CONFORMATION OF 2'-O-METHYL CYTIDINE, A MODIFIED FURANOSE COMPONENT OF RIBONUCLEIC ACIDS.

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The crystal structure of an orthorhombic form of 2'-O-methyl cytidine was determined from three dimensional X-ray diffraction data. The two molecules in each asymmetric unit have C2-endo C3-exo puckered furanose rings. This differs from the C3-endo puckering observed for cytidine (1) and it may have some relevance to the kinks that appear at the two 2'-O-methylated nucleotides in the anticodon phosphate ester backbone of the phe tRNA structure (2). This work and other studies (3,4) show that the presence of a 2'-O-methyl group does not prevent the furanose moiety from adopting its most commonly observed configurations. 2'-O-methyl nucleotides make up a small percentage of the residues in HnRNA, rRNA, tRNA and mRNA and therefore their conformational nuances are of interest.

INTRODUCTION

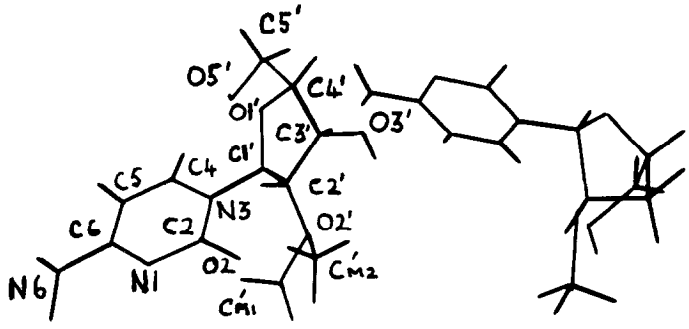
Furanose methylation occurs on the 45S RNA, or a related primary transcription unit, in all eukaryotes so far examined. The commencement of post transcriptional processing of the nuclear precursor of rRNA in HeLa cells has been shown to depend on methylation (5), over 90-percent of which occurs on the furanose groups (6). Although approximately 50-percent of the ribonucleotides are lost in this processing event, the 2'-O-methyl nucleotides are retained in some organisms studied (7,8). The apparent relationship between 2'-O-methyl nucleotide content and metabolic stability of certain segments of RNA has

led to the suggestion that it might be part of a cellular mechanism for processing RNA molecules.

2'-O-methyl cytidylic acid is present in a number of tRNA sequences, nearly always at the 5'-end of the anticodon loop. Further interest in the role of 2'-O-methylation has been generated by the recent report of the three dimensional structure of tRNA. In this structure the phosphate ester backbone kinks at residues 32 and 34, the only 2'-O-methyl nucleotides present. In the structure determination of tRNA and larger nucleic acid molecules the resolution and quality of the X-ray data is not so good that atoms can be identified in the electron density maps. It is from high resolution single crystal analyses of compounds, like 2'-O-methyl cytidine, that conformation is revealed in atomic detail and useful model building information becomes available.

METHODS

The methylation of cytidine at the 2'-hydroxyl site by treatment with diazomethane has been described (9). It was purified on a Dowex 1 (OH^-) column and eluted with 30-percent aqueous methanol. The eluted fraction containing chromatographically homogeneous 2'-O-methyl cytidine was concentrated under vacuum at 37°C to approximately 0.5M. Crystals of 2'-O-methyl cytidine formed after 24 hours at room temperature. This nucleoside crystallizes in the space group $P2_12_12_1$ with $\underline{a}$ = 8.742(5), $\underline{b}$ = 8.673(4) and $\underline{c}$ = 31.537(15) Å. There are eight molecules of nucleoside in the unit cell and thus two crystallographically independent molecules in the asymmetric unit. Intensities for 2460 unique reflections were measured on a Picker four circle diffractometer using molybdenum Kα radiation. Trial coordinates for all 36 non-hydrogen atoms were obtained by direct methods using the computer program MULTAN (10). Coordinates for the non-hydrogen atoms and their anisotropic temperature factors were refined in a least-squares fashion.



OmC1 OmC2

Figure 1.

The two crystallographically independent, but similar, 2'-O-methyl cytidine conformations. Both positions of the statistically distributed methyl group are shown for OmC1.

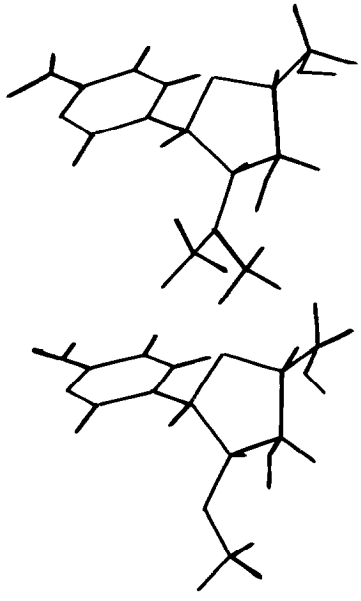


Figure 2.

The two molecules of 2'-O-methyl cytidine arranged (using interactive computer graphics) to be in the same relative orientation so that they may be easily compared.

Hydrogen atoms were selected as peaks in difference Fourier maps. The final R index (= Σ $||Fo| - |Fc|| / \Sigma |Fo|$) for all reflections is 0.069.

RESULTS

Figure 1 shows the two crystallographically independent 2'-O-methyl cytidine molecules, OmC1 and OmC2. Figure 2 shows the two molecules after manipulation on an interactive computer graphics system and this view clearly demonstrates how alike the molecules are. Both molecules have furanose rings in anti orientation to the bases. The dihedral angle about N3C1' that defined this conformation may be expressed as χ [C2N3C1'C2'] (11) or as ϕCN [C4N3C1'O1'] (12) where the atomic numbering scheme is that of figure 1. χ (and ϕCN) for OmC1 is 112.9° (and 47.4°) and for OmC2 is 108.2° (and 43.6°). These χ angles are close to the average value 112°, for C2-endo furanose rings, arrived at by Arnott and Hukins (11) in their survey of nucleosides and nucleotides. The furanose rings are indeed puckered C2-endo C3-exo, with atoms C2' and C3' displaced 0.41Å and -0.23Å for OmC1 and 0.45Å and -0.16Å for OmC2, from the C1'O1'C4' plane with respect to C5'. Other parameters that describe the furanose conformation (13) are listed in Table I. The arrangement of atoms about the C4'C5' bond is gauche, gauche. This means that the orientation of the C5'O5' bond about C4'C5' with respect to the ring bonds C3'C4' and C4'O1' is gauche (∿60°),

Table I. Furanose Conformational Angles

Molecule	τ0	τ1	τ2	τ3	τ4	P	τ
OmC1	-16.0°	34.2°	-38.2°	30.1°	-8.8°	174.5°	38.4°
OmC2	-17.8	33.6	-36.9	27.6	-6.5	171.3	37.3
C2-endo values from (11)	-23.5	36.9	-35.9	22.9	0.2	162.0	38.4

τ0-τ4 are the dihedral angles about the furanose ring bonds O1'C1', C1'C2', C2'C3', C3'C4' and C4'O1', respectively. P and τ are the "phase angle" of pseudorotation and the "degree of pucker." (13)

gauche (∿-60°) respectively. ϕ_{oo}[O5'C5'C4'O1'] is -64.9° for OmC1 and -67.0° for OmC2. ϕ_{oc}[O5'C5'C4'C3'] is 55.6° for OmC1 and 52.6° for OmC2. Another conformational angle of importance is σ[O3'C3'C4'C5']. This dihedral angle has averaged values of 83°, 147° and 156° for C3-endo, C2-endo and C3-exo furanose puckers respectively (11). OmC1 with σ at 151.0° and OmC2 with σ at 150.1° show both C2-endo and C3-exo character but, they are closer to C2-endo furanose puckers, hence their description as C2-endo C3-exo.

The main interest in this structure determination centers on the methoxy group orientation and its conformational implications, if any. The methyl group is oriented with respect to C1', C3' and H2' about the O2'C2' bond. We define τMH [Cm'O2'C2'H2'], τMC1 [Cm'O2'C2'C1'] and τMC3 [Cm'C2'O2'C3']. These three angles are -31.1°, 94°, 207° for OmC1 (66-percent Cm1' in figure 1) 36.6°, 161.7°, 274.7° for OmC1 (33-percent Cm2' in figure 1)and 39.7°, 167.3°, 279.8° for OmC2. These staggered positions are clearly illustrated in figures 1 and 2. τMH for 2'-O-methyl adenosine is 23° for molecule A and 28° for Molecule B. (3) The pattern of hydrogen bonds in the crystal structure was studied using three dimensional interactive computer graphics. A noticeable feature is the lack of base-base interactions. Intermolecular hydrogen bonding is largely between bases and furanose rings. Much of the donor and acceptor potential is realized, with all hydrogens that are covalently bonded to nitrogens and oxygens participating in hydrogen bonding. C4 is close to O1' and O5' in each molecule.

DISCUSSION AND CONCLUSION

In table II 2'-O-methyl cytidine is compared with cytidine and 2'-O-methyl adenosine. The different furanose puckerings observed in the 2'-O-methylated molecules show that this modified nucleoside is flexible enough

Table II. Comparison of OmC with Two Related Nucleosides (g = gauche, t = trans)

Molecules	ϕCN	Conformation about C4'C5'		Pucker	τMH
		ϕ_{oo}	$\phi_{oc}(\xi)$		
Cytidine (1)	18.4°	g	g	C3-endo	----
2'-O-methyl cytidine					
Molecule 1	47.4	g	g	C2-endo C3-exo	-31° and 37°
Molecule 2	43.6	g	g	C2-endo C3-exo	40
2'-O-methyl adenosine (3)					
Molecule A	14.5	g	g	C3-endo C2-exo	26
Molecule B	0.5	g	t	C2-exo C3-endo	28

to assume various conformations in different crystalline environments. These conformations might be useful in model building large nucleic acid molecules which contain 2'-O-methylated residues. For example, they may be pertinent to the observed backbone kinks at the 2'-O-methylated nucleotides in the anticodon loop of phe tRNA (2). Of the 2'-methoxy staggers (τMH) in table II the one most compatible with RNA double helical geometry is the -31° observed in OmC1. The 2'-O-methylated nucleotides are not invariant residues in tRNA but there is some indication that this modification influences the propensity of a given base to participate in double helical or base paired configurations (4). At least one 2'-O-methyl nucleotide is in the wobble base position.

It is hoped that this analysis and further structural studies will contribute to a conformational hypothesis that might explain both the specificity of furanose methylation and a possible biological role.

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