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The aminoacyl-tRNA decoding site (A site) is a molecular switch in the ribosome guaranteeing high translation fidelity. The secondary structure of the A site is conserved except at a few nucleotides between bacteria, mitochondria and eukaryotic cytoplasm. X-Ray analyses have revealed that tertiary structures of the three types of A sites are surprisingly different [1,2]. Therefore, on the basis of the many crystal structures obtained, it is suggested that these three main cell types possess different dynamical states and barriers in the molecular switches controlling translational fidelity, underlying the different evolutionary pressure on decoding. Aminoglycosides are highly effective antibacterial drugs that decrease translation accuracy by binding to the "on" state of the bacterial A-site molecular switch. On the other hand, toxicity to human resulting from the clinical use of aminoglycoside has been considered to originate from the binding of these drugs to the mitochondrial and cytoplasmic A site. Our X-ray analyses have revealed that the binding modes of aminoglycosides to the mitochondrial and cytoplasmic A sites, in which aminoglycosides bind to the "off" states of the A sites, are surprisingly different from those found in the bacterial A site [3].

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Keywords: ribosomes, RNA structure, antibiotic binding

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Crystal structure of Z-DNA d(CGCGCG) complexed with Ca^{2+} ion , and Mg^{2+} ion

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In solution, negatively charged DNA was neutralized by positively charged metal ions such as monovalent Na^+, K^+ and divalent Ca^{2+}, Mg^{2+}. These cations are essential for DNA folding and sometimes decide the helical conformation of DNA by hydrogen bonding networks. We determined crystal structures of DNA hexamer d(CGCGCG) with very high concentration of Mg^{2+} and Ca^{2+} ions by new temperature control technique we developed. Two kinds of DNA crystal were obtained from crystallization solution containing 500mM $MgCl_2$ and 500mM $CaCl_2$ by annealing from 338K to 293K, respectively. X-ray experiment was carried out using synchrotron radiation at BL38B1 in SPring-8. X-ray diffraction data were collected at high resolution (1.2 Å for $MgCl_2$ and 1.1 Å for $CaCl_2$). These crystals belong to new crystal form $P3_2$ (a=b=18.5 Å, c=72.7 Å) and left-handed DNA helixes were stacked along a long c-axis in $MgCl_2$ crystal. That's entirely different from a usual crystal ($P2_12_12_1$, a=17.9 Å, b=30.8 Å, c=43.7 Å) obtained from 20mM, 50mM and 250mM Na^+ solution. Initial phases of $MgCl_2$ crystal were determined by molecular replacement with the program of AMoRe, and structural refinement and model building were carried out with the program of Refmac 5.0 and Coot 0.2, respectively. 68 H_2O molecules and 8 Mg^{2+} binding sites were determined with R=23.3 % and R_{free}=26.9%. Phosphate groups of Z-DNA were connected with the other phosphate group of neighboring Z-DNA via two Mg^{2+} ions. This structural motif would be utilized in storing DNA in compact room with high salt condition.

Keywords: metal ions in biology, DNA crystallography, DNA structure

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X-Ray analyses of DNA duplexes stabilized by bicyclic-C residues

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Base-modified nucleic acids are being evaluated for applications in biotechnology and as therapeutic agents. We found that a nucleotide carrying 7,8-dihydropyrido[2,3-*d*]pyrimidin-2(3H)-one, which is a cytosine derivative with a propene attached at the N4 and C5 atoms (hereafter bicyclic-C or X), increases the stability of DNA duplexes [1]. To establish the conformational effects of X on DNA and to obtain insight into the correlation between the structure and stability of X-containing DNA duplexes, the crystal structures of $[d(CGCGAATT\text{-}X\text{-}GCG)]_2$ (GX9) and $[d(CGCGAAT\text{-}X\text{-}CGCG)]_2$ (AX8) have been determined at 2.9 Å resolutions, respectively. The global and local conformations of the X-containing duplexes and the unmodified duplex are very similar. The X and the counter G bases form a pair in the canonical Watson-Crick geometry, similar to the previously reported GX9* [2]. On the other hand, the final omit map of AX8 suggests that X forms two types of pairs with the counter A residue, one is a wobble type and the other is a Watson-Crick like as the first example. In the former pairing, the adenine base must be protonated, while in the latter, X or A might adopt an imino form through tautomerization. Another interesting finding is that the base stacking interactions at the base pair steps containing the X residues are significantly changed. These changes in stacking areas, however, explain the increased stability of X-containing DNA duplexes.

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Keywords: DNA structure, base modification, DNA stability

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X-ray structure of A and B-DNA under high hydrostatic pressure (up to 2 GPa)

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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