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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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