flexible and tolerant of the conformational changes important for molecular recognition.

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CONFORMATIONAL VARIABILITY OF RNA BACKBONE

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As shown by ribozyme and especially ribosome structures solved in last few years, molecules of RNA form complicated 3D folds which have no match among known DNA structures but their complexity is quite comparable to that of protein folds. Complicated RNA folds are enabled by a high flexibility of the nucleotide backbone but little is known about its conformational behavior. A well refined structure of the large ribosome subunit 50S at 2.4A, NDB structure RR0033 (PDB ID 1JJ2), Ban *et al.* Science **289**, 905 (2000), provides a database of over 2700 nucleotides. This work analyzes conformations of these nucleotides by a combination of Fourier averaging and clustering techniques.

Majority of all nucleotides of RR0033, about 70%, are in the A-type conformation, this main conformational type can be further classified into three subclasses. The remaining 30% of nucleotides with other than A-type conformations were analyzed in a greater detail. The backbone torsion angles for each nucleotide were grouped into eight sets of three angles with the main emphasis on the torsions around the two phosphodiester bonds, O3*-P (torsion zeta) and P-O5* (alpha). Each set of three torsions results in a 3D distribution of points in a parametric torsional space and this distributions was Fourier transformed into densities of nucleotide conformations. Peak positions (maxima) of these maps confine the most probable (di)nucleotide conformations.

Nucleotides belonging to the same peaks in several torsional 3D maps have similar geometry. Such nucleotides were grouped and compared in Cartesian (real) 3D space. In such a way, twelwe types of highly untypical (non-A) nucleotide conformations were identified and their Cartesian coordinates determined. These untypical nucleotide conformations can be useful in e.g.refinement process and are available upon request.

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STRUCTURE AND DYNAMICS OF RIBOSOMAL 5S RNA AND ITS COMPLEX WITH RIBOSOMAL PROTEIN L25

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Ribosomal 5S RNA (5S rRNA) is an integral component of the large ribosomal subunit in all known organisms with the exception of the small ribosomes of fungal and animal mitochondria. The 5S rRNA of *Escherichia coli* (*E. coli*) interacts with ribosomal proteins L5, L18 and L25 and enhances protein synthesis by stabilization of the ribosome structure but its exact role in protein synthesis is still not known. 5S rRNA contains internal loop - **Loop E**. The Loop E is a salient example of a uniquely structured non-Watson-Crick motif, as it contains seven consecutive non-Watson-Crick base pairs, including wobble G.U base pair and substantial cross-strand purine stacking. This unique duplex architecture together with adjacent sequence helix IV form binding site for ribosomal protein L25.

To understand the structure and function of internal Loop E and interaction between 5S rRNA Loop E and ribosomal protein L25, we have carried out set of molecular dynamics simulations.

Initial structures were directly taken from x-ray crystallography - crystal structure of 5S rRNA Loop E (*E. coli*) [1] and crystal structure of ribosomal protein L25 complexed with the 5S rRNA fragment [2]. Another studied structure was chloroplast Loop E for which there is no atomic resolution structure yet available and which is sufficiently different from bacterial Loop E motifs in sequence, but evolutionarily related to it. Model of chloroplast Loop E was proposed based on homology modeling [3], initial structure for this model was bacterial Loop E, mutation of three base pairs was performed based on the isosteric mutation.

Main focus of our investigation was to study of the structure, dynamics, hydration and cation binding of non-Watson-Crick base pairs and interaction between ribosomal protein L25 and 5S rRNA Loop E. Another aim of this study was to test the usefulness of the MD technique in

evaluating the dynamics and energetics of molecular models of RNA motifs constructed by phylogenetic analysis and isostericity principles.

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THE EFFECT OF WATER SOLVENT ON THEORETICAL NMR SPIN - SPIN COUPLINGS IN DNA: IMPROVEMENT OF CALCULATED VALUES BY APPLICATION OF TWO SOLVENT MODELS

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The NMR indirect spin - spin coupling constants calculated in DNA base pairs are compared with one-bond $^1J(X,H)$, $^1J(C,X)$ and two-bond 2J(X,H), (X = C, N) coupling constants measured in DNA hairpin molecule d(GCGAAGC) [1]. The two theoretical models of solvent: explicit and Polarized Continuum, were introduced. Both explicit and PCM solvent model provide a similar improvement of the calculated J constants when compared to the experimental data. The mean absolute deviation between the calculated and experimental couplings is significantly reduced by solvent inclusion, from 1.7 to 1.1 Hz for guanine, from 2.4 to 0.6 Hz for cytosine, and from 2.3 to 1.6 Hz for adenine. The strongest solvent shift was calculated for the $^1J(C,H)$ coupling constants, particularly for the (C8,H8) coupling in guanine (6.1 Hz) and adenine, and the (C5,H5) and (C6,H6) couplings in cytosine. These changes in $^1J(C,H)$ coupling seem to correspond to the charge transfer from water bonding and lone pair orbitals to the guanine anti-bonding C8-H8, C8-N7, and C8-N9 orbitals. From the close agreement between the calculated and experimental coupling constants we can deduce that the C-H group of the hairpin bases is extensively hydrated even when interaction with solvent is rather weak and non - specific.

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LECTURES - MARCH 15

STRUCTURAL BASIS OF HIV-1 AND HIV-2 PROTEASE INHIBITION BY A MONOCLONAL ANTIBODY

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Since the demonstration that the Human Immunodeficiency Virus protease (HIV PR) is essential in the viral life cycle [1], this enzyme has become one of the primary targets for antiviral drug design. With the objective of probing the structural stability of HIV PR and the eventual design of potential inhibitors directed to regions other than the active site, we have examined the effects of anti-HIV-1 PR monoclonal antibodies (mAbs) on the catalytic activity of the protease [2, 3]. Monoclonal antibody mAb1696, although raised against the HIV-1 PR, inhibits the catalytic activity of both the HIV-1 and HIV-2 enzymes with inhibition constants in nanomolar range [3] and cross-reacts with peptides comprising the N-terminus of the HIV protease (residues 1 to 7). The N-terminal region is essential for dimerization of monomers and thus forming the active HIV PR dimer.

To investigate further the mechanism of HIV PR inhibition by mAb1696, a recombinant single-chain Fv fragment (scFv) that contains haeavy and light chain variable domains of mAb1696 joined by a flexible linker have been prepared and used for crystallographic studies [4]. Although the crystallization of scFv1696 complexed with the HIV proteases has not yet been successful, the formation of these complexes has been demonstrated in solution. As an alternative approach to studying the antigenic recognition by the mAb 1696 at the structural level, we have crystallized complexes of scFv1696 with the respective epitope peptide fragments of the HIV protease. The three-dimensional structure of the complex formed between scFv1696 and epitope peptide PQITLWQRR (corresponding to the N-terminus of HIV-1 PR) has been solved and refined at 2.70 A resolution [4]. The structure of the complex formed between scFv1696 and epitope peptide PQFSLWKR (corresponding to the N-terminus of HIV-2 PR) has been solved and refined at 1.88 A resolution.

Interactions of peptides with scFv1696 have been analyzed and compared in both structures. On the basis of the interactions seen in the complex, the cross-reactivity between mAb1696 and the HIV-1 and HIV-2 protease and their N-terminal peptides can be explained. The broad reactivity between mAb 1696 on one side and the HIV-1 PR and HIV-2 PR derives from the invariance or conservation