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X-Ray analysis of 2′,3′-lyxoanhydrothymidine, a conformationally restricted inhibitor of retroviral reverse transcriptases

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2′,3′-Lyxoanhydrothymidine (LAT), a conformationally restricted inhibitor of retroviral reverse transcriptases, has been studied by X-ray analysis. The unit cell contains two crystallographically independent molecules A and B. Their sugar moieties have an identical structure: an 04′-*endo* pucker of the furanose cycle and a *trans* conformation about the exocyclic C4′–C5′ bond. The conformations of A and B molecules differ with respect to the *N*-glycosidic bond: χA(04 'Cl' N1C2) = −121.9° which is typical of a common *anti* conformation whereas χB (04'Cl'N1C2) = 121.2° corresponds to a rare high-*syn* conformation. All the conformation properties of LAT molecules stem from the presence of an epoxide cycle in their molecules.

X-ray analysis; 2′,3′-Lyxoanhydrothymidine; Reverse transcriptase; DNA biosynthesis

1. INTRODUCTION

It would be expedient to comparatively analyse the active centers of different DNA polymerases in order to comprehend the structure and functioning of these enzymes involved in the genetic apparatus. Moreover, such an analysis would help to constitute selective inhibitors of different DNA polymerases, HIV reverse transcriptase (HIV RT) inclusive.

There are several hypotheses which account for the different specificity of DNA polymerases from various sources. One hypothesis holds that retroviral RT has the lowest specificity for the substrate analogs of DNA chain elongation as compared with other DNA polymerases. That is why nucleoside analogs which are not recognised by other DNA polymerases, for instance, nucleotide analogs with bulky substituent in the 3′-position, can be incorporated by RT into the 3′-terminus of a growing DNA chain [1,2].

Other authors believe that the stereo-electronic properties of the azido group in 3′-azido-2′,3′-dideoxynucleoside 5′-triphosphates make these compounds specific for the interaction with RT [3,4]. The third hypothesis proposes that the conformation of nucleoside 5′-triphosphates is principally responsible for the specificity of their action, namely the conformation of a pentafuranose cycle and the orientation associated with it of a substituent at C5′ and of a nucleic acid base. As was shown by the analysis of structures in crystals for a number of nucleosides, compounds active against HIV and other retroviruses have a 3′-*exo* conformation whereas the conformation of inactive compounds is 3′-*endo* [5].

We found in 1988–1989 that 2′,3′-lyxoanhydronucleoside 5′-triphosphates could act as termination substrates of retroviral RTs and mammalian terminal deoxynucleotidyltransferase, but had no effect on DNA synthesis catalyzed by other mammalian DNA polymerases [6,7]. The termination properties of other DNA elongation substrate analogs with the restricted conformational flexibility of a sugar residue were also studied: 2′,3′-riboanhydronucleoside 5′-triphosphates [6–8], 2′,3′-*O*-isopropylidenecytidine 5′-triphosphate [6,7], 2′,3′-dideoxy-2′,3′-didehydronucleoside 5′-triphosphates [9]. All the data reported in the above communications show that compounds with a restricted conformational flexibility of a pentafuranose cycle may be considered as selective or non-selective termination substrates. Owing to their conformational rigidity, such compounds can offer great promise in studying the active centre of DNA polymerases.

This communication presents the results of X-ray study of 2′,3′-lyxoanhydrothymidine (LAT).

2. MATERIALS AND METHODS

LAT was synthesised as in [10]. Crystals used for X-ray analysis were obtained from a saturated LAT solution in ethanol by slowly evaporating the solvent at an ambient temperature. Their space group

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is P1, and the cell dimensions are: a = 5.321(3), b = 9.974(1), c = 10.407(1) Å, α = 73.55(1), β = 77.41(3), γ = 88.21(2)°, V = 516.7 Å^3, Z = 2, i.e. two crystallographically independent LAT molecules (A and B) are present in the cell.

The dimensions of the unit cell and the intensities of a three-dimensional set of reflections were measured on a CAD-4F diffractometer (ω/θ scan technique, $1 < \theta < 60°$, CuK_α radiation, a graphite monochromator). The experimental data were corrected for the Lorentz and polarization factors. The intensities of 1862 independent reflections with $I > 3\sigma(I)$ were used in the structural study. The structure was determined by direct methods and refined by the full-matrix least-squares method in the anisotropic approximation for C, N and O atoms. The position of most H atoms were located on the difference Fourier maps, the coordinates of some atoms were calculated geometrically. Refinement converged to R = 3.8%. All the calculations were made with the SDP programmes [11].

3. RESULTS AND DISCUSSION

Fig. 1 presents two crystallographically independent LAT molecules with the atomic numbering accepted in this work, and shows the orientation of thermal ellipsoids of non-hydrogen atoms. The bond lengths and the bond angles are given in Table I.

One can see from Table I that all the geometrical parameters for both molecules coincide quite well within the range of σ–3σ, with the exception of the exocyclic bond angles at glycosidic bond. The same situation was observed in the 3′-azido-2′,3′-dideoxythymidine (AZT) crystallographically independent molecules whose conformation was different with respect to the glycosidic bond [4].

It would be also relevant to note that, with the exception of the C2′–C3′ bond, all the bond lengths coincide within the range of σ–3σ in LAT and natural thymidine molecules [12]. The C2′–C3′ bond in the epoxide cycle is longer by 10σ than a similar bond in thymidine (1.523 Å). Moreover, the epoxide cycle in LAT molecules makes the endocyclic angles C1′C2′C3′ and C2′C3′C4′ of furanoside cycles greater by 2.4° and 6.3°, on the average, as compared to those in thymidine.

The endocyclic bond angles of epoxide cycles range from 58.4° to 61.5° and the C3′-O2′,3′ bonds of both molecules are by 0.023 Å longer than the C2′-O2′,3′ bonds.

The incorporation of the furanose ring C2′ and C3′ atoms into the epoxide cycle restricts the conformational flexibility of the sugar moiety. As a result, the furanose ring has the same conformation in the crystallographically independent LAT molecules. The phase angles of pseudorotation P are 98.9 and 97.6° for A and B molecules, respectively, which corresponds to the O4′-*endo*-C1′-*exo*-pucker of the sugar. Here, the deviations of O4′ atoms from the planes of C2′, C3′ and C4′ atoms in both molecules (0.308 Å for A and 0.368 Å for B) are nearly 3 times greater than those of C1′ atoms (0.115 Å for A and 0.111 Å for B). If mean-square planes are drawn through all the carbon atoms of the furanose rings, the maximal deviation of the carbon atoms will be 0.026 Å and the O4′ atoms will be situated on the same side of the planes as atoms N1 and C5′ at a distance of 0.397 Å and 0.452 Å in molecules A and B, respectively. Within the limits of this accuracy (0.026 Å), the conformations of carbohydrate cycles are described as O4′-*endo*, which coincides with the conformation of natural substrates in complexes with *E. coli* DNA polymerase I according to NMR data [13].

The furanose ring in LAT molecules are somewhat flattened as compared with 2′-deoxynucleosides. Indeed, the maximal amplitudes of pseudorotation for LAT (Ψ_mA = 29.2° and Ψ_mB = 33.1°) are lower than for thymidine (Ψ_m = 38.2° [14]).

The conformations of LAT A and B molecules are also identical with respect to the exocyclic C4′–C5′ bond. The torsion angles O5′C5′C4′C3′ are −167.7° for A and −177.5° for B molecule, which corresponds to the *gauche-trans* orientation of C5′–O5′ bond relative to furanose.

One may conclude therefore that the carbohydrate moieties of two crystallographically independent LAT

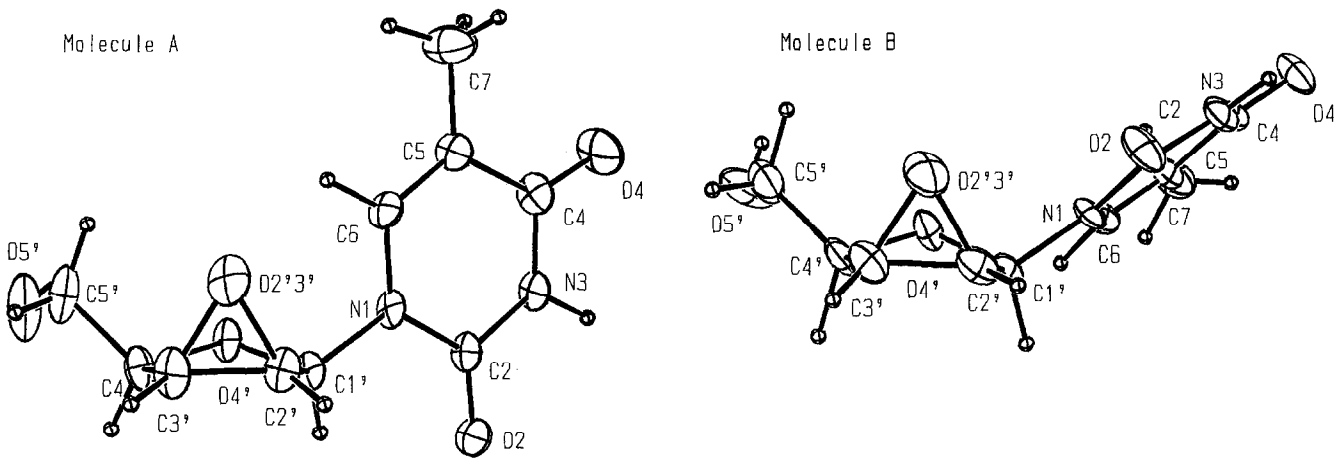

Fig. 1. The structure of A and B molecules of 2′,3′-lyxoanhydrothymidine; the thermal ellipsoids correspond to 50% probability.

Table I

Bond distances (Å) and bond angles (°) with ESDs in parentheses

Bond	Molecule		Bond	Molecule	
angle	A	B	angle	A	B
N1–C2	1.375(5)	1.391(6)	C1′–C2′	1.516(7)	1.509(8)
N1–C6	1.389(7)	1.379(7)	C1′–O4′	1.422(5)	1.437(5)
N1–C1	1.461(6)	1.462(6)	C2′–O2′,3′	1.423(6)	1.418(7)
C2–O2	1.218(7)	1.214(7)	C2′–C3′	1.458(6)	1.463(7)
C2–N3	1.374(7)	1.368(7)	C3′–O2′,3′	1.447(7)	1.441(6)
N3–C4	1.395(8)	1.384(8)	C3′–C4′	1.502(7)	1.501(8)
C4–O4	1.223(7)	1.221(6)	C4′–C5′	1.500(7)	1.500(7)
C4–C5	1.447(6)	1.462(6)	C4′–O4′	1.455(6)	1.445(6)
C5–C7	1.509(9)	1.495(9)	C5′–O5′	1.444(8)	1.413(8)
C5–C6	1.340(7)	1.346(7)			
N1 C1′ C2′	116.4(4)	119.7(4)	C2 N1 C6	121.5(4)	121.1(4)
N1 C1′ O4′	108.1(4)	105.1(4)	C2 N1 C1′	117.8(4)	123.6(5)
C2′ C1′ O4′	105.3(3)	104.7(4)	C6 N1 C1′	120.5(3)	115.3(4)
O2′,3′ C2′ C1′	113.7(4)	114.5(5)	N1 C2 O2	123.1(4)	124.0(4)
C1′ C2′ C3′	105.3(4)	105.0(4)	N1 C2 N3	114.8(5)	113.7(5)
O2′,3′ C2′ C3′	60.3(3)	60.0(3)	O2 C2 N3	122.0(4)	122.3(4)
C2′ O2′,3′ C3′	61.1(3)	61.5(3)	C2 N3 C4	127.0(4)	129.0(4)
O2′,3′ C3′ C2′	58.6(3)	58.4(3)	N3 C4 O4	119.2(4)	120.8(4)
C2′ C3′ C4′	108.6(4)	108.2(5)	N3 C4 C5	115.0(5)	114.2(4)
O2′,3′ C3′ C4′	111.5(5)	112.3(5)	O4 C4 C5	125.9(6)	125.0(5)
C3′ C4′ C5′	114.0(5)	115.3(5)	C4 C5 C7	117.7(5)	119.6(4)
C3′ C4′ O4′	103.9(4)	103.8(3)	C4 C5 C6	118.6(5)	117.7(5)
C5′ C4′ O4′	109.2(4)	108.5(4)	C7 C5 C6	123.6(4)	122.7(4)
C1′ O4′ C4′	108.8(3)	107.7(4)	N1 C6 C5	123.1(4)	124.3(4)
C4′ C5′ O5′	112.7(5)	110.5(5)			

molecules coincide both in their geometrical dimensions and conformations. However, A and B molecules differ in their conformation about the *N*-glycosidic bond. Just as in the structure of AZT, although the crystallographically independent LAT molecules fall within the region of *anti* conformations, the values of their torsion angles lie at the opposite ends of this region. The torsion angle χ(O4′C1′N1C2) in the A molecule is −121.9° which is typical of a usual *anti* conformation while, in the B molecule, it equals 121.2° and corresponds to a rare high-*syn* conformation. It is noteworthy that the high-*syn* conformation is found mainly in the molecules of 2,2′-anhydronucleosides where it is rigidly fixed [15].

As can be seen from [3–5] the conformational flexibility both of the furanose ring and about the glycosidic bond, typical of natural nucleosides, is preserved in the molecules of AZT, a selective inhibitor of retroviruses. That is why, in the DNA synthesizing complex, AZT molecules after their intracellular triphosphorylation can easily acquire a conformation required for the formation of a productive complex. The situation is different for the inhibitor of retroviral RTs with a restricted conformational flexibility. The sugar moiety in the LAT molecules has a rather rigid structure with the furanose ring O4′-*endo* puckered. At the same time, the LAT molecules preserve a conformational flexibility about the *N*-glycosidic bond which, however, some restricted by the presence of an epoxide cycle. As a result, two conformations are realised: a usual *anti* conformation (molecule A) and high-*syn* conformation (molecule B).

The structural data obtained in this work imply that RTs and terminal deoxynucleotidyltransferase can yield a productive complex with substrate analogs in which the carbon atoms of the furanose ring have a flattened conformation and the O4′ atom occupies an O4′-*endo* position. An *anti* conformation typical of the A molecule is realized most probably about the *N*-glycosidic bond in the substrate since the LAT 5′-phosphate residue is incorporated into the 3′-terminus of DNA in a system with DNA polymerase instead of thymidine 5′-phosphate. This residue forms a regular Watson-Crick pair with the template [6,7], which is possible only provided the base has *anti* conformation. All this is a reason for subjecting the active centres of DNA polymerases to structural analysis.

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