CONFORMATION OF THE tRNA MINOR CONSTITUENT DIHYDROURIDINE

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1. Introduction

The rare nucleoside dihydrouridine could be traced in all the transfer RNAs sequenced so far except *E. Coli* $tRNA^{Tyr}$ I $su^{-}(su^{+})$ [III] [1,2]. According to the cloverleaf model [3] it occurs in non-helical regions of the tRNA secondary structure and is regularly located in the so-called "dihydrouridine loop". As part of our studies on the structural properties of natural and modified nucleic acid constituents we have performed X-ray analysis of dihydrouridine below described (fig. 1).

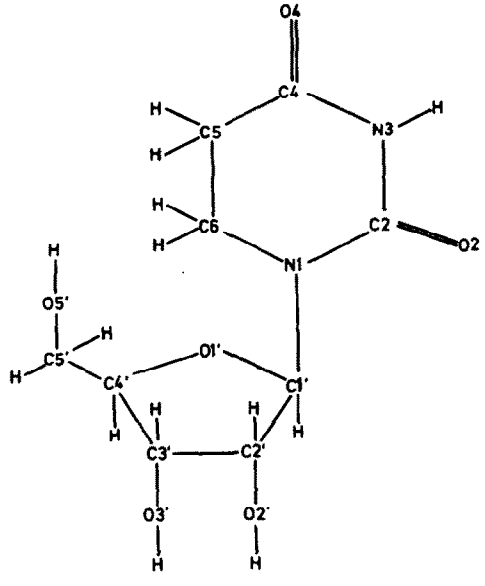

Fig. 1. Chemical formula of dihydrouridine.

2. Materials and methods

Dihydrouridine (purchased from P-L Biochemicals Inc., Milwaukee, Wisc. USA) crystallizes from aqueous *n*-butanol in the orthorhombic space group $P2_12_12_1$ with cell dimensions $a = 11.779$ Å, $b = 8.150$ Å, $c = 23.068$ Å. One asymmetric unit contains two molecules of the nucleoside and one molecule of water, corresponding to observed and calculated densities of 1.51 g/cm^3 and 1.53 g/cm^3, resp. We collected about 2000 intensity data on an automatic Siemens diffractometer with Ni-filtered Cu radiation and converted them to normalized structure factors (*E*'s). The structure was determined by direct methods involving a cyclic application of the tangent formula [4] combined with multiple solution techniques [5]. A three-dimensional *E*-map computed with the phases derived from the most consistent solution clearly revealed the positions of all the 35 non-hydrogen atoms within the asymmetric unit. After three cycles of isotropic full matrix least squares refinement the reliability factor is 11.2%. At this stage the following structural features are clear.

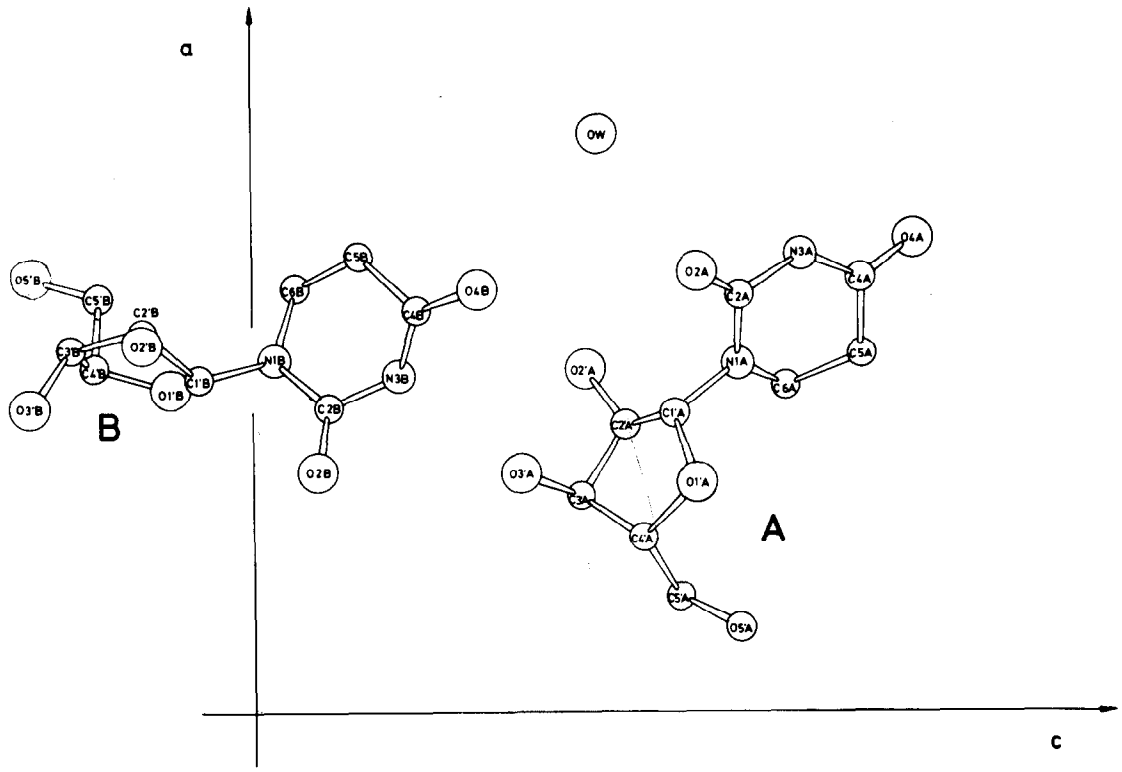

Fig. 2. Projection of an asymmetric unit onto the *a*, *c* plane. The water molecule is indicated by OW.

3. Results and discussion

3.1. *Conformation of dihydrouridine*

The heterocycle of a nucleoside can take two preferred conformations with respect to the sugar moiety which are called *syn* and *anti* [6], if the oxygen atom O2 is pointing towards or away from the ribose. The dihydrouridine molecules both are in *anti* conformation with torsional angles C2′–C1′–N1–C2 = 112° for molecule A and = 135° for molecule B. We have not used the torsional angle C2′–C1′–N1–C6 [7] which normally serves to define the conformation about the glycosidic bond C1′–N1 because of heterocycle pucker (see below). (In the latter definition the angles C2′–C1′–N1–C6 would be approximately −112° and −45° for molecules A and B, resp. which corresponds to the *anti* range.)

3.2. *Geometry of the heterocycles*

Bond angles and distances within the dihydrouracil residues are similar for both molecules and in agreement with data obtained for dihydrouracil [8]. Both C2–O2 and C4–O4 distances are indicative for a keto, keto structure as commonly found in uridine and thymidine nucleosides.

The heterocycles are puckered, i.e. atoms C5 and C6 are deviating from the plane through atoms N1, C2, N3, C4. These deviations are +0.16 Å and −0.53 Å for atoms C5 and C6 in molecule A but −0.04 and +0.63 Å in molecule B. Atoms C6 and C2′ are on the same side of the plane through N1, C2, N3, C4 in molecule A but on opposite sides on molecule B. Another description for the pucker of the heterocycle is the torsional angle C4–C5–C6–N1 which is −52° for molecule A and +52° for molecule B. Thus the pucker of the heterocycles is different in molecules A and B and one has to assume that both puckering modes are present in solution as well.

3.3. *Geometry of the ribose moieties*

The geometrical data for the ribose moieties are in overall agreement with structural data obtained for other ribonucleosides [10]. The pucker of the ribose is best described as C2′-endo in both cases, i.e. atoms C1′, C3′, C4′, O1′ are nearly coplanar and atom C2′ is out of this plane by about 0.5 Å and on the same side as C5′. The similar pucker for both riboses is also evident from the torsional angles O2′–C2′–C3′–O3′ which are close to 40° for molecules A and B.

Two other comformational parameters necessary to describe ribose structures are the dihedral angles O5′–C5′–C4′–O1′ (ρ_{OO}) and O5′–C5′–C4′–C3′ (ρ_{OC}) [9] which indicate the position of atom O5′ with respect to the ribose ring. These angles are $\rho_{OO} = 54°$ (*gauche*) and $\rho_{OO} = 169°$ (*trans*) for molecule A and $\rho_{OO} = 178°$ (*trans*) and $\rho_{OC} = 65°$ (*gauche*) for molecule B. These conformations both are rather unexpected since in ribonucleotides the gauche, gauche conformation with the atom O5′ located above the ribose is most common.

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