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Cyclohexene Oligonucleotides: Structure of the L-CeNA Sequence GTGTACAC

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Cyclohexene nucleic acids (CeNA) contain a cyclohexene ring instead of the normal β-D-2'-deoxyribose. The cyclohexene oligonucleotide GTGTACAC was synthesized using phosphoramidite chemistry and standard protecting groups [1].

CeNA is stable against enzymatic degradation and induces RNaseH activity. CeNA also forms more stable duplexes with RNA than its natural analogues [2] [3].

Crystals of GTGTACAC were obtained at 289K by the hanging-drop vapour-diffusion technique. The crystals diffract to 1.7 Å resolution and belong to the trigonal space group R3 with unit-cell parameters a = 41.434 and c = 66.735 Å.

The structure of a fully modified GTGTACAC sequence with left handed CeNA building blocks is presented. Particular interests concern the puckering of the sugar moiety, helical parameters and the hydration of the double helix.

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Keywords: nucleic acids, oligonucleotides, antisense

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Detection of 8-hydroxy-2'deoxyadenosine and 8-hydroxy-2'deoxyguanosine by Avidin

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By using x-ray crystallography, isothermal titration calorimetery and fluorescence spectroscopy this study shows that avidin binds the oxidised nucleosides 8-hydroxy-2'deoxyadenosine and 2'deoxyguanosine.

Oxidised bases are endogenously present in the nuclear and mitochondrial DNA of most tissue types. An increase in the cellular concentration of these oxidised bases is an important marker for degenerative diseases such as Alzheimer's disease, aging and for carcinogenesis. Free radical attack, predominately by the ·OH radical, is one of the main causes of oxidative DNA damage.

Avidin is a basic, glycosylated protein found in egg white. Its biological role remains unclear although bacterial growth inhibition and a role in reproduction have been proposed. It is known that avidin binds biotin (vitamin H) with very high affinity ($10^{15}M^{-1}$). Avidin forms a tetramer with each monomer formed from an 8-stranded anti-parallel β-barrel. With biotin bound, the loop between strands β3 and β4 is in an ordered conformation. However in the absence of biotin (or, as found in this study, the presence of 2'deoxyguanosine or 8-hydroxy-2'deoxyguanosine) this loop is disordered in the crystal structure. This study shows that 8-hydroxy-2'deoxyadenosine and 2'deoxyguanosine bind in the same hydrophobic pocket as biotin but with an affinity in the μM range. The lower affinity of this interaction correlates with the β3/ β4 loop remaining disordered in the crystal structure.

It is hoped that these studies will lead to a robust and reliable assay system for the detection of oxidised bases in DNA

Keywords: avidin, 8-hydroxy-2'deoxyadenosine, 8-hydroxy-2'deoxyguanosine

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Unveiling the DNA Strand Transfer-mechanism of Relaxase TrwC

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The three-dimensional crystal structure of the relaxase domain of TrwC in complex with DNA (25-mer oligonucleotide), recently reported [1], showed that the protein has a metal binding site at the active site, in which, three histidine residues (His150, His161 and His163) and a water molecule coordinate a metal cation. The nature and role of this metal in the strand transfer-mechanism it is not clear. It was suggested that it could play an important role polarizing the scissile phosphate or stabilizing the transition state. Further structural information is needed to understand its function and unveil the enzymatic mechanism.

In the present work we discuss the results obtained with TrwC-DNA crystals, soaked with different metals: Cu^{2+}, Ni^{2+}, Mg^{2+} and Mn^{2+}. We also report the successful cocrystallization and structure determination of the protein with longer oligonucleotide sequences, that include the scissile bound (27 and 29-mer oligonucleotides), achieved by introducing a mutation in the catalytic residue Tyr18 (Y18F).

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Keywords: DNA-binding protein, metal bound-structure, DNA-strand transfer-mechanism

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Crystal Structure and Function of Human Spindlin1

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Spindlin1 (Spin), is an abundant maternal transcript present in the unfertilized egg and 2-cell, but not 8-cell stage embryo. It associates with the meiotic spindle and is phosphorylated by MOS/ MAP kinase pathway in a cell-cycle-dependent fashion. Our study indicated that spin is localized into the cell nuclei and associated with the DNA binding activity. Although experimental results indicate that this protein family includes important players in meiosis and early embryogenesis, their biochemical function is largely unknown.

Here, spin has been cloned, overexpressed and purified, and crystals have been obtained using hanging-drop vapor-diffusion technique. Diffraction data sets up to 2.2 Å (crystal form 2) were collected, and single-wavelength anomalous diffraction (SAD) was used for phasing. The refined structure exhibits a new fold with no obvious similarity to those of other proteins with known three-dimensional (3D) structure. A model of spin-DNA binding is proposed based on the presented structure.

Keywords: spindlin1, DNA-binding, SAD

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The Structure of λ O Protein Fragment Provides Insights About Replisome Assembly

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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