X-ray structure of cytidine-5'-O-dimethylphosphate. Novel stacking between the ribosyl O(2') hydroxyl oxygen atom and the base

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ABSTRACT

The anionic oxygen atoms of the phosphodiester backbone of RNA and DNA are particularly susceptible to esterification by many mutagenic and carcinogenic alkylating agents. To better understand the geometric, electronic and conformational properties of the alkylated sugar phosphate moiety, the X-ray structure of the phosphotriesterified nucleotide, cytidine-5'-O-dimethylphosphate ($C_{11}H_{18}N_3O_8P$), was undertaken. The compound crystallizes in the monoclinic space group $P2_1$, with unit cell parameters of $a = 5.741(2)$, $b = 11.625(1)$, $c = 11.425(1)$ Å, $\beta = 94.43(2)^\circ$. The structure was solved by direct methods and refined by block-diagonal least-squares technique to an R index of 0.034 ($R_w = 0.046$). The D-ribofuranosyl ring is in the 4T_1 twist conformation ($P = 13.1(2)^\circ$, $\tau = 36.7(2)^\circ$) and the conformation about the C(1')-N(1) glycosyl bond is *anti* ($\chi_{CN} = 8.3(2)^\circ$). The four P-O bond lengths are significantly shorter than those of the nonalkylated nucleotides. The three sets of phosphodiester linkages, (w'_A, w_A) , (w'_B, w_B) and (w'_C, w_C) , take the (g, t), (t, g) and (g, t) conformations, respectively. There is no base-base or alkyl-base stacking, however, a novel intermolecular stacking is found between the ribosyl O(2') hydroxyl oxygen atom and a neighboring pyrimidine ring. This hydroxyl-base stacking interaction may have implications in the stabilization of the tertiary and quaternary structure of ribonucleic acids and nucleic acid-protein complexes.

INTRODUCTION

The attack by many mutagenic and carcinogenic alkylating agents on RNA and DNA often results in the alkylation of the sugar phosphate backbone.¹⁻⁶ Several studies characterizing the biochemical and conformational properties of the triesterified sugar phosphate backbone have been carried out using phosphotriesterified oligonucleotides. It has been shown that, due to the nonionic nature of the triesterified phosphate group, such alkylated nucleotides are able to easily cross cell membranes.^{7,8} Miller *et al.* have demonstrated that a single ethylphosphotriester group on a decadeoxyoligonucleotide template significantly lowers its rate and extent of polymerization by DNA polymerase I.⁹ It has also been shown that the triesters d-Tp(Et)Tp(Et)Cp(Et)A and d-Tp(Et)Gp(Et)G are able to form hydrogen bonded complexes with their complementary sequences -UpGpApA (of the anticodon region of yeast and

E. coli tRNA^{Phe}), and -CpCpA (of the 3' amino accepting terminus of tRNA^{Phe}), respectively.¹⁰

The finding of hydrogen bonded complex formation combined with spectroscopic studies of phosphotriesterified polymers^{6,11} clearly demonstrates that esterification of the sugar phosphate backbone does not interfere with the formation of a right-handed Watson-Crick double helical structure, i.e. the assumption of a (g^-, g^-) sugar phosphate backbone conformation. On the other hand, phosphotriesterified nucleotides not involved in base pairing display increased conformational flexibility in the sugar phosphate backbone. Circular dichroism studies on the dinucleotide phosphotriesters dAp(Et)T,⁶ dTp(^{Me}_{Et})T¹¹ and dAp(^{Me}_{Et})A¹¹ revealed that they exhibit decreased base-base stacking interactions as compared to the dinucleotides dApT, dTpT and dApA, respectively, indicating that the sugar phosphate backbone is usually not in a helical (g^-, g^-) conformation. Furthermore, NMR experiments on the dideoxyligonucleotide ethyl phosphotriesters, dIp(Et)I and dAp(Et)A, have revealed that the ethyl groups undergo shielding by the purine rings,¹² which requires the alkylated sugar phosphate group to take a non (g^-, g^-) conformation.

Recently, we determined the X-ray structure of the first free (noncyclic) phosphotriesterified nucleotide, adenosine-5'-O-diethylphosphate, and found that, in addition to the significant changes in the electronic and geometric properties of the phosphate group, none of its three combinations of phosphodiester linkages assumed a (g^-, g^-) conformation.¹³ It was also found that one ethyl group was involved in intramolecular stacking with the adenine base. To gain additional insight into the conformational, electronic and geometric properties of the triesterified sugar phosphate backbone, we have determined the crystal and molecular structure of the phosphotriesterified pyrimidine nucleotide cytidine-5'-O-dimethylphosphate. From the structural and conformational analyses, we show that methylation (like ethylation) of the phosphate moiety results in geometric and electronic perturbations of the phosphate group and a lack of preference for the (g^-, g^-) phosphodiester conformation. Furthermore, the crystal packing reveals a novel intermolecular stacking between the O(2') ribosyl hydroxyl oxygen atom and a pyrimidine ring, and its structural significance is discussed.

EXPERIMENTAL

A crystal of cytidine-5'-O-dimethylphosphate (FW = 351.26), crystallized from water and air dried ($D_m = 1.52 \text{ g cm}^{-3}$, $D_c = 1.534 \text{ g cm}^{-3}$, $Z = 2$), with dimensions 0.5 x 0.3 x 0.22 mm, was chosen for intensity data collection on

an Enraf-Nonius CAD4 diffractometer using Zr-filtered Mo K_{α} radiation ($\lambda = 0.7107 \text{ \AA}$). The unit cell parameters $a = 5.741(2)$, $b = 11.625(1)$, $c = 11.425(1) \text{ \AA}$, $\beta = 94.43(2)^{\circ}$, $V = 760 \text{ \AA}^3$, were refined using 25 automatically centered reflections. Of a total of 2777 unique reflections, measured to a 2θ value of 64° , 2458 reflections with intensities greater than $1.5\sigma(I)$ were used in the structural analysis. Three reflections monitored throughout data collection (every 2 hours of X-ray exposure) revealed negligible decay. An absorption correction employing an empirical ϕ curve as well as Lorentz and polarization corrections were applied to the intensities.

STRUCTURAL DETERMINATION AND REFINEMENT

The structure was solved by application of the multisolution tangent formula technique using the computer program MULTAN.¹⁴ The initial E map, computed for the phase set with the lowest residual, revealed 21 of the 23 nonhydrogen atoms. The remaining nonhydrogen atoms were located in the following Fourier synthesis. The atomic parameters of the nonhydrogen atoms were refined by block-diagonal least-squares technique employing anisotropic thermal parameters. Subsequent difference Fourier syntheses revealed all 18 hydrogen atoms, whose atomic positions were refined by block-diagonal least-squares employing isotropic thermal parameters. Three hydrogen atoms, H(03'), H2(C7) and H2(C8), failed to converge during refinement. The H(03') atomic position was fixed at the coordinates obtained for it from the initial difference Fourier map, while the atomic coordinates of the H2(C7) and H2(C8) hydrogen atoms were fixed from geometric considerations. The block-diagonal least-squares refinement converged at $R = \sum ||F_o| - |F_c|| / \sum |F_o| = 3.4$ ($R_w = 4.6$).¹⁵ The function minimized was $\sum w(|F_o| - |F_c|)^2$ where a modified counting statistics weighting scheme¹⁶ was used in which $w = 1/[\sigma^2(F) + (0.02F_o)^2]$. The average shift/error ratios for the atomic parameters were 0.002 for the nonhydrogen atoms, with a maximum value of 0.018 for O(3'), and 0.05 for the hydrogen atoms.

Scattering factors for the nonhydrogen atoms were taken from Cromer and Waber¹⁷ and those for the hydrogen atoms were from Stewart *et al.*¹⁸

RESULTS AND DISCUSSION

An ORTEP¹⁹ drawing indicating the atom numbering and overall molecular conformation is given in Figure 1. The fractional positional and isotropic thermal parameters for all atoms are presented in Table 1.²⁰ The bond lengths and bond angles for the nonhydrogen atoms are listed in Table 2.

Figure 1. ORTEP drawing of cytidine-5'-O-dimethylphosphate. The nonhydrogen atoms are represented by 50% probability ellipsoids while the hydrogen atoms are drawn as spheres of arbitrary size.

The ribofuranosyl, glycosyl and sugar phosphate backbone torsion angles are given in Table 3.

Ribosyl, Glycosyl and Base Geometry and Conformation

The ribofuranose ring of cytidine-5'-O-dimethylphosphate is found in the 3T_2 twist conformation,²¹ one of the preferred sugar puckers, where the phase angle (P) and amplitude (τ_m) of pseudorotation²² are $13.1(2)^\circ$ and $36.7(1)^\circ$, respectively. This contrasts to the sugar pucker of the ribofuranose ring of the other known noncyclic phosphotriester, adenosine-5'-O-diethylphosphate,¹³ which exhibits the alternative preferred conformation, the C(2')-endo.²³ The conformation about the C(4')-C(5') bond is gauche⁺ ($46.5(3)^\circ$).

The glycosyl bond length, N(1)-C(1'), and bond angle, N(1)-C(1')-O(4'), are $1.487(3)\text{\AA}$ and $108.7(2)^\circ$, respectively. The conformation about the glycosyl bond is anti with a value of $8.3(2)^\circ$ for χ_{CN} (O(4')-C(1')-N(1)-C(6)), which is smaller than the anti χ_{CN} value ($69.0(3)^\circ$) of adenosine-5'-O-diethylphosphate.¹³

The values of the bond lengths and angles for the pyrimidine ring are the same as those reported for the standard neutral cytosine ring,²⁴ from which the largest deviations in bond length and angle are $0.007\text{\AA}$ and 0.8° , respectively. The equation describing the least-squares plane through the six atoms of the pyrimidine ring, which is flat, is $-0.721 X + 0.399 Y - 0.566 Z = 3.87$ where X, Y and Z are measured in angstroms along the crystallographic a, b and c axes, respectively. The exocyclic atoms O(2) and N(4)

Table 1. Atomic Parameters for Cytidine-5'-O-dimethylphosphate. Fractional Positional Parameters are Multiplied by 10^3 for Nonhydrogen Atoms and 10^4 for Hydrogen Atoms.

$B_{eq} = \frac{4}{3} \sum_i \sum_j B_{ij} a_i \cdot a_j$				
Atom	x	y	z	B_{eq} or B
P	34695(8)	83831(0)*	-341(3)	2.22(1)
O(1)	19757(27)	77480(15)	7155(13)	3.31(3)
O(3)	55566(28)	90490(15)	5975(13)	3.44(3)
C(7)	67415(45)	85633(26)	16478(23)	4.19(6)
O(4)	21945(31)	93299(16)	-8451(14)	3.73(4)
C(8)	9044(53)	102310(28)	-3585(24)	4.76(7)
O(5')	46333(24)	76487(14)	-9742(12)	2.98(3)
C(5')	33513(33)	67650(17)	-16401(16)	2.64(4)
C(4')	43371(30)	66329(15)	-28188(15)	2.15(3)
C(3')	69950(31)	65690(17)	-27973(15)	2.35(3)
O(3')	79356(31)	54663(16)	-25577(14)	3.86(4)
C(2')	74311(29)	69991(16)	-40276(15)	2.19(3)
O(2')	70229(25)	60714(14)	-48162(12)	3.03(3)
C(1')	55192(30)	79015(16)	-42490(14)	2.20(3)
O(4')	36945(21)	76166(13)	-35402(12)	2.49(3)
N(1)	63805(26)	90739(13)	-39294(12)	2.19(3)
C(2)	79301(32)	95871(16)	-46594(14)	2.32(3)
N(3)	87629(29)	106504(15)	-43696(13)	2.53(3)
C(4)	80537(31)	112069(17)	-34406(14)	2.35(3)
C(5)	64590(36)	106987(18)	-27012(15)	2.67(4)
C(6)	56567(33)	96452(17)	-29872(15)	2.49(4)
O(2)	84784(30)	90636(15)	-55360(12)	3.36(3)
N(4)	88824(35)	122595(17)	-32071(15)	3.31(4)
H1(5')	370(4)	606(3)	-117(2)	2.7(5)
H2(5')	158(4)	694(2)	-184(2)	2.1(4)
H(4')	352(4)	596(3)	-315(2)	2.7(5)
H(3')	766(4)	707(2)	-216(2)	1.5(4)
H(03')**	793(0)	508(0)	-318(0)	4.0(0)
H(2')	896(4)	724(2)	-409(2)	2.6(5)
H(02')	826(7)	605(5)	-508(3)	7.4(12)
H(1')	494(5)	794(2)	-508(2)	1.6(4)
H(5)	576(5)	1113(3)	-207(2)	3.4(6)
H(6)	456(4)	929(3)	-251(2)	3.0(5)
H1(N4)	853(6)	1265(4)	-245(3)	5.1(8)
H2(N4)	961(4)	1256(3)	-368(2)	2.5(5)
H1(C7)	566(4)	841(3)	225(2)	3.6(6)
H2(C7)**	735(0)	783(0)	147(0)	4.0(0)
H3(C7)	811(5)	917(4)	183(3)	5.3(8)
H1(C8)	144(6)	1062(4)	56(3)	6.5(9)
H2(C8)**	-43(0)	922(0)	-3(0)	4.9(0)
H3(C8)	-16(7)	1055(5)	-105(4)	9.7(14)

* The y atomic coordinate of the phosphorus atom was fixed during refinement.

** The atomic coordinates of these hydrogen atoms were fixed from geometric considerations after failure to converge during refinement.

Table 2. Nonhydrogen Bond Lengths (Å) and Angles (°) for Cytidine-5'-O-dimethylphosphate.

N(1)-C(2)	1.399(2)	N(1)-C(1')	1.487(2)	C(4')-C(5')	1.509(3)
C(2)-N(3)	1.357(3)	C(1')-O(4')	1.412(2)	C(5')-O(5')	1.446(3)
C(2)-O(2)	1.233(2)	C(1')-C(2')	1.525(3)	O(5')-P	1.561(2)
N(3)-C(4)	1.334(2)	C(2')-O(2')	1.413(2)	P-O(1)	1.459(2)
C(4)-N(4)	1.333(3)	C(2')-C(3')	1.531(2)	P-O(3)	1.556(3)
C(4)-C(5)	1.422(3)	C(3')-O(3')	1.410(3)	O(3)-C(7)	1.448(3)
C(5)-C(6)	1.340(3)	C(3')-C(4')	1.526(3)	P-O(4)	1.582(3)
C(6)-N(1)	1.357(2)	C(4')-O(4')	1.441(2)	O(4)-C(8)	1.421(4)
N(1)-C(2)-N(3)	118.1(2)	O(4')-C(1')-C(2')	107.5(2)		
N(1)-C(2)-O(2)	119.2(2)	C(1')-C(2')-O(2')	109.7(2)		
O(2)-C(2)-N(3)	122.6(2)	O(2')-C(2')-C(3')	107.8(2)		
C(2)-N(3)-C(4)	120.8(2)	C(2')-C(3')-O(3')	112.8(2)		
N(3)-C(4)-N(4)	118.7(2)	O(3')-C(3')-C(4')	114.5(2)		
N(3)-C(4)-C(5)	121.4(2)	O(4')-C(4')-C(5')	109.4(2)		
N(4)-C(4)-C(5)	119.9(2)	C(3')-C(4')-C(5')	115.7(2)		
C(4)-C(5)-C(6)	117.4(2)	C(4')-C(5')-O(5')	109.4(2)		
C(5)-C(6)-N(1)	121.3(2)	C(5')-O(5')-P	121.5(2)		
C(6)-N(1)-C(2)	120.8(2)	O(5')-P-O(1)	115.5(2)		
C(6)-N(1)-C(1')	121.8(2)	O(5')-P-O(3)	103.6(2)		
C(2)-N(1)-C(1')	117.3(2)	O(5')-P-O(4)	100.4(2)		
N(1)-C(1')-O(4')	108.7(2)	O(1)-P-O(3)	116.5(2)		
N(1)-C(1')-C(2')	111.6(2)	O(1)-P-O(4)	115.4(2)		
C(1')-C(2')-C(3')	102.1(2)	O(3)-P-O(4)	103.3(2)		
C(2')-C(3')-C(4')	101.7(2)	P-O(3)-C(7)	119.0(2)		
C(3')-C(4')-O(4')	105.1(2)	P-O(4)-C(8)	121.0(2)		
C(4')-O(4')-C(1')	110.2(2)				

Table 3. Selected Torsion Angles(°) of Cytidine-5'-O-dimethylphosphate.

χ_{CN}	O(4')-C(1')-N(1)-C(6)	8.3(2)
τ_o	C(4')-O(4')-C(1')-C(2')	3.4(2)
τ_1	O(4')-C(1')-C(2')-C(3')	-24.6(2)
τ_2	C(1')-C(2')-C(3')-C(4')	34.9(2)
τ_3	C(2')-C(3')-C(4')-O(4')	-34.0(2)
τ_4	C(3')-C(4')-O(4')-C(1')	19.6(2)
ψ'_A	O(3')-C(3')-C(4')-C(5')	83.3(3)
ψ_A	C(3')-C(4')-C(5')-O(5')	46.5(3)
ϕ_A	C(4')-C(5')-O(5')-P	151.4(2)
ω_A	C(5')-O(5')-P-O(3)	170.4(2)
ω'_A	O(5')-P-O(3)-C(7)	-93.0(2)
ω_B	C(5')-O(5')-P-O(4)	-83.0(2)
ω'_B	O(5')-P-O(4)-C(8)	-178.6(3)
ω_C	C(7)-O(3)-P-O(4)	162.6(2)
ω'_C	O(3)-P-O(4)-C(8)	-71.8(3)
ω_D	C(5')-O(5')-P-O(1)	41.8(2)

are within the plane as is the substituent atom C(1').

Sugar Phosphate Geometry

The dimethylation of cytidine-5'-monophosphate results in the geometric perturbation and neutralization of its sugar phosphate backbone. The phosphoester bonds, P-O(3), P-O(4) and P-O(5'), are 1.556(3), 1.582(3) and 1.561(2) Å, respectively, and are shorter than the average phosphoester bond found in the nucleotide monophosphates (1.60 Å). When the three bond lengths are averaged, their mean P-O distance (1.566 Å) is slightly longer than the averaged phosphoester bond length of the phosphotriesterified nucleotide, adenosine-5'-O-diethylphosphate (1.558 Å).¹³ The nonesterified P-O(1) bond (1.459(2) Å), on the other hand, is slightly shorter than the corresponding P-O bond observed for adenosine-5'-O-diethylphosphate (1.464(2) Å), but falls within the range of values for the nonesterified P-O bond lengths of all phosphotriesterified nucleotides (1.44(1) Å to 1.466(2) Å).^{13,25,26} The shortened P-O(1) bond length reveals a strong double bond character, which is further reflected by the values of the O(5')-P-O(1) (115.4(2)°), O(3)-P-O(1) (116.5(2)°) and O(4)-P-O(1) (115.4(2)°) bond angles. The remaining phosphoester bond angles, O(3)-P-O(4), O(3)-P-O(5') and O(4)-P-O(5') are 103.3(2)°, 103.6(2)° and 100.4(2)°, respectively, and fall within the range of values observed for the phosphodiester themselves.

The Phosphodiester Backbone Conformation-(w',w)

The triesterification of the sugar phosphate backbone results in three sets of phosphodiester linkages; $w'_A[O(5')-P-O(3)-C(7)]$, $w_A[C(5')-O(5')-P-O(3)]$; $w'_B[O(5')-P-O(4)-C(8)]$, $w_B[C(5')-O(5')-P-O(4)]$; $w'_C[O(3)-P-O(4)-C(8)]$, $w_C[C(7)-O(3)-P-O(4)]$ (Table 3). (w'_A, w_A) takes a (g^-, t) phosphodiester conformation, which is the same as that observed for the (w'_A, w_A) phosphodiester linkage of the phosphotriester adenosine-5'-O-diethylphosphate.¹³ (w'_C, w_C), which describes the phosphodiester conformation between the C(7) and C(8) methyl groups, is also found in the (g^-, t) conformation and differs from the (t,t) conformation found for the analogous phosphodiester linkage between the ethyl groups of adenosine-5'-O-diethylphosphate.¹³ (w'_B, w_B) assumes a (t, g^-) conformation, which is similar to the phosphodiester conformations found in the crystal structures of the single stranded dinucleotides UpA²⁷ and pTpT.²⁸ Again, as in the other noncyclic phosphotriester, adenosine-5'-O-diethylphosphate, none of these phosphodiester linkages assumes the (g^-, g^-) conformation, which is favored by right-handed polynucleotide helices.²³

Figure 2. A crystal packing diagram of six molecules viewed from a general position displaying some of the hydrogen bonding scheme. The hydrogen bonding and short nonbonded contacts are given in Tables 4 and 5.

Hydrogen Bonding and Molecular Packing

A packing diagram, illustrating some of the intermolecular hydrogen bonding, is given in Figure 2. Hydrogen bonding is centered about the N(4) amino protons, which form hydrogen bonds to neighboring O(1) and O(2) oxygen atoms (Table 4), and the O(2') hydroxyl proton which bonds to the pyrimidine ring nitrogen atom N(3) (2.703(2)Å). The H(03') proton may be involved in an intramolecular hydrogen bond to the O(2') oxygen atom where the H(03')-O(2') distance is 2.223(1)Å and the O(3')-H(03')-O(2') angle is 114.8(2)°. However, the failure of H(03') to converge during refinement makes the participation of H(03') in hydrogen bonding uncertain. The O(3') hydroxyl

Table 4. Hydrogen Bond Lengths (Å) and Angles (°).

<u>A-H...B</u>	Symmetry Code and Translation for <u>B</u>	<u>A-H</u>	<u>H...B</u>	<u>A...B</u>	<u>A-H...B</u>
N(4)-H1(N4)...O(1)	(2,x+1,y,z)	1.01(4)	2.03(3)	2.980(3)	156(4)
N(4)-H2(N4)...O(2)	(2,x+2,y,z-1)	0.79(3)	2.28(3)	3.016(3)	154(3)
O(2')-H(02')...N(3)	(2,x+2,y-1,z-1)	0.79(4)	1.92(4)	2.703(2)	167(5)
C(8)-H1(C8)...O(3')	(2,x+1,y,z)	1.16(4)	2.29(3)	3.358(3)	152(3)

Table 5. Shorter Intermolecular Contacts (Å).

A...B	Symmetry Code and Translation for B	
O(2')...N(1)	(2,x+1,y-1,z+1)	3.291(2)
O(2')...C(2)	(2,x+1,y-1,z+1)	3.341(2)
O(2')...N(3)	(2,x+1,y-1,z+1)	3.415(2)
O(2')...C(4)	(2,x+1,y-1,z+1)	3.404(2)
O(2')...C(5)	(2,x+1,y-1,z+1)	3.370(3)
O(2')...C(6)	(2,x+1,y-1,z+1)	3.289(2)
O(2')...C(2)	(2,x+2,y-1,z+1)	3.464(2)
O(2')...O(2)	(2,x+2,y-1,z+1)	3.481(2)
O(2')...N(4)	(2,x+2,y-1,z+1)	3.655(3)
C(7)...O(2)	(1,x,y,z+1)	3.345(3)
C(7)...N(4)	(2,x+2,y-1,z)	3.330(4)
C(7)...O(1)	(1,x-1,y,z)	3.399(3)
C(8)...C(5)	(1,x-1,y,z)	3.594(3)
C(8)...O(1)	(2,x,y-1,z)	3.370(4)

oxygen atom does, however, make a C-H...O hydrogen bond to the H(1) proton of the C(8) methyl group (Table 4).

In addition to hydrogen bonding, the molecular packing is characterized by the van der Waals interactions between the C(7) methyl carbon atom and the N(4), O(2) and O(1) atoms (Table 5), where the shortest contact is with N(4) (3.330(4)Å). The C(8) methyl carbon atom has van der Waals contacts with the C(5) carbon atom (3.594(3)Å) and the O(1) oxygen atom (3.370(4)Å). There are no methyl-methyl nonbonded contacts or methyl-base stacking interactions.

Hydroxyl-Base Stacking - A Half Sandwich Structure

Base-base stacking interactions are not present in the crystal structure of cytidine-5'-O-dimethylphosphate. However, a novel intermolecular stacking is found between the O(2') hydroxyl oxygen atom of the ribofuranose ring and a neighboring pyrimidine ring (Figures 3a & b), which resembles the sugar ring oxygen-base stacking interactions observed in many crystal structures of nucleic acid constituents.^{27,29-34} In this structure the hydroxyl oxygen atom sits below the pyrimidine ring allowing its lone pair electrons to interact with the π electron cloud of the base (Figure 3a). From its nearly central position the O(2') hydroxyl atom makes favorable nonbonded contacts with all the ring atoms (Table 5), the strongest of which are with the C(6) carbon (3.289(2)Å) and N(1) nitrogen (3.291(2)Å) atoms and the weakest with the N(3) nitrogen atom (3.415(2)Å). The H(O2') hydroxyl proton is not involved in stacking with the ring (see below).

The hydroxyl-base stacking interaction is similar to the water-base

Figure 3. Projections perpendicular (a) and parallel (b) to the base plane displaying the intermolecular O(2') hydroxyl-pyrimidine ring stacking. The dashed lines between the O(2') oxygen atom and the base represent intermolecular contacts while the dashed line from H(03') to the N(3) nitrogen atom represents hydrogen bonding (see Tables 4 and 5).

(preferably a purine) stacking interactions found in some of the so-called half sandwich structures.³⁵⁻³⁹ In these structures the water molecule is "sandwiched" by a base on one side and two neighboring bases on the other side such that they form a "window" around it. Also, the stacking water is not involved in hydrogen bonding to the stacked base. In our structure the O(2') hydroxyl group replaces the water molecule. The window here is formed by a neighboring pyrimidine ring and the ribofuranose ring to which the stacking hydroxyl atom is covalently bonded (Figure 3b). The O(2') hydroxyl group, like the stacking water, is not involved in hydrogen bonding to the stacked base, rather, the H(02') hydroxyl proton forms a hydrogen bond to the N(3) nitrogen atom of the "window" base (see Table 4 and Figure 3b).

Structural Implications of the O(2') Hydroxyl Group in RNA

The O(2') hydroxyl group apparently plays several roles in RNA structure. First, the 2'OH group has been shown to stabilize the C(3')-endo sugar pucker and anti glycosyl conformation in ribonucleotides by forming water mediated hydrogen bonds to the N(3) nitrogen atom of purines or the O(2) carbonyl oxygen atom of pyrimidines.⁴⁰ Further, the O(2') ribosyl hydroxyl group strengthens the sugar phosphate backbone structure of yeast tRNA^{phe} by hydrogen bonding to some of the 3'-side sugar ring oxygen atoms and phosphate group oxygen atoms (both possibly water mediated).⁴¹⁻⁴³ We here observe that the O(2') hydroxyl oxygen atom stacks with a neighboring pyrimidine base. It is not unreasonable to assume that such stacking interactions could exist in vivo and play a role in stabilizing the tertiary structures of ribonucleic acids, such as messenger and ribosomal RNA. It is also

Figure 4. An overlay of cytidine-5'-O-dimethylphosphate (light molecule) and adenosine-5'-O-diethylphosphate (dark molecule), which have been homologized through their N(1,9)-C(1')-O(4')-C(5')-O(5')-P fragments. The terminal carbon atoms of the ethyl groups of adenosine-5'-O-diethylphosphate have been removed for the sake of clarity. It is seen that the triesterified nucleotides exhibit the same preferred conformational traits (C(3')-endo, anti, g or C(2')-endo, anti, g) as the nucleotides themselves.

possible that the ribosyl O(2') hydroxyl group could stack with the ring atoms of the aromatic amino acids tyrosine, phenylalanine and tryptophan. This stacking interaction could be important in the quarternary structures of nucleic acid-protein complexes, e.g., ribosomes. The above interaction properties of the 2'OH hydroxyl group of RNA appear responsible for conferring both rigidity (or lack of polymorphism⁴⁰) to RNA helices as compared to DNA helices, and flexibility to RNA, i.e., by generating a variety of nonhelical structures such as loops and bends.

SUMMARY

The molecular and crystal structure of the triesterified nucleotide cytidine-5'-O-dimethylphosphate is presented. As was observed in the crystal structure of the first noncyclic triesterified mononucleotide, adenosine-5'-O-diethylphosphate, the phosphate of the title triester undergoes significant perturbations in its electronic, geometric and conformational properties. An overlay of these nucleotide phosphotriesters, which were homologized through their N(1,9)-C(1')-O(4')-C(5')-O(5')-P fragments, shows that their alkylated sugar phosphate backbones are conformationally similar (Figure 4) and that none of the phosphodiester linkages takes the (g^- , g^-) conformation displayed by right-handed polynucleotide helices,²³ although sterically

allowed. Alkylation of the phosphate moiety apparently does not disturb the preferred geometries and conformations of the ribofuranose rings and bases as the phosphotriesters display C(2')-endo and C(3')-endo sugar puckers and anti glycosyl conformations adopted by the nucleotides themselves.²¹ Further, the sugar puckers and glycosyl conformations are correlated in a manner similar to that of the nonalkylated nucleotides, i.e. C(2')-endo-high anti (found in adenosine-5'-O-diethylphosphate) and C(3')- endo-low anti (found in cytidine-5'-O-dimethylphosphate).²¹

A novel intermolecular stacking, which is analogous to that observed in the half-sandwich structures, is found between the O(2') hydroxyl oxygen atom and the pyrimidine ring. Although the biological significance of such stacking is unknown it could play a role in the stabilization of the tertiary and quarternary structures of some macromolecules.

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