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How life emerged on Earth remains one of the great Mysteries for mankind. Molecules with backbones forming stable double helices, held by self-association, and capable of auto-replication - and more precisely nucleotides held by Watson-Crick base pairings - were considered as the seminal building blocks of life. Many scenarios involving extreme conditions were described, all of them dealing with the extreme pressure conditions of the "primary soup" that was present on earth at prebiotic stages. High-pressure molecular crystallography (HPMX) investigation of DNA was undertaken using crystals of the d(GGTATACC) octamer, in the range 0.2-2 GPa. This sequence crystallizes in the hexagonal P61 space group and is particularly interesting because it includes in a A-DNA crystal matrix, the B-form of DNA, leading us to simultaneously monitor the two forms of DNA under pressure. The 3D structure of d(GGTATACC) was recorded at ambient pressure, 0.55, 1.09 and 1.39 GPa and refined at 1.6 Å resolution. Fiber diagrams of the embedded B-DNA that superpose to the diffraction pattern of the A-DNA were analyzed from ambient pressure to up 1.9 GPa. A large axial compression of the DNA is observed (11 % at 1.39 GPa). The average base-step varies in A-DNA from 2.92 down to 2.73 Å, and in B-DNA from 3.40 to 3.10 Å. Surprisingly, in the case of A-DNA, the geometry of Watson-Crick base parings remains essentially invariant in the domain of pressure up to 1.39 GPa. Above 1.4 GPa, the crystal structure irreversibly deteriorates while the B-DNA fiber diagram still persists above 2 GPa. The remarkable stability and adaptation of d(GGTATACC) to high pressure is clearly associated with the base-paired double helix topology of the molecule by which it behaves as a molecular spring.

Keywords: DNA, high pressure,, macromolecular crystallography

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Conformational flexibility of cyclohexene residues

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Cyclohexene nucleic acids (CeNAs) are developed as antisense drugs, which selectively bind with the messenger RNA to inhibit protein synthesis in human cells. CeNAs have proven to interact more effectively with RNA than its natural DNA and RNA analogues, and are able to introduce RNaseH activity. The flexibility of the cyclohexene moiety allows incorporation of the CeNA residues in both A- and B-type DNA, as the ring can adopt the $^2H_3$ or the $^3H_2$ half-chair conformations. We present here the structure of a fully modified CeNA duplex GTGTACAC (space group *R*32, 1.53 Å resolution, R = 15.8%). All CeNA residues adopt the $^3H_2$ conformation mimicking the C3'-endo conformation of natural A-DNA. In a second structure a CeNA residue is incorporated in the Dickerson dodecamer (space group $P222_1$, 1.9 Å resolution, R = 22.7%). To maintain the global B-type helix of the dodecamer, the C3' atom of the CeNA sugar ring is pushed into the ^2E envelope conformation, close to the expected $^2H_3$ conformation. In addition three $Co(NH_3)_6$ complexes stabilise the CeNA residue and crystal packing. These two recent structures indeed prove the flexibility of the CeNA ring making these CeNA building blocks ideal candidates for antisense therapy.

Keywords: nucleic acid crystallography, antisense, cyclohexene nucleic acid

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Structural insight on the mechanism of regulation of the MarR family of proteins

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MarR transcription factors are diverse in nature and some of them are known to regulate multiple antibiotic resistance (MAR) in bacteria. They make up a large family of proteins characterized by a winged-helix DNA binding domain. Organic solvents and antibiotics can trigger an antibiotic resistant state which ultimately results in limited influx and increased efflux of these compounds into the cell. The multiple antibiotic resistance (mar) operon is regulated by a MarR transcriptional regulator and the products of this operon are known to mediate the MAR phenotype via regulation of more than 60 genes. Antibiotics and organic compounds bind to the MarR protein and induce a conformational change, a known mechanism of its regulation. Salicylate is a common inducer of MAR and inhibitor of MarR. This study presents for the first time the structure of a MarR family member from Methanobacterium thermoautotrophicum in the presence of salicylate. Salicylate binding revealed a large conformational change in its DNA binding lobe that renders it inactive to bind DNA. Salicylate binds two asymmetric sites on the M. thermoautotrophicum MarR non-cooperatively, as will be shown here through DNA binding and thermal denaturation assays. This provides insight into how transcription factors of the MarR family regulate the effective amount of multiple antibiotic resistance inducers. Understanding of this inactivation mechanism and comparative analysis with E.coli MarR can improve our understanding of multiple antibiotic resistance and can open the road to the development of new antimicrobial agents.

Keywords: transcription factor structure, antibiotic resistance, bound ligand interactions

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Activity regulation of the transcription factor Ets-1 by DNA-mediated homo-dimerization

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The Ets-1 (E26 transforming specific sequence) proto-oncoprotein is a member of the Ets family of transcription factors that share a unique DNA binding domain termed ETS domain which recognizes specifically a GGAA/T core element. The function of the Ets-1 transcription factor is regulated by two autoinhibitory regions that flank ETS domain. Previous data revealed the mechanism for auto-inhibition of a monomeric Ets-1 on DNA response elements with a single Ets-1 binding site. Here, we present the X-ray structure of the Ets-1/DNA/Ets-1 complex formed on the sromelysin-1 promoter element containing two palindromic head to head Ets1-binding

sites. The structure reveals a ternary complex in which protein homo-dimerization is mediated by the specific arrangement of the two Ets-1 binding sites and demonstrates how Ets-1 transcription factor dimerizes by forming a central protein/DNA interface that involves several residues from a loop connecting the N-terminal autoinhibitory region and the ETS domain. Ets-1 variants, in which residues involved in protein-protein interaction are mutated, lose the ability for DNA-mediated dimerization and stromelysin-1 promoter transactivation. The X-ray structure of the Ets-1/DNA/Ets-1 complex formed on the stromelysin-1 promoter shows for the first time how Ets-1 transcription factor can function as a homodimer contrary to previous structures where Ets-1 was bound to DNA as a monomer or formed complexes with other transcription factors on DNA. Thus, our data unravel the molecular basis of the ability of Ets-1 to function as a facultative dimeric transcription factor and play an important role in the transcription regulation of the stromelysin-1 promoter.

Keywords: Ets-1, transcription regulation, protein-DNA complexes

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Structure of the FOXO3a-DBD/DNA complex suggests the effects of post-translational modification

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FOXO3a is a transcription factor of the FOXO family. The FOXO proteins participate in multiple signaling pathways, and their transcriptional activity is regulated by several post-translational mechanisms, including phosphorylation, acetylation and ubiquitination. Because these post-translational modification sites are located within the C-terminal basic region of the FOXO DNA-binding domain (FOXO-DBD), it is possible that these post-translational modifications could alter the DNA-binding characteristics. To understand how FOXO mediate transcriptional activity, we report here the 2.7 Å crystal structure of the DNA-binding domain of FOXO3a (FOXO3a-DBD) bound to a 13-bp DNA duplex containing a FOXO consensus binding sequence (GTAAACA). Based on a unique structural feature in the C-terminal region and results from biochemical and mutational studies, our studies may explain how FOXO-DBD C-terminal phosphorylation by protein kinase B (PKB) or acetylation by cAMP-response element binding protein (CBP) can attenuate the DNA-binding activity and thereby reduce transcriptional activity of FOXO proteins. In addition, we demonstrate that the methyl groups of specific thymine bases within the consensus sequence are important for FOXO3a-DBD recognition of the consensus binding site.

Keywords: FOXO3a, winged/helix, DNA complex

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Structural basis for human mitochondrial DNA polymerase processivity

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Replication of mitochondrial DNA (mitoDNA) is an essential process for maintenance of the molecule which encodes a subset of components for oxidative phosphorylation. The replication is conducted by a nuclear-coded DNA polymerase gamma (Polg) which also has exonuclease activity as proofreading function and dRP lyase activity as repair function. Interestingly, mutations in Polg can cause an impaired mitoDNA replication, which are implicated in human mitochondrial diseases. In addition, human Polg is the target of adverse reactions of anti-HIV reagents and is in part responsible for drug toxicities. To illustrate the structural basis for mitoDNA replication and facilitate rational design of antiviral drugs, we determined crystal structure of human Polg holoenzyme to 3.2 Å resolution. The structure revealed heterotrimer architecture of the enzyme with a monomeric catalytic subunit (Polg A) and a dimeric accessory subunit (Polg B). The two subunits form extensive interaction thereby providing a novel mechanism for high processivity of DNA replication. Polg A folds into three distinct domains, a polymerase (pol) and a nuclease (exo) domains, as well as a spacer domain sandwiched between the above two domains. While the pol and exo domains present high homology with those of other members of DNA Pol I family, the spacer domain shows a unique fold where a large area of subunit interaction are formed. The structure of the spacer domain also provides an explanation for its ability to coordinate the enzymatic activities of pol and exo domains as well as increasing processivity of Polg A. The structural information of Polg would set the stage of further understanding the mechanism for mitoDNA replication as well as mitochondrial toxicity of anti-HIV drug.

Keywords: DNA polymerase gamma, processivity, mitochondria

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Crystal structure of the HRDC domain of human Werner syndrome protein, WRN

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Werner syndrome is a human premature aging disorder characterized by chromosomal instability. The disease is caused by the functional loss of WRN, a member of the RecQ-helicase family that plays an important role in DNA metabolic pathways. WRN contains four structurally folded domains comprising an exonuclease, a helicase, a winged-helix, and a helicase-and-ribonuclease D/C-terminal (HRDC) domain. In contrast to the accumulated knowledge pertaining to the biochemical functions of the three N-terminal domains, the function of C-terminal HRDC remains unknown. Recently we determined the crystal structure of the human WRN HRDC domain (Kitano et al. J. Biol. Chem. 2007; 282 2717-28). The domain forms a bundle of alpha-helices similar to those of Saccharomyces cerevisiae Sgs1 and Escherichia coli RecQ. Surprisingly, the extra ten residues at each