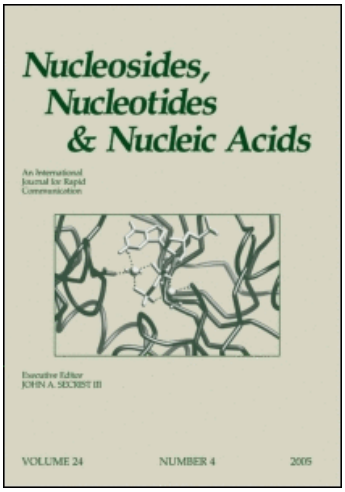

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The Solution NMR Structure of 2'-O-Methyl CGCGCG RNA Duplex

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THE SOLUTION NMR STRUCTURE OF 2'-O-METHYL CGCGCG RNA DUPLEX

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Abstract : Complete assignments of nonexchangeable protons in ^{1}H NMR spectra of 2'-O-methyl-CGCGCG complemented by its analysis of ^{13}C and ^{31}P NMR spectra revealed A-RNA double helical structure in low salt solution.

Description of the structure and dynamics of CG tracts in double helical RNAs is far from complete. For CGCGCG duplex the A-RNA structure in a low salt solution was proposed[1-3]; but due to severe overlapping of ribose signals in the NMR spectra, their full assignments were difficult. Till present, our attempts to get well diffracting monocrystals from this duplex have been unsuccessful. Very recently, crystals of tittle duplex were obtained[4]. It has been proved that RNA can adopt a left handed double helical structure[5,6]. Results of NMR study of "Z" form of CGCGCG duplex were already presented[7].

This communication is part of our studies concerning structural features of CG tracts and their modified counterparts. They include also monitoring "A" => "Z" transition as a model event for their evaluation. Here we would like to present the structure of the 2'-O-methyl-CGCGCG RNA duplex as the basis for the full interpretation of the parent CGCGCG data. This should allow for the evaluation of the role of 2'-hydroxyl function in respect to their ability to intra- and intermolecular hydrogen bonding network formation.

2'-O-Methyl-CGCGCG (2'-O-[^{13}C]Me labeling on selected cytidine residues) was obtained *via* chemical synthesis[8] using support-aided phosphoramidite method and its 300/500MHz NMR studies were conducted (Varian) under low salt conditions (150mM NaCl). Resonance assignments of non exchangeable protons have been achieved using various 2D NMR techniques: DQFCOSY, NOESY, HETCOR, HMQC. The chemical shifts and coupling constants of carbon atoms concerning all methyl groups, C8, C6, C5, C1' have been determined using HMQC spectra. All five phosphorus atoms of 2'-O-methyl-CGCGCG were assigned based on 2D heteronuclear correlated ^{31}P-^{1}H spectra.

Results of NMR spectroscopy and molecular computations are as follow:

1. All the signals of non-exchangeable protons have been assigned (apart from H1' rest of 2'-O-methyl-ribose protons are crowded in 3.8-4.8 ppm region) (TABLE) and most vicinal coupling constants measured,
2. The pattern of NOE connectivities between H1' and H6/H8 protons and results of ^{31}P NMR spectra proved the double helical structure,

TABLE. ^1H chemical shifts (δ, ppm) of 2'-O-Me-CGCGCG RNA duplex.

Nucl	H8/H6	H5	H1'	H2'	H3'	H4'	H5'	H5''	OCH_3
C1	8.05	6.01	5.67	4.25	4.59	4.23	4.00	3.90	3.67
G2	7.84	-	6.03	4.25	4.74	4.44	4.38	4.17	3.82
C3	7.74	5.30	5.72	4.30	4.54	4.41	4.38	4.13	3.65
G4	7.58	-	5.94	4.16	4.55	4.40	4.34	4.10	3.77
C5	7.67	5.25	5.70	4.07	4.48	4.34	4.35	4.06	3.62
G6	7.55	-	5.94	3.76	4.31	4.19	4.25	4.04	3.55

3. Assignment of H2' protons have been made based on NOESY spectrum. Interresidue distances between H2' and H6/H8 protons and vicinal couplings $J_{1',2'}$ (<2 Hz) and $J_{3',4'}$ (8-10 Hz) clearly indicate A-RNA structure of the helix. Additionally, the ^{13}C NMR spectra of aromatic region reveal great similarity between carbon chemical shifts for both parent riboCGCGCG and 2'-O-methyl- CGCGCG duplex,
4. Analysis of vicinal coupling constants based on two state model[9] allows to describe the 2'-O-methyl-ribose ring puckering (P=0-10^o , Φ_N =41^o) as pure (N) for all units except for G(6) for which 15% of S conformer was present. For residues C1,G2,G4,G6 the backbone angles γ were determined as γ^+ (gg) from measurements of $J_{4',5'}$ and $J_{4',5''}$.
5. NOE interactions between 2'-O-methyl groups and both intra-and inter-residue to H6/H8, and intra-residue to sugar protons have been found using NOESY spectrum. They allow to introduce first step of DG constrains to a canonical A-RNA structure during restrained molecular mechanics calculation (IrisIndigo2, Biosym Tech. software). In this very stable (as reflected by temperature-dependent measurements) A-RNA structure, 2'-O-Me groups are directed towards a minor groove. Further calculations are in progress.

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