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CONFORMATIONAL SPACE OF NUCLEIC ACIDS

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Past years brought up an information explosion of nucleic acid (NA) structures, mainly due to X-ray crystallography of large RNA molecules as ribosomal particles or various ribozymes. Several research groups including ours have systematically analyzed conformational space of RNA [1] and DNA [2]. These studies have laid firm foundation of description of NA structures at detailed dinucleotide level. Here we discuss the main features of observed RNA and DNA conformers and compare conformational behavior of RNA and DNA nucleotides.

Both DNA and RNA occur mostly in right-handed double helical forms, DNA in the B-form and RNA in the A-form. The vast majority of DNA dinucleotides form a bundle of similar conformers, which transform to one another in an almost continuous fashion. A number of B-type conformers concentrate around the "canonical" B-DNA, called BI and they transform by gradual torsional rotations to another major B-form, so called BII-DNA, and to the A-DNA form. B and A forms are connected via several A-to-BI sub-states with mixed structural properties including sugar puckers intermediate between the major C2'-endo and C3'-endo as O4'-endo and C1'-exo puckers. Bimodality of sugar puckers clearly pronounced in RNA is not observed in DNA and deoxyribose undergoes plastic, almost continuous transformation. The whole region B- and A-DNA conformers can be regarded as one broad right handed double helical form.

Several conclusions regarding sequence preferences for certain DNA conformers, as a preference of YR steps for adopting the BII-form, high propensity of the CG step for mixed A/B conformations, can be drawn and will be discussed in greater detail. Shown will also be how wrapping of DNA around histone proteins in a nucleosome-core particle is attained by a fairly regular alteration of BI and BII conformers, occasionally substituted by deformed BI or combined B/A conformers

In contrast to DNA, widely diverse RNA conformations seem to form isolate islands in the conformational space. The extra hydrogen bond donor and acceptor, the hydroxyl -O2'H at the ribose ring, stabilizes conformations that lead to bulges, loops, and consequently to RNA molecules globally folded in three dimensional space. When RNA is disrupted from its most stable AI form, it "jumps" to conformations incompatible with the rigid right handed helix. DNA, with its numerous, closely related conformers, is "soft", whereas RNA, with fewer, but conformationally very different, conformers, is "rigid" but "brittle". The definition of isolated RNA conformers [2], currently with about fifty distinct 'rotamers', allows for formulation of classification schema. The two-letter schema proposed by Richardson *et al* [2] is based on approximate values for all 7 torsion angles between two ribose sugars; the first part of the modular conformer name is based on values of torsions , used are numbers, the other part, described by letters, is based on . Sugar-to-sugar unit in the most frequent RNA conformation, A-form, is described as 1a. An advantage of the schema is that it can be used in RNA structural bioinformatics. The tw-character backbone conformer names can be alternated with the base sequence to provide an information-rich string description of the combined linear sequence and structure in RNA molecules. For instance the UNCG tetraloop [3] can be described by the modular string N**1a**U**1z**N**2[**C**6n**G**1a**N.

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PRELIMINARY STRUCTURE CHARACTERIZATION OF DHAA MUTANTS FROM *RHODOCOCCUS RHODOCHROUS*

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Haloalkane dehalogenases (EC 3.8.1.5) are members of the / -hydrolase fold family and catalyze hydrolytic conversion of a broad spectrum of hydrocarbons to corresponding alcohols [1]. These enzymes are potentially important biocatalysts for industrial and bioremediation applications. Besides a wide range of haloalkanes, DhaA can slowly convert serious industrial pollutant 1,2,3-trichloropropane (TCP) [2]. Three mutants marked as DhaA04, DhaA14 and DhaA15 were constructed to study importance of tunnels connecting buried active site with the surrounding solvent for the enzymatic activity.

All mutant proteins were crystallized using a sitting-drop vapor-diffusion technique [3]. Grow conditions were optimized [4] and crystals were used for synchrotron diffraction measurements at the beamline X11 of a DORIS storage ring at the EMBL Hamburg Outstation.

Diffraction data for DhaA04, DhaA14 and DhaA15 mutants were collected to the high resolutions of 1.23 Å, 0.95 Å and 1.15 Å, respectively. Crystals of DhaA04 belong to the orthorhombic space group $P2_12_12_1$ while crystals of second two mutants DhaA14 and DhaA15 to the triclinic space group $P1$. The known structure of the haloalkane dehalogenase from *Rhodococcus* species (PDB code 1bn6) [5] was used as a template for the molecular replacement. Currently, structures of the DhaA mutant proteins are in the process of being further refined and interpreted.

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GALACTONOLACTONE INHIBITING THE ACTIVE SITE OF -GALACTOSIDASE FROM *ARTHROBACTE*R SP. C2-2. CRYSTAL STRUCTURE AT 2.2Å RESOLUTION

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The three-dimensional structure of enzyme beta-galactosidase from an Antarctic bacterium *Arthrobacter* sp. C2-2 with bound inhibitor has been determined at a resolution of 2.2 Å.

The enzyme -galactosidase (EC 3.2.1.23) belongs to the enzyme class called glycosylases which catalyze the hydrolysis of the terminal beta-D-galactosyl of beta-D-galactosides. It is attractive for research and industry because of its wide range of biotechnological applications (to treat lactose intolerance, to prevent crystallization in sweet products, to increase its sweetening power, to simplify fermentation during production of soured milk products, to modify the freezing point of ice creams, etc.).

The psychrotrophic bacterium *Arthrobacter* sp. C2-2 was isolated in the Antarctic area and this enzyme is active at low temperature, which is interesting property for food processing applications. Unlike, more known -galactosidase from *Escherichia coli*, which form tetramers, the -galactosidase from *Arthrobacter* sp. C2-2 forms hexamers with molecular weight of 660 kDa. Each monomer consists of five domains and contains 1023 residues. The active site is localized in the TIM barrel domain in the center of each monomer. The active site contains the pair of catalytic residues Glu442 and Glu521. The molecule of galactonolactone was found locked in the deep binding mode near the catalytic residues Glu442 and Glu521. Undoubtedly, the position of inhibitor closely simulates the transition state of galactose before the second step of enzymatic reaction (i.e. the release of product or the transglycosylation reaction).

X-ray diffraction data were collected at the source of synchrotron radiation ESRF in Grenoble. The data were processed using HKL-2000. The crystal belongs to space group $P2_1$ with unit cell parameters a = 140.3 Å, b = 205.5 Å, c = 140.5 Å, = = 90°, = 102.5°. The structure is refined by REFMAC.

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STUDIUM MAGNETISMU A MAGNETICKÝCH STRUKTUR POMOCÍ SYNCHROTRONOVÉHO ZÁŘENÍ

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Až do roku 1972, kdy pánové F. de Bergevin a M.Brunel [1] poprvé experimentálně pozorovali magnetický příspěvek rozptylu rtg. záření, bylo určení magnetické struktury látek doménou rozptylu neutronů. Neutrony mají pro studium magnetismu, resp. magnetických struktur velmi vhodné vlastnosti - zejména vlastní magnetický moment (spin). Energie neutronů používaných pro rozptylové experimenty (~1 100 meV) je srovnatelná s energií excitací v pevných látkách, např. fononů, magnonů, excitonů a pod. Jsou tedy velmi vhodné i pro inelastické experimenty. Dnes už máme více možností jak studovat magnetické vlastnosti látek. Jaké požadavky máme na záření, vlnění, vhodná pro studium magnetismu?
Požadujeme:

- vhodnou vlnovou délku
- citlivost k magnetickému uspořádání