RNP particles. Similar RNA binding characteristics with preference for U-rich, single-stranded regions make them excellent models for understanding the binding of eukaryotic Sm proteins to their RNA targets. Moreover, the fact that in Archaea maximally two distinct Sm proteins interact with RNA as opposed to seven in Eukaryotes greatly facilitates crystallographic studies. Here we report the 2.2 Å crystal structure of Pyrococcus abyssii Sm protein (PA-Sm) in complex with a uridine heptamer. PA-Sm forms a heptameric ring structure similar to that seen in other archaeal Sm core complexes. We find that the RNA is binding at two different protein sites: at the first site, inside the central cavity, highly conserved residues form stacking and specific hydrogen bonding contacts with the uracil bases. Similar contacts were found in an A.fulgidus Sm1/U_5 complex (Törö et al., EMBO J. 20, 2293-03, 2001) and may be present in human SmG. At the second site, located at the surface of the heptamer, the RNA interacts with an aromatic residue (Y34) as well as amino acids from the N-terminal region. These results are used to draw a model of the association of eukaryotic Sm proteins with their Sm sites and surrounding sequence. Further analysis of the protein-protein and protein-RNA associations will be performed to elucidate the *in vivo* function of archaeal Sm proteins.

Keywords: ARCHAEA, RIBONUCLEOPARTICLE, SM PROTEINS

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MONOMERIC AND DIMERIC CRYSTAL STRUCTURES OF AN ENGINEERED MONOMERIC TRIOSEPHOSPHATE ISOMERASE

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Triosephosphate isomerase (TPI) is a member of the (α/β)-barrel 8-fold superfamily (TIM-Barrel), this fold shows a wide variety of enzymatic activities with different reaction mechanisms. Wild type TPI from *Trypanosoma brucei* is a dimer, however a monomeric variant of this enzyme (MonoTIM) has been obtained by shortening and redesigning loop 3, an important structural component of dimerization for the native enzyme, in order to destabilize the dimers (Borchert et al. (1993). Structure, 3:205-213). MonoTIM retained one thousandth of the activity, as well as good solubility and fold stability. MonoTIM has been further engineered by directed evolution schemes to obtain a mutant with a 44-fold improvement in catalytic efficiency (Saab-Rincon et al., 2001. Protein Engineering, 3(14):149-155). We crystallized this mutant and obtained two crystal types of different space groups ($P1$ and $P2_12_12_1$) using the vapor diffusion in hanging drop method. Both crystals grew under the same chemical conditions, while the only difference was the temperature (18 and 30 °C). We refined both crystal structures. $P1$ crystal structure shows a independent monomeric particle, while $P2_12_12_1$ crystals showed the mutant molecule arranged in dimers with a dimerization surface totally different from the wild type TPI dimers. An FPLC gel filtration run shows that the protein exists in solution in a monomer-dimer equilibrium. A structural analysis of both crystal structures confirmed that the dimeric form of the mutant is an inactive enzyme while the active form is the monomer.
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Keywords: MONOTIM DIRECTED EVOLUTION SCHEMES MONOMER DIMER EQUILIBRIUM