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Bacteriophage λ replication initiator, known as λ O protein, is one of the two replication proteins encoded by the bacteriophage's own genome. Its role in initiating bacteriophage DNA replication has been firmly established. Crystals of λ O protein N-terminal fragment, belonging to space group $p2_12_12$ and diffracting to 2.5 Å, were obtained and solved by using anomalous dispersion of selenium atoms. There are two λ O dimers in the asymmetric unit, related to each other by a two-fold non-crystallographic symmetry operator. Genetic data and molecular surface area considerations seem to indicate that the interface(s) between these dimers could be physiologically important and not just a crystallization artifact.

Despite very low homology at the amino-acid sequence level (11% for the structurally aligned regions), the DNA-binding region of λ O displays great similarity to the CAP DNA-binding domain. This structural homology, in combination with genetic data, indicates that DNA-binding mode for λ O protein is similar to the one present in CAP.

Keywords: macromolecular crystallography, replication, protein–DNA interaction

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Structural Study of the Nucleolin~G-quartet Complex

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Nucleolin is a very abundant protein of molecular mass 70 kDa that localizes to the nucleolus. It is often used as a cell proliferation marker. Nucleolin interacts with oligonucleotides such as ribosomal RNA [1]. A 40 kDa-fragment of this protein named P40 has been purified to homogeneity. Nucleolin full-length as well as P40 interact with short DNA segments structured in G-quartet [2]. G-quartets are known as potential therapeutic agent against carcinogenesis.

Various G-rich sequences have been structured and tested in interaction with P40 by "Band-shift". Crystallisation trials have been performed for the most stable complexes. Thus, a crystallization condition has been obtained for an expected P40-4(TG4T) complex. Crystals of dimensions 200 x 100 x 20 μm^3 diffract up to 2 Å in house. They belong to space group P1 with unit-cell parameters a=29Å b=32Å c=35Å α=66° β=72° γ=83°. Such small cell volume corresponds to G-quartet only. X-ray data have been collected and the structure has been solved by molecular replacement, using as model the structure of the same G-quartet obtained in another crystal form [3]. Crystallisation trials on other complexes are under way.

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Keywords: human proteins, DNA and protein crystallography, anticancer drug structural study

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Crystal Structure of DNA Polymerase from Bacteriophage M2

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DNA polymerases are a group of enzymes that use single-stranded DNA as a template for the synthesis of the complementary DNA strand and are a central player in DNA repair and replication. The many known DNA polymerases can be classified into six families based on phylogenetic relationships: families A, B, C, D, X and Y. DNA polymerase from bacteriophage M2 (M2 DNA polymerase) is classified into B-family DNA polymerase. M2 DNA polymerase replicates the genome of bacteriophage M2. The enzyme can start to synthesize DNA using a primer-protein as a primer and does not require DNA/RNA primers. We are involved in structural analysis of M2 DNA polymerase to clarify the relationship of its structure and function. Crystals of M2 DNA polymerase were obtained by hanging drop vapor diffusion technique. The crystal belongs to the space group of $P6_122$ and the unit cell parameters are $a = b = 97.0$, $c = 292.1$ Å and $\gamma = 120°$. All X-ray data was collected at SPring-8 BL41XU. Crystal structure of M2 DNA polymerase was determined by multiple isomorphous replacement method with anomalous scatterings using Hg and Pt derivatives at 3.0 Å resolution. Phase calculation was perfomed with the programs *SOLVE* and *RESOLVE*. The structure was refined with the programs *CNS* and *REFMAC*.

Keywords: protein structures, DNA polymerases, DNA replication

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The Role of Water in Protein-DNA Complexes from high Resolution X-ray Crystallography

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Sac7d is a small, abundant, non-specific DNA-binding protein of the hyperthermophilic archaeon *Sulfolobus acidocaldarius*. Crystal structures of different Sac7d mutants in complex with DNA have been analyzed at high resolution (1.45 Å – 2.25Å). The high resolution structures of Sac7d-DNA complexes afford good opportunity to study protein-DNA-water interactions in detail. Four recurring well-ordered water molecules are observed in the buried cavity located between protein and DNA surfaces near the intercalation site. They fill up the cavity and enable close packing of protein-DNA interface. These four water molecules are always present at the interface of Sac7d and DNA, although with varying arrangements in different complexes. The buried cavity between Sac7d V26F/M29F and DNA becomes narrower than that in wt-Sac7d due to the phenylalanine/base stacking interaction between Phe26 and G3 base. The four bridging water molecules rearrange in the longitudinal direction along helix axis to match the shapes of the protein-DNA interface.

An important question is how do Sac7d/Sso7d bind to DNA in a sequence-general manner. This water-filled cavity is important in that it allows G-C base pairs to be bound without steric problems due to the additional N2 amino group, thus permitting the proteins its sequence-general binding to DNA.

It is interesting to note that bridging water molecules play an important role in modulating the non-sequence-specific binding of Sac7d by acting as filler, whereas they play an entirely different role as specific linkers between protein and DNA in defining the sequence specificity in the *Trp* repressor-DNA recognition.

Keywords: water, hydrogen bond, protein-DNA interaction

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Crystal Structure of PriB – a Primosomal DNA Replication Protein of *E. coli*

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PriB is one of the *Escherichia coli phi*X-type primosome proteins which are required for assembly of the primosome, a mobile multi-enzyme complex responsible for the initiation of DNA replication. Here we report the crystal structure of the *Escherichia coli* PriB at 2.1 Å resolution by multi-wavelength anomalous diffraction using a mercury derivative [1]. The polypeptide chain of PriB is structurally similar to that of single-stranded DNA-binding protein (SSB). However, the biological unit of PriB is a dimer, not a homo-tetramer like SSB. Electrophoretic mobility shift assays demonstrated that PriB binds single-stranded DNA and single-stranded RNA with comparable affinity. We also show that PriB binds single-stranded DNA with

certain base preferences. Base on the PriB structural information and biochemical studies, we propose that the potential tetramer formation surface and several other regions of PriB may participate in protein-protein interaction during DNA replication. These findings may illuminate the role of PriB in *phi*X-type primosome assembly.

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Keywords: replication, primosome, single-stranded DNA binding protein

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Structure of a Dimeric Single-stranded DNA Binding Protein from *Thermus aquaticus*

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Single-stranded DNA binding (SSB) proteins are involved in DNA replication, repair and recombination. While not showing pronounced sequence specificity they bind strongly to single-stranded DNA (ssDNA) but not to double-stranded DNA(Meyer und Laine 1990). This results in stabilizing the ssDNA, preventing hairpin formation and holding it in a suitable conformation for the action of other proteins involved in e.g. DNA replication. There exist several structural classes of SSB proteins ranging from monomers, homodimers, heterotrimers to homotetramers which all have oligonucleotide/oligosaccharide binding folds (OB-fold) in common(Murzin 1993; Suck 1997). One of these classes is formed by the homotetrameric SSB proteins which occur in eubacteria like *E. coli* and in eukaryotic mitochondria. These proteins contain one OB-fold per monomer resulting in four DNA binding sites in each homotetramer. Recently, SSB proteins were identified in the bacterial Thermus group that share homologies to the tetrameric SSB proteins, but the monomers are twice the size compared to those of the homotetrameric SSBs. These proteins contain two OB-folds per monomer and it could be shown that they form dimers in solution(Dabrowski et al., 2002; Eggington et al., 2004). Thus, the principle of four DNA binding sites per functional unit also is conserved in these bacterial SSB proteins. In this work we have expressed, crystallized and solved the structure of the SSB protein from the thermophilic bacterium *Thermus aquaticus*. New insights, based on the structural information, will be discussed in the context of the SSB function in thermophilic bacteria.

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Keywords: SSB, replication, flexible-regions

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Crystal Structure of SUMO1-conjugated Thymine DNA Glycosylase

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SUMO (Small ubiquitin-like modifier) is post-translationally attached to many proteins and regulates the function or localization of modified proteins. Thymine DNA glycosylase (TDG), which plays an integral role in base excision repair of G-U or G-T mismatch DNA, is shown to be modified by SUMO-1 or SUMO-2/3. SUMO modification of TDG promotes the TDG dissociation from its product DNA containing an abasic site. However, the molecular mechanism of the SUMOylation-induced DNA release of TDG has not been revealed yet. Here we determined the crystal structure of SUMO-1 modified TDG central region (SUMO1-TDG). The structure revealed that TDG and SUMO-1 interact with each other through both covalent and non-covalent interactions, and these interactions likely induce the structural rearrangement of the C-terminal region of TDG. This induced structural change includes the alpha-helix formation, which protrudes from the body of the protein and seems to make a steric crash with DNA bound to TDG. Thus, this steric clash seemingly enhances the release of the product DNA from TDG. Results from biochemical assays using a series of TDG mutants support this structure and function model for molecular mechanism of SUMOylation-dependent TDG dissociation from DNA.

Keywords: SUMO, DNA repair, protein modification

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Crystal structure of *Deinococcus Radiodurans* RecO

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The RecFOR pathway has been shown to be essential for DNA repair through the process of homologous recombination in bacteria and, recently, to be important in the recovery of stalled replication forks following UV irradiation. RecO, along with RecR, RecF, RecQ and RecJ, are principal actors in this fundamental DNA repair pathway.

Here we present the three-dimensional structure of RecO from the extreme-radiation resistant bacterium, *Deinococcus radiodurans*. The crystal structure of *D. radiodurans* RecO (drRecO) reveals possible binding sites for DNA and for the RecO-binding proteins within its three discrete structural regions: an amino-terminal oligonucleotide/oligosaccharide binding (OB) domain, a helical bundle and a Cys_4 zinc finger motif. Furthermore, drRecO was found to form a stable complex with RecR and to bind both ssDNA and dsDNA. Mutational analysis confirmed the existence of multiple DNA binding sites within the protein.

Keywords: DNA repair, zinc finger motif, X-ray crystal structure

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Crystal Structure of SUMO2-conjugated Thymine DNA Glycosylase

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SUMO (Small ubiquitin-like modifier) is post-translationally attached to many proteins and regulates the function or localization of modified proteins. TDG (Thymine DNA glycosylase), which plays an important role in base excision repair of G-U or G-T mismatch DNA, is shown to be modified by SUMO-1 or SUMO-2/3. SUMO modification of TDG promotes the TDG dissociation from its product DNA containing an abasic site. However, the molecular mechanism of