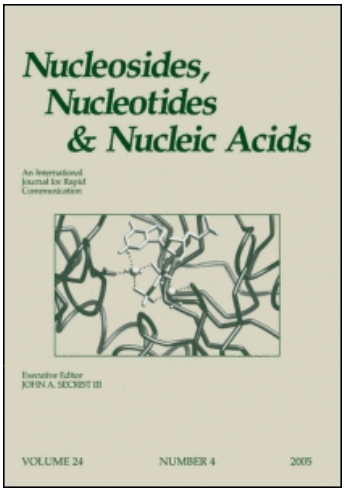

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STRUCTURAL FEATURES OF 2',3'-RIBOANHYDROADENOSINE, A CONFORMATIONALLY RESTRICTED TERMINATION SUBSTRATE OF DNA POLYMERASES

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ABSTRACT: 2',3'-Riboanhydroadenosine (raA), a conformationally restricted inhibitor of some DNA polymerases, has been studied by X-Ray crystallography. It crystallizes in space group P1 with unit cell parameters: a=4.834(1); b=6.893(1); c=15.942(2)Å; α=90.51(1); β=97.16(2); γ=89.27(2)°; V=527.1Å^3 and with two independent molecules (A and B) in the cell. The conformation of A and B molecules about the glycosidic bond is different. In the A molecule, the glycosidic torsion angle χA is 56.8° and corresponds to *syn* conformation; in the B molecule, χB=-170.8°, which corresponds to the nucleoside in *anti* conformation. The sugar rings of both molecules are slightly puckered (0.1Å), C1' being *exo* in A and C4'-*endo*-O4'-*exo* in B. The conformation of A and B molecules about the exocyclic bond C4'-C5' is *gauche*$^+$. The observed similarities in some structural and biochemical properties of 2',3'-riboanhydronucleosides and 2',3'-dideoxy-2',3'-didehydronucleosides are discussed.

INTRODUCTION

The study of 2',3'-riboanhydroadenosine 5'-triphosphate (raATP) as a substrate analogue of DNA polymerases was commenced by Abboud *et al* [1]. These authors found that, in the presence of a synthetic primer-template, [poly-d(A-T)], *E.coli* DNA polymerase I, Klenow fragment incorporated mononucleotide residue of raATP into the 3'-end of this polynucleotide. The modified polynucleotide was then covalently bound to the enzyme and, as a result, the latter was inactivated. A similar action mechanism was proposed for other DNA polymerases.

Later findings have demonstrated that raATP acts as a termination substrate in the presence of an oligodeoxynucleotide primer when natural DNA serve as a template: raATP is incorporated into the 3'-end of the primer chain, which does not bind covalently to DNA polymerase. This

occurs when the process is catalyzed by DNA polymerase I, Klenow fragment, rat liver DNA polymerase β, avian myeloblastosis virus (AMV) reverse transcriptase, and calf thymus terminal deoxynucleotidyl transferase. By contrast, calf thymus DNA polymerase α does not catalyze the incorporation of raATP into the 3'-end of a nascent DNA chain [2]. The authors of [2] hold that the additional epoxide ring of raATP makes the conformation of its sugar residue similar to that of natural dNTP substrates in a [DNA polymerase + primer-template + dNTP] complexes [3].

In 1989 Catalano and Bencovic used a heterodeoxynucleotide primer-template to demonstrate that raATP acted as a termination substrate for DNA polymerase I , Klenow fragment, i.e. was incorporated into the 3'-terminus of the template and formed no covalent bonds with the enzyme [4]. However, the rate of enzyme dissociation from the complex [DNA polymerase + template-terminated primer] was far lower as compared with complexes with DNA terminated by other substrates analogues. Human placenta DNA polymerase α was shown to be capable of raATP covalent binding in the presence of poly(dA).oligo(dT) but not in the presence of poly(dG).oligo(dC) [5]. Here, raATP did not react with the primer either with complementary or noncomplementary templates.

The results of [2,4] appear more indicative of the actual situation because natural heteronucleotide templates were used in these works. Therefore, the role of raATP as a termination substrate for a number of DNA polymerases may be taken as a fact. It would be of interest to study the conformation of either raATP or its precursor, 2',3'-riboanhydroadenosine (raA) for the following reasons: the limited number of its possible conformations on the one hand and the participation of this compound in the formation of a productive complex with the DNA synthesizing system on the other make it possible to furnish information about the conformation of substrates in DNA synthesizing complexes and about the topography of active centers in DNA polymerases. This communication presents the results of raA X-ray analysis.

EXPERIMENTAL

RaA was synthesized as in [2]. Crystals for X-ray analysis were grown from a saturated raA solution in methanol by slowly evaporating the solvent at an ambient temperature. The space group was P1, and the

unit cell parameters were as follows: a=4.834(1); b=6.893(1); c=15.942(2)Å; α=90.51(1); β =97.16(2); γ =89.27(2)^{o}; V=527.1Å^{3}; Z=2.

The unit cell contains two crystallographically independent raA molecules, A and B. The parameters of the unit cell and the intensities of reflections were measured with a CAD-4F diffractometer (the ω/θ scan technique, CuK_{α} radiation, a graphite monochromator). A full reciprocal sphere was measured and the intensities of reflections were averaged in order to reduce possible experimental errors. The intensities of 1376 independent reflections with $I > 3\sigma$ (I) were used in the work. The experimental data were corrected for the Lorentz and polarization factors. The structure was determined by direct methods and refined by the full-matrix least-squares method with anisotropic temperature factors for C, N and O atoms. The positions of H atoms were located on difference Fourier maps and refined in isotropic approximation. The refinement converged at R=4.1%. All the calculations were made with the SDP programs [6]. The coordinates of atoms and their thermal parameters are listed in Table 1.

RESULTS AND DISCUSSION

Figure 1 presents two crystalographically independent raA molecules with the atomic numbering accepted in this work, and shows the orientation of thermal ellipsoids for non-hydrogen atoms. The bond lengths and bond angles of A and B molecules are given in Table 2.

The geometrical dimensions of both molecules are similar within 3σ, and the differences found for certain bonds, which exceed this can be attributed to crystal quality.

The mean values of bond lengths and bond angles for the nucleotide bases in the raA molecules are within 2σ of those for neutral adenine [7]. The bases are nearly planar.

The mean geometrical dimensions of the carbohydrate moieties are close to those in the structure of 2',3'-lyxoanhydrothymidine (laT) [8]. Just as in laT, the C2'-C3' bonds in the epoxide ring of raA are shorter by ca. 10σ-12σ than the corresponding bonds in the natural nucleoside [9]. The shortening of these bonds makes the adjacent bond angles C1'-C2'-C3' and C2'-C3'-C4' greater by approximately 6^{o}.

The furanose cycles of raA A and B molecules have a slightly different conformation. In the A molecule, the phase angle of pseudorotati-

TABLE 1.

Positional parameters ($x10^4$ for C,O,N, $x10^3$ for H) and their estimated standard deviations in raA structure. Starred atoms were refined isotropically.

	Molecule A				Molecule B			
Atom	x	y	z	B_{equ}	x	y	z	B_{equ}
N1	7913(8)	4051(5)	-18(2)	3.19(7)	2175	3516	8131	3.00(7)
C2	6780(9)	4861(6)	645(3)	3.18(8)	3319(10)	2710(6)	7487(3)	3.11(8)
N3	5135(7)	4123(5)	1138(2)	3.00(7)	5052(8)	3472(5)	6995(2)	2.87(7)
C4	4481(9)	2281(6)	920(2)	2.70(8)	5686(8)	5310(6)	7238(2)	2.49(7)
C5	5355(9)	1288(6)	233(3)	2.92(8)	4737(9)	6301(6)	7916(2)	2.65(8)
C6	7096(9)	2234(6)	-248(2)	2.77(8)	2915(9)	5336(6)	8372(2)	2.69(8)
N7	4283(9)	-567(5)	178(2)	3.66(8)	5840(8)	8152(5)	7978(2)	3.29(7)
C8	2920(10)	-710(7)	832(3)	3.9(1)	7351(10)	8261(6)	7355(3)	3.30(9)
N9	2931(8)	977(5)	1309(2)	3.01(7)	7326(7)	6588(5)	6884(2)	2.60(6)
N6	8165(8)	1413(6)	-901(2)	3.64(8)	1842(8)	6165(5)	9029(2)	3.33(7)
C1'	1928(10)	1054(6)	2127(3)	3.23(8)	8860(8)	6179(6)	6154(2)	2.65(7)
C2'	4088(10)	1050(8)	2873(3)	4.3(1)	6906(10)	5996(6)	5348(3)	3.19(8)
O2'3'	2744(10)	695(6)	3638(2)	5.51(9)	8502(8)	5544(4)	4662(2)	4.05(7)
C3'	3467(10)	2634(8)	3433(3)	3.9(1)	7606(10)	7541(6)	4800(3)	3.30(9)
C4'	1008(10)	3739(7)	2996(3)	3.8(1)	9804(10)	8721(6)	5260(3)	3.15(8)
O4'	277(7)	2770(5)	2190(2)	3.97(6)	10648(6)	7699(4)	6047(2)	3.17(6)
C5'	1536(10)	5866(8)	2845(3)	4.8(1)	8881(10)	10778(7)	5421(3)	3.55(9)
O5'	4095(8)	6235(5)	2558(2)	4.91(8)	6178(7)	10774(5)	5697(2)	4.43(7)
HC2	737(10)	618(8)	75(4)	3(1)*	269(10)	147(10)	734(4)	3(1)*
HC8	180(10)	-195(9)	99(4)	3(1)*	840(10)	942(10)	722(4)	3(1)*
H1N6	702(10)	54(9)	-109(4)	4(1)*	74(10)	530(9)	934(4)	3(1)*
H2N6	971(10)	205(9)	-115(4)	4(1)*	235(10)	748(10)	914(4)	4(1)*
HC1'	72(10)	-19(10)	218(4)	4(1)*	991(10)	507(9)	632(4)	3(1)*
HC2'	606(10)	18(9)	289(4)	3(1)*	516(10)	529(9)	534(4)	3(1)*
HC3'	526(10)	325(9)	379(4)	3(1)*	624(10)	792(10)	426(4)	4(1)*
HC4'	-58(10)	355(9)	335(4)	4(1)*	1148(10)	906(9)	493(4)	3(1)*
H5'1	148(10)	657(10)	338(4)	5(2)*	887(10)	1154(9)	489(4)	4(1)*
H5'2	-3(10)	638(10)	241(4)	4(1)*	1029(10)	1140(9)	587(4)	4(1)*
HO5'	464(10)	575(10)	201(4)	4(1)*	614(10)	1187(9)	612(4)	4(1)*

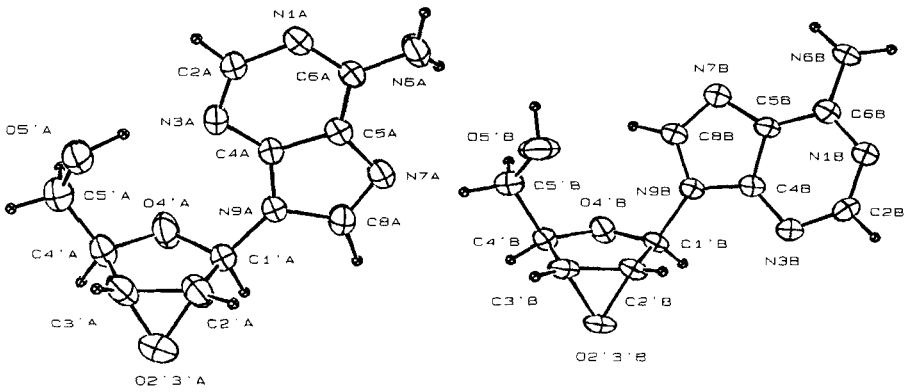

FIGURE 1. The structure of arA A and B molecules. O, N and C atoms are represented by thermal ellipsoids with 50% probability. The drawing was made using the ORTEP program.

on PA=122.8° and the degree of pucker ψ_mA=8.1°, which corresponds to the C1'-*exo*($_{C1'}$,E) conformation of the sugar. The C1' atom is displaced from the plane of C2', C3', C4' and O4' atoms by 0.114 Å.

The furanose ring in the B molecule has the conformation of a symmetrical twist: C4'-*endo*-O4'-*exo* ($^{C4'}_{O4'}$,T). The phase angle of pseudorotation PB=250.8° and the degree of pucker ψ_mB=9.1°. The C4' and O4' atoms are displaced from the plane of C1', C2' and C3' atoms by 0.075 and 0.059 Å, respectively . Such a minor displacement of the atoms, as well as the low values of degree of pucker, indicate that the furanose rings are rather flattened in both raA molecules. If mean-squares planes are drawn through all the atoms of the furanose rings, the maximal deviations of the atoms from them will be 0.046 Å in the A molecule and 0.049 Å in the B molecule. Therefore, within these limits, the furanose rings may be considered as planar. A similar flattening of the furanose rings occurs in 2',3'-dideoxy-2',3'-didehydronucleosides (d_4N) which, just as raA, can act as nonspecific termination substrates [10,11].

The epoxide rings of raA A and B molecules make dihedral angles of 105° and 102° with respect to the furanose rings; the mean values of their bond lengths and bond angles are in range of 1.449 to 1.460Å and 59.6 to 60.4° respectively.

TABLE 2.

Bond distances (Å) and bond angles (°) in A and B molecules of raA

Bond / Angle	Molecule A	Molecule B	Bond / Angle	Molecule A	Molecule B
N1 – C2	1.361(6)	1.338(5)	O2'3' – C3'	1.437(7)	1.461(5)
N1 – C6	1.352(5)	1.349(4)	C3' – C4'	1.503(7)	1.464(6)
C2 – N3	1.298(6)	1.333(6)	C4' – O4'	1.449(5)	1.456(5)
N3 – C4	1.344(5)	1.349(5)	C4' – C5'	1.518(7)	1.510(6)
C4 – C5	1.393(6)	1.395(6)	C5' – O5'	1.399(7)	1.430(6)
C4 – N9	1.377(5)	1.365(5)	C2 – HC2	0.96(6)	0.93(6)
C5 – C6	1.381(6)	1.389(6)	C8 – HC8	1.07(6)	1.00(7)
C5 – N7	1.384(6)	1.387(5)	N6 – H1N6	0.85(6)	0.98(7)
C6 – N6	1.336(6)	1.345(5)	N6 – H2N6	1.00(7)	0.95(7)
N7 – C8	1.307(7)	1.308(6)	C1' – HC1'	1.06(7)	0.93(6)
C8 – N9	1.383(6)	1.371(5)	C2' – HC2'	1.12(6)	0.98(6)
N9 – C1'	1.447(6)	1.478(5)	C3' – HC3'	1.07(6)	1.05(6)
C1' – C2'	1.482(6)	1.503(5)	C4' – HC4'	1.01(7)	1.05(7)
C1' – O4'	1.428(6)	1.392(5)	C5' – H5'1	0.99(7)	1.00(7)
C2' – O2'3'	1.475(7)	1.445(6)	C5' – H5'2	1.02(6)	1.02(6)
C2' – C3'	1.456(7)	1.453(6)	O5' – HO5'	0.99(7)	1.01(6)
C2 –N1 –C6	116.6(4)	118.3(3)	C8 –N9 –C1'	122.4(4)	127.5(3)
N1 –C2 –N3	130.2(4)	129.6(4)	N9 –C1' –C2'	116.2(4)	111.4(3)
C2 –N3 –C4	111.6(4)	110.5(4)	N9 –C1' –O4'	109.7(3)	110.0(3)
N3 –C4 –C5	124.9(4)	125.9(4)	C2' –C1' –O4'	106.7(4)	107.0(4)
N3 –C4 –N9	129.4(4)	128.4(4)	C1' –C2' –O2'3'	109.0(4)	109.1(3)
C5 –C4 –N9	105.7(3)	105.7(3)	C1' –C2' –C3'	108.3(4)	106.4(4)
C4 –C5 –C6	118.0(4)	117.5(4)	O2'3' –C2' –C3'	58.7(3)	60.5(3)
C4 –C5 –N7	110.4(4)	109.7(4)	C2' –O2'3' –C3'	60.0(3)	60.0(2)
C6 –C5 –N7	131.6(4)	132.8(4)	C2' –C3' –O2'3'	61.3(3)	59.4(3)
N1 –C6 –C5	118.4(4)	118.1(3)	C2' –C3' –C4'	107.4(4)	108.9(3)
N1 –C6 –N6	117.7(4)	119.5(4)	O2'3' –C3' –C4'	111.9(4)	113.0(4)
C5 –C6 –N6	123.7(4)	122.4(4)	C3' –C4' –O4'	105.9(4)	105.4(4)
C5 –N7 –C8	104.7(4)	104.9(3)	C3' –C4' –C5'	114.8(4)	113.4(4)
N7 –C8 –N9	113.3(4)	112.9(4)	O4' –C4' –C5'	109.2(4)	111.5(4)
C4 –N9 –C8	105.8(4)	106.8(4)	C1' –O4' –C4'	111.1(3)	111.5(2)
C4 –N9 –C1'	130.6(3)	125.7(3)	C4' –C5' –O5'	114.5(5)	109.7(4)

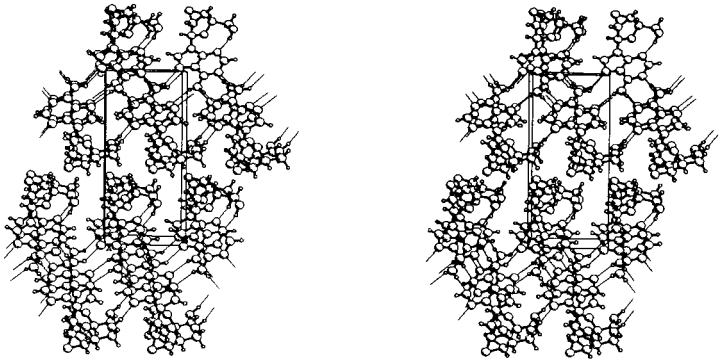

FIGURE 2. A stereoview of arA molecular packing along the a axis. Hydrogen bonding is shown with thin lines. Picture was done with the PLUTO program.

TABLE 3.

Hydrogen-bond distances and angles

Donor atom	Acceptor atom	Position of acceptor atom	Distance		Angle(°)
			D...A(Å)	H...A(Å)	D - H...A
O5'A	N3A	x,y,z	2.775(5)	1.82(7)	161(6)
N6A	N1B	x+1,y,z-1	3.017(4)	2.03(7)	168(5)
N6A	N7B	x,y-1,z-1	3.000(5)	2.23(6)	149(6)
N6B	N1A	x-1,y,z+1	2.979(5)	2.02(7)	167(5)
N6B	N7A	x,y+1,z+1	3.047(5)	2.24(6)	141(5)
O5'B	N3B	x,y+1,z	2.868(5)	1.90(7)	162(6)

The conformation of both molecules with respect to the exocyclic bond C4'-C5' is *gauche*$^+$, the torsion angle γ(O5'-C5'-C4'-C3') is 42.9^o in A and 43.9^o in B.

The mutual orientation of the furanose rings and bases is however different in A and B molecules. The torsion angle χ(O4'-C1'-N9-C4) is 56.8^o in the A molecule and corresponds to the *syn* conformation with respect to the glycosidic bond. The *syn* conformation is additionally stabilized by the intramolecular hydrogen bond O5'A-H...N3A whose length is 2.775 Å. The torsion angle χB is -170.8^o in the B molecule, which corresponds to *anti* conformation. The conformational flexibility of raA molecules with respect to the N-glycosidic bond is made easier by the flattened furanose rings, which decreases steric hindrances between the base and furanose atoms.

Figure 2 illustrates the packing of raA molecules in a unit cell. The principle of hydrogen bond saturation is realized in the structure, i.e. hydrogen bonding involves all H atoms potentially capable to form them. The geometrical parameters of hydrogen bonds are listed in Table 3. O5'A-HO5'A...N3A is the only intramolecular hydrogen bond, while the other bonds are intermolecular ones. The hydrogen bonded molecules form infinite bilayers parallel to the plane (ab). Nucleic bases are buried inside the layers and involved in stacking interaction, sugar moieties comprise their surface. The layers are held together by van der Waals interactions.

This X-ray analysis indicates that raA molecules have flattened furanose rings and are conformationally flexible with respect to the N-glycosidic bond. Similar structural properties have been reported for d_4N molecules which, like raA, are conformationally restricted compounds acting as strong, nonspecific termination substrates for a number of DNA polymerases [10,11]. Since both the conformation and the biological activity of these compounds are similar, we can draw a conclusion that they mimic the conformation of 2'-deoxyribose rings of natural substrates in DNA synthesizing complexes. This conclusion is in good agreement with the results of Ferrin and Mildvan [3], who used the method of NMR in solution to detect flattened furanose rings of dNTP substrates in [DNA polymerase + template + dNTP] complexes.

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