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Nucleic Acid Conformation: Crystal Structure of a Naturally Occurring Dinucleoside Phosphate (UpA)

THEORIES of the molecular structure of nucleic acids have so far been based on evidence from the crystal structures of monomeric units such as nucleosides and mononucleotides, the interpretation of diffraction patterns of oriented nucleic acid fibres and molecular model building[1-6]. Such approaches can help to suggest structures of periodic molecules such as helices, but they are insufficient for predicting and understanding nonrepetitive structures such as the loops in transfer RNA (tRNA), presumably associated with many of the functions of tRNA. To understand the geometry of nucleic acids and possible constraints on their conformation, it is therefore essential to know the detailed conformation of the sugar residues and the conformational relationship between the sugar residue, the base and the phosphate group[7-9]. The simplest molecule which contains this information is a 3′5′-dinucleoside phosphate. We now report the structure of uridine-3′,5′-adenosine phosphate (UpA). This is the first naturally occurring dinucleoside phosphate whose crystal structure has been determined by X-ray diffraction. The only other dinucleoside phosphate with known crystal structure is adenosine-2′,5′-uridine phosphate[10], but it does not have the naturally occurring 3′5′ sugar phosphate linkage.

Powdered UpA was purchased from Gallard Schlesinger, New York. The crystals grew on slow evaporation as long beautiful needles from acidic (10^{-3} M HCl) aqueous solution. There are two crystallographically independent UpA molecules and one water molecule in the asymmetric portion of the unit cell. The crystal data are given in Table 1. There are four UpA and two water molecules in a unit cell. The asymmetric unit contains seventy-nine non-hydrogen atoms with a molecular weight of 1084.8 daltons. 2,640 unique reflexions were measured with the $\theta/2\theta$ scan technique on a Hilger–Watts automatic four circle diffractometer with CuKα radiation.

The positions of the phosphorous atoms were obtained by a comparison of a Patterson synthesis of all data with one calculated using data of resolution greater than 1.5 Å. The high resolution Patterson synthesis distinguishes the phosphorus–phosphorus vectors from the other large peaks in the Patterson synthesis which result from overlap of lighter atom vectors. Patterson superpositions based on the phosphorus positions revealed the orientations of the two independent phosphate groups. Successive cycles of Fourier refinement revealed the locations of all seventy-nine atoms. Several cycles of least squares refinement with isotropic thermal parameters reduced the disagreement index*, R, from 0.29 to its current value of 0.12. In retrospect, direct method approaches and rotation search techniques were partially successful.

Table 1 Crystal Data

$a = 16.91$ Å
$b = 12.37$ Å
$c = 11.25$ Å
$\beta = 95.95°$

ρ (calculated) $= 1.65$ g/cm^3, ρ (measured) $= 1.63$ g/cm^3
Space group (monoclinic) $P2_1$

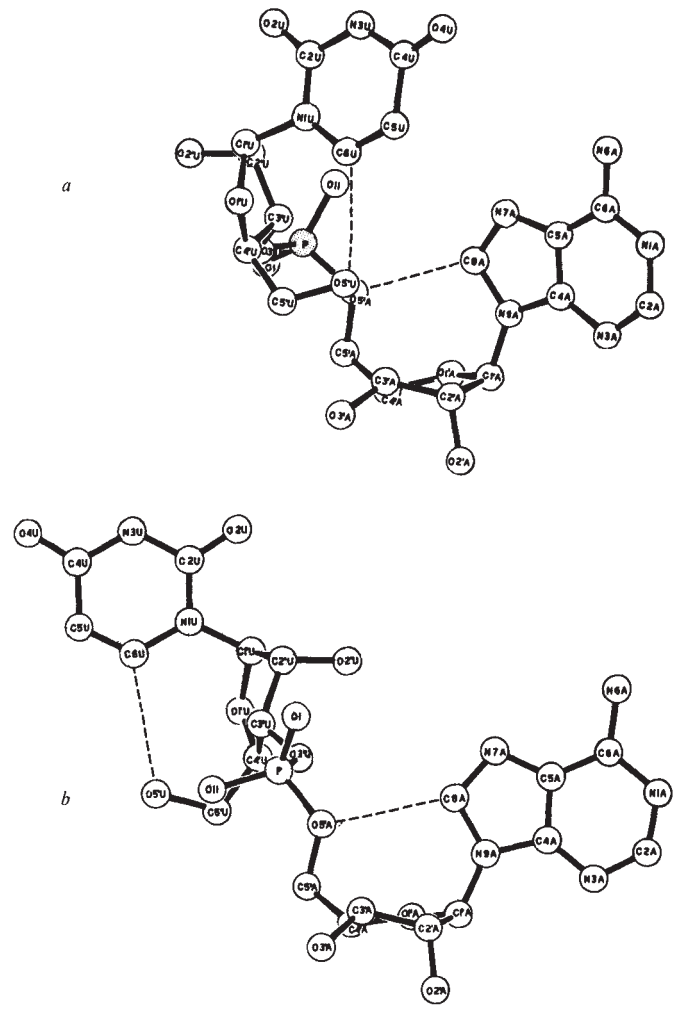

Fig. 1 UpA 1 (*a*) and UpA 2 (*b*) with similar views of the ribose of the adenosine portions. The atoms of the phosphate groups are shaded. The dotted lines represent intramolecular hydrogen bonds. The atom labels are given by three symbols. The first is the atom type, the second is the atom number and the third designates whether the atom is in the adenosine (A) or uridine (U) portion of the molecule. The ′ is on the atoms belonging to the sugar residues.

Only structural features of general interest will be described here; the structural details, conformational analysis, atomic coordinates, thermal parameters and structure factors will be published later.

The two crystallographically independent molecules exhibit markedly different conformations as shown in Fig. 1. The conformation of UpA 1 (Fig. 1*a*) appears deceptively similar to models of helical nucleic acids. The two bases are approximately parallel and in the anti-conformation[11] with respect

* The disagreement index is defined as $\frac{\Sigma||F_{obs}|-|F_{calc}||}{\Sigma|F_{obs}|}$ where F_{obs} and F_{calc} are observed and calculated structure factors.

to the ribose sugars. Both sugar residues are in the 3′ endo conformation. However, the conformation about the phosphate diester linkage O(3′U)-P-O(5′A) is very different from any proposed repetitive helical nucleic acid structure as shown in Fig. 2*a* and *b* which make a comparison of UpA 1 and an RNA helical structure. In a helical nucleic acid structure all the sugar residues must be aligned similarly with respect to the helix axis as in Fig. 2*b*. On UpA 1 the sugar residues are oppositely aligned. This conformation allows a sharp bend to occur in a single strand of RNA. It is therefore likely that the conformation of UpA 1 or its minor variations will be found in the loop structure of RNA such as the anticodon loop of tRNA.

The extended appearance of UpA 2 (Fig. 1*b*) does not resemble any of the proposed helical nucleic acid structures. But, it can be approximately converted into a right handed RNA helical structure with eleven base pairs per turn by rotation around the P→O(3′U) bond of about 110° as can be seen by comparing Fig. 2*b* and *c*. As in UpA 1 the bases are in nearly parallel planes and are in the anti-conformation with respect to the sugars. The sugar residues are in the 3′ endo conformation. The bond lengths and angles of the two molecules are similar and show no unusual characteristics.

UpA 1 can be approximately converted to UpA 2 by the following operations. (1) Rotate about P→O(5′A) directed bond by 175°. This rotation allows the fingers in Fig. 2*a* to point in the same direction. (2) Rotate about P→O(3′U) directed bond by 81°. This rotation makes the orientation of the hands in Fig. 2*a* similar to those in *c*.

There is no base pairing between the adenines and the uracils of the type proposed by Watson and Crick[1] or found by Hoogsteen[12]; all the adenines lie in one plane, the uracils in another, and the planes are parallel with a separation of 3.4 Å. The adenine of UpA 1 is hydrogen bonded to the adenine of UpA 2 in the same manner as in polyadenylic acid[6] (Fig. 3*a*). The uracils form hydrogen bonded dimers; N(3U) of UpA 2 donates a hydrogen to O(2U) or UpA 1 and N(3U) of UpA 1 donates a hydrogen to O(4U) of UpA 2 (Fig. 3*b*). This type of uracil–uracil base pairing, in which there is no local two-fold axis perpendicular to the plane of the hydrogen bonds, has not been previously observed.

From considerations of hydrogen bonding and the *p*H of crystallization, it is probable that there is a proton bonded to N(1A) of both adenines as in polyadenylic acid[6]. The phosphate group is in a monoanionic form thus making both molecules zwitter ions.

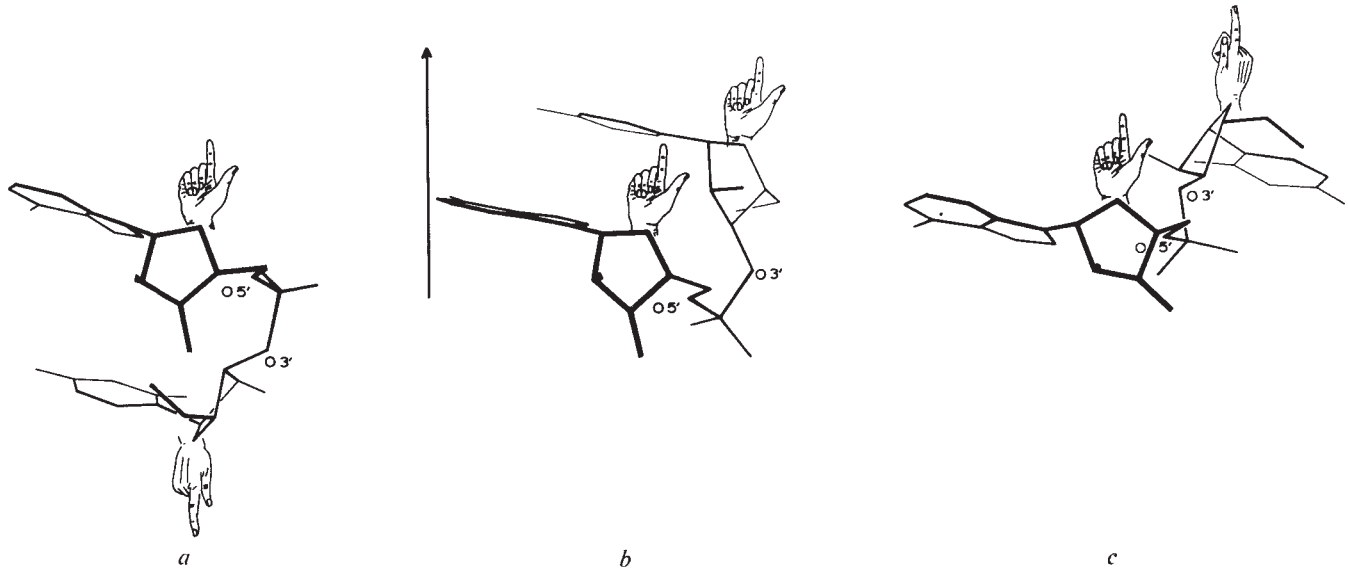

Fig. 2 Views of (*a*) UpA 1; (*b*) RNA with eleven base pairs per turn (RNA 11); and (*c*) UpA 2 oriented so that the bases of the adenosine portions are similarly aligned. In order to clarify the conformation differences among the molecules we have attached a hand to the ribose ring oxygen. Note that both fingers in *b* are pointing in the direction of the helix axis which is shown by an arrow. In UpA 1 (*a*) they point in opposite directions and in UpA 2 (*c*) both fingers are pointing in the same direction. Note carefully the orientation of the hands. A right handed rotation of 110° of the uridine portion of *c* will align the molecule so that it looks like RNA 11.

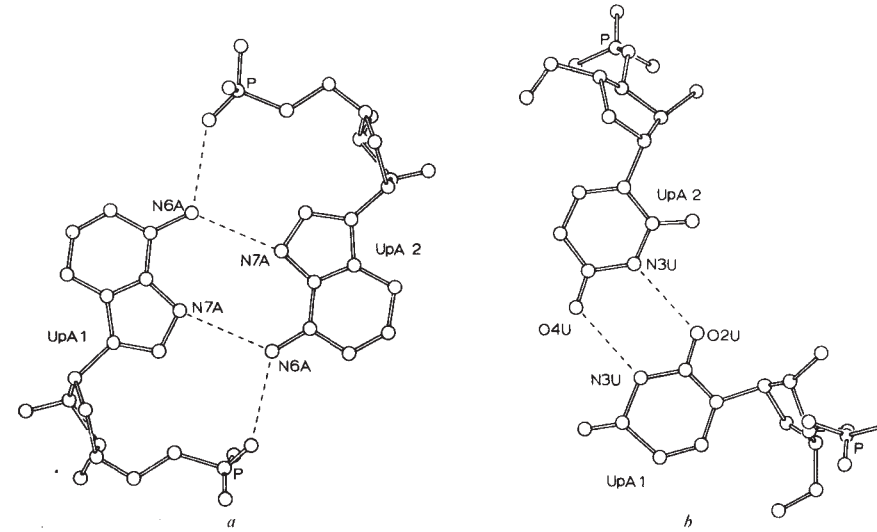

Fig. 3 Base pairing of the (*a*) adenines and (*b*) uracils in the crystal structure of UpA. The dotted lines indicate the possible intermolecular hydrogen bonds.

One of the unexpected features in the structure is that there are possible hydrogen bonds with distances of about 3.1 Å between O(5′) of the adenosine riboses and C(8) of the adenines, and between the O(5′) of the uridine riboses and C(6) of the uracils (Fig. 1). All four nucleosides have this feature, which was noted by Sutor[13] and observed in many crystal structures of nucleosides and mononucleotides and also in the uridine portion of adenosine-2′,5′-uridine phosphate[10]. From deuterium experiments[14] and proton magnetic resonance measurements[15] the hydrogen on C(8) or purines and C(6) of pyrimidines are known to be partly acidic and to interact with the phosphoester oxygen in solution.

Although the two UpA molecules look wildly different, the most striking feature of the structure is the fact that all four independent nucleosides have very similar conformations: (1) all four bases are in the anti-conformation; (2) all four sugars are in 3′ endo conformations and (3) all four nucleosides have possible intramolecular hydrogen bonds between O(5′) and the bases. If these constraints are general, the conformational degrees of freedom of nucleotides in nucleic acids are much more limited than has previously been assumed, and the phosphodeister bond contains the two major conformational parameters in nucleic acid structures.

We thank Alexander Rich, Jenny Glusker and Cyrus Levinthal for encouragement and discussions, Lou Katz and Reidar Bornholdt for programming assistance, and Joyce Dargay and Ann Chomyn for technical assistance. We thank Hilger and Watts Ltd, London, and General Electric Co. for the use of automatic diffractometers, and the Graphics Facility for Interactive Displays (Graffidi Labs) and the Computer Center at Columbia University for the use of their computers.

This research was supported in part by grants from the US Public Health Service, the National Science Foundation, and the American Cancer Society, and by an appropriation from the Commonwealth of Pennsylvania.

NADRIAN C. SEEMAN
JOEL L. SUSSMAN

Department of Biological Sciences,
Columbia University,
New York NY 10027

HELEN M. BERMAN

The Institute for Cancer Research,
Philadelphia,
Pennsylvania 19111

S. H. KIM

Department of Biology,
Massachusetts Institute of Technology,
Cambridge,
Massachusetts 02139

Received May 24, 1971.

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Action of Heparin on Platelet Electrophoretic Mobility

THE aggregating agents adenosine diphosphate (ADP) and noradrenaline induce a biphasic change in human platelet electrophoretic mobility[1]. Small concentrations (0.005 mg ml.$^{-1}$ and 0.05 mg ml.$^{-1}$) increase platelet mobility after incubation for 10 min; high concentrations (0.05 mg/ml.) induce a decrease in mobility. The same change also occurs in pig[2] and rabbit[3] platelet mobilities. Hampton and Mitchell[4] showed that the addition of 1–50 U of heparin to plasma has no effect on the biphasic mobility of human platelets. Nevertheless, measurements of the electrophoretic mobility of pig platelets after intravenous infusion with heparin have led to *in vitro* studies of both pig and human platelets, which I shall now describe.

The apparatus used was a horizontal, cylindrical microelectrophoresis chamber (Rank Bros., Bottisham, England) as described by Bangham *et al.*[5] but with a capacity of 1 ml. and with solid silver chloride electrodes. All measurements were made at 25° C and expressed in μm s^{-1} V^{-1} cm^{-1}. The apparatus was calibrated with washed human erythrocytes[6]. The current and voltage were checked before and after each run to ensure there was no alteration in the "electrical length" of the cell, and an average result was obtained from ten readings taken in each direction. All blood samples were centrifuged at 1,000 r.p.m. for 20 min and the platelet rich plasma (PRP) was diluted 1 : 10 with saline[2]. Samples for the *in vitro* study were withdrawn by venipuncture from normal, control pigs, mixed with the necessary volume of 3.8% sodium citrate (as an anticoagulant) and treated in a similar manner. Heparin (Boots) was added to the PRP before dilution with saline, giving final concentrations of 0.008, 0.016, 1, 5 and 10 U ml.$^{-1}$ Suspensions of human platelets were prepared in the same way from samples withdrawn from the median cubital vein of five healthy, non-smoking (R. I. H., manuscript in preparation) male volunteers, after they had eaten only a light, uncooked breakfast.

Microelectrophoresis of pig platelets obtained by indwelling carotid cannula, 5 min after an intravenous infusion of 500 U of heparin, resulted in a lack of biphasic response after incubation with both ADP and noradrenaline. Response was still lacking when platelets were withdrawn 15 h after the administration of heparin, but mobility had returned to normal after 24 h. The addition of small concentrations (0.008–1.0 U ml.$^{-1}$ of heparin to pig PRP *in vitro* also abolished the

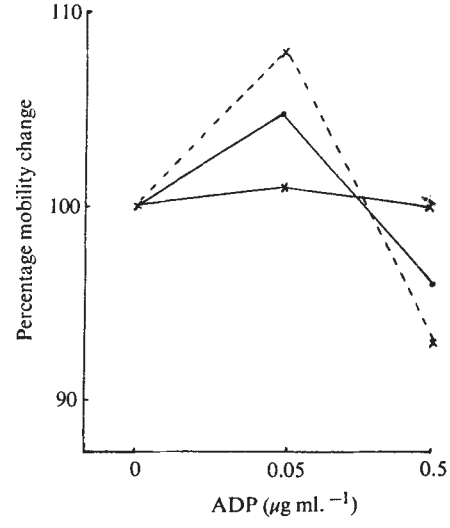

Fig. 1 Effect of heparin on pig platelet electrophoretic mobility. × - - - ×, Control; ○—○, heparin (0.16 U ml.$^{-1}$), ●—●, heparin (5.0 U ml.$^{-1}$).