

# Crystal and Molecular Structure of a Naturally Occurring Dinucleoside Monophosphate. Uridyl-(3'-5')-adenosine Hemihydrate. Conformational "Rigidity" of the Nucleotide Unit and Models for Polynucleotide Chain Folding†

J. Rubin, T. Brennan, and M. Sundaralingam\*

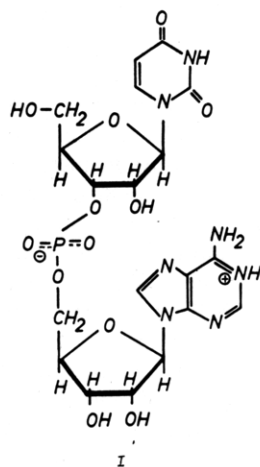
**ABSTRACT:** The crystal structure of a naturally occurring dinucleoside monophosphate, uridyl-(3'-5')-adenosine (UpA), has been determined using three-dimensional X-ray intensities. UpA crystals are monoclinic, space group  $P2_1$ , with unit cell parameters  $a = 16.961$  (6),  $b = 12.350$  (2),  $c = 11.245$  (3) Å, and  $\beta = 95.88$  (2)°. There are two independent molecules of UpA and a molecule of water per asymmetric unit. The structure was solved by a combination of Patterson, Fourier, and tangent refinement methods. All 50 hydrogen atoms were located in difference-Fourier maps. Structure refinement was carried out by the full-matrix least-squares method to a final residual of 4.0% using 3187 observed reflections. The standard deviations in bond distances (angles) range from 0.002 to 0.004 Å (0.2°) for the phosphodiester groups and from 0.006 to 0.011 Å (0.3 to 0.6°) for the base and sugar residues. The UpA molecules exist as zwitterions; the adenine base is protonated at N(1) while the phosphate is negatively charged. The molecular dimensions of the nucleoside and nucleotide units are generally in good agreement with the values found in the corresponding monomer structures. Similarly, the nucleotide moieties are in the preferred conformation as found for the mononucleotide structures: anti glycosyl torsion angle and gauche,gauche conformation about the C(4')-C(5') bond. All four riboses exhibit the same puckering, C(3')-endo, C(2')-exo ( $^3T_2$ ), which is one of the two preferred conformations

found for the sugars. However, the two UpA molecules are markedly different in their overall conformations due mainly to the differences in the rotations about the P-O(5') and P-O(3') ester bonds. UpA1 exhibits the (-)gauche,trans conformation for the phosphodiester group while UpA2 exhibits the (+)gauche,(+)gauche conformation. Both conformations are different from the (-)gauche,(-)gauche conformation found for the phosphodiester group in the accurately analyzed double-helical polynucleotide structures. The conformation of UpA2 provides a model for the formation of hairpin loops in polynucleotides as, for example, the anticodon loop of tRNAs. The structure of UpA taken in conjunction with the available 5'-nucleotide structures indicates that tertiary folding of a polynucleotide chain is achieved primarily through conformational changes in the phosphodiester function. The Watson and Crick complementary base pairing is not observed in this structure. Rather, the crystal structure may be considered as made up of alternate sheets of self-paired uracil and adenine bases lying on the 402 planes with an average separation of 3.4 Å between planes. Both base-base and base-ribose O(1') interactions are found in UpA. The water molecule, which lies close to the plane of the uracil bases, is trapped in a pocket where it is hydrogen bonded to five oxygen atoms of adjacent UpA molecules. The structure demonstrates both intramolecular and intermolecular C-H...O hydrogen bonding.

It might be said that modern molecular biology had its beginnings when the double helical structure of DNA was elucidated by Watson and Crick (1953a). The structure of DNA immediately lent itself to an understanding of gene replication (Watson and Crick, 1953b). What knowledge we have of the secondary and tertiary structures of helical nucleic acids (as, for example, A-DNA (Fuller *et al.*, 1965), B-DNA (Langridge *et al.*, 1960), C-DNA (Marvin *et al.*, 1961), and RNA-11 (Arnott *et al.*, 1967) has been derived largely from X-ray diffraction studies of nucleic acid fibers and of nucleo-

side and nucleotide single crystals in combination with model building. Detailed stereochemical data on the constituents of nucleic acids provide the main basis for constructing molecular models of nucleic acids and polynucleotides. Consequently, considerable interest has been generated for the study of nucleoside and nucleotide crystal structures by single crystal X-ray diffraction techniques. Such studies on the monomers have resulted in a number of rules governing the stereochemistry of the constituents of the nucleic acids and polynucleotides (Sundaralingam, 1969, and references therein). In our continuing studies on the structures of the nucleic acid constituents, we now report the molecular and crystal structure of the naturally occurring dinucleoside monophosphate, uridyl-(3'-5')-adenosine, UpA (I). A preliminary communication on this work has been published (Rubin *et al.*, 1971). An independent study of the same crystal structure has also been published (Seeman *et al.*, 1971). UpA represents an essential link between the structural studies of the single crystal monomers and the polynucleotide fibers because it provides direct information on the conformation about the internucleotide 3',5'-phosphodiester linkage that is present in all naturally occurring polynucleotides (Brown and Todd,

† From the Department of Biochemistry and Laboratory of Molecular Biology, College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wisconsin 53706. Received February 22, 1972. We gratefully thank the National Institutes of Health of the U. S. Public Health Service for support of this research through Grants GM 17378 and GM 18455 and the Wisconsin Alumni Research Foundation for partial support of the computing. Supplementary material (structure factor data) will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036, by referring to code number BIOL-3112. Remit check or money order for \$3.00 for photocopy or \$2.00 for microfiche.



1952; Dekker *et al.*, 1953). The value of the study of UpA is further enhanced by the presence in the crystal structure of two independent molecules of UpA having very different overall conformations but similar conformations for the nucleotide moieties. This information on the apparent conformational rigidity of the nucleotide unit (with respect to rotation about the glycosyl bond, the C(4')-C(5') bond and the C(5')-O(5') bond) and the conformational flexibility in the phosphodiester group provides the basis for a better understanding of the stereochemistry of polynucleotides.

Moreover, the excellent quality of the UpA crystals has made possible the measurement of an accurate set of X-ray intensities. As a result, we have been able to refine the atomic parameters of the 79 nonhydrogen atoms to about the same precision as those of the mononucleotides, and to locate unequivocally the positions of all the hydrogen atoms present in the dinucleosides. This has allowed a detailed description of the hydrogen-bonding scheme which will undoubtedly be also important in understanding the tertiary structures and functions of nucleic acids.

## Experimental Section

**Crystal Data.** A purified sample of UpA was obtained from Zellstoffabrik Waldhof, Mannheim, West Germany. The compound crystallized readily from saturated methanol-water (4:1) solutions (pH 3.2) forming large clear prismatic crystals (Figure 1). Preliminary oscillation and Weissenberg photographs indicated the crystal system to be monoclinic. The systematic absences,  $0k0$ ,  $k = 2n + 1$ , indicated the space group  $P2_1$ ,  $P2_1/m$  being ruled out since the dinucleoside is optically active. The cell constants were determined by a least-squares refinement of the  $2\theta$  values of 12 medium-angle reflections carefully measured on a diffractometer. The relevant crystal data are given in Table I. The experimental crystal density was in agreement with the calculated density assuming four formula weights of UpA ( $C_{19}H_{24}N_7O_{12}P$ ) in the unit cell, that is, two independent molecules of UpA per asymmetric unit. However, during the structure analysis two water molecules were also located in the unit cell. Therefore the asymmetric unit of structure is composed of two UpA molecules and one water molecule.

Three-dimensional X-ray intensity data were collected by the  $\theta$ - $2\theta$  scan technique with a scan speed of  $2^\circ/\text{min}$  on a Picker automated four circle diffractometer using Ni-filtered Cu radiation ( $\lambda = 1.5418 \text{ \AA}$ ). Approximately 4000 independent reflections were recorded up to  $2\theta_{\text{max}} = 127^\circ$  using  $3^\circ$  scans for each reflection and 10-sec background counts on

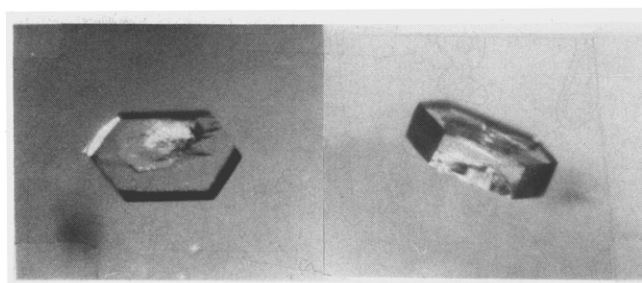


FIGURE 1: Photomicrographs of a crystal of UpA in two orientations showing the crystallographic axis. The approximate crystal dimensions are  $0.5 \times 0.35 \times 0.15 \text{ mm}$ . For the X-ray work the crystal was mounted about the  $a$  axis.

either side of the peak. A reflection was considered observed if the intensity  $I > 1.5\sigma(I)$  where  $\sigma(I) = [I_{\text{scan}} + I_{\text{bgd}}]^{1/2}$ . On this basis there were 3187 observed reflections which constituted 80% of the scanned reflections, and 60% of the total available in the sphere of reflection ( $\lambda(\text{Cu K}\alpha) = 1.5418 \text{ \AA}$ ). The intensities were corrected for the usual Lorentz and polarization effects, but neither absorption nor extinction corrections were applied.

**Structure Solution.** The two independent phosphorus atoms in the structure were located through an analysis of gradient-sharpened origin removed three-dimensional Patterson functions using  $E_0^2 - 1$  values as coefficients, where  $E$  is the normalized structure amplitude. The Patterson function of the space group  $P2_1$  contains only one Harker section located at  $v = 1/2$  representing the vectors  $(2x, 1/2, 2z)$  between each atomic position  $(x, y, z)$  and its screw axis related one  $(\bar{x}, 1/2 + y, \bar{z})$ . The Harker section of the UpA Patterson function computed using all intensity data (Figure 2a) revealed one predominant peak at  $u = 0.35$ ,  $w = 0.70$ . A second Patterson map was calculated employing only the higher angle reflections with  $\sin \theta/\lambda \geq 0.5$ . Such a map should indicate more strongly the phosphorus-phosphorus vectors due to the relatively large X-ray-scattering factors for phosphorus at high angles. In fact, the latter technique had been used

TABLE I: Crystal Data for UpA.

Crystal System:	Monoclinic
Space group:	$P2_1$
Unit Cell Dimensions	
$a$ (Å)	$16.961 \pm 0.006$
$b$ (Å)	$12.350 \pm 0.002$
$c$ (Å)	$11.245 \pm 0.003$
$\beta$ (deg)	$95.88 \pm 0.02$
Observed density ( $\text{g cm}^{-3}$ )	1.625 (by flotation in $\text{CCl}_4\text{-CHBr}_3$ )
Calculated density ( $\text{g cm}^{-3}$ )	1.626 (assuming four formula units of $\text{C}_{19}\text{H}_{24}\text{N}_7\text{O}_{12}\text{P}$ )
	$1.651 (4(\text{C}_{19}\text{H}_{24}\text{N}_7\text{O}_{12}\text{P}) + 2\text{H}_2\text{O})^a$
Number of molecules of UpA per asymmetric unit	2, plus a molecule of water

<sup>a</sup> The presence of the water molecule was shown in the difference-Fourier refinement (see text).

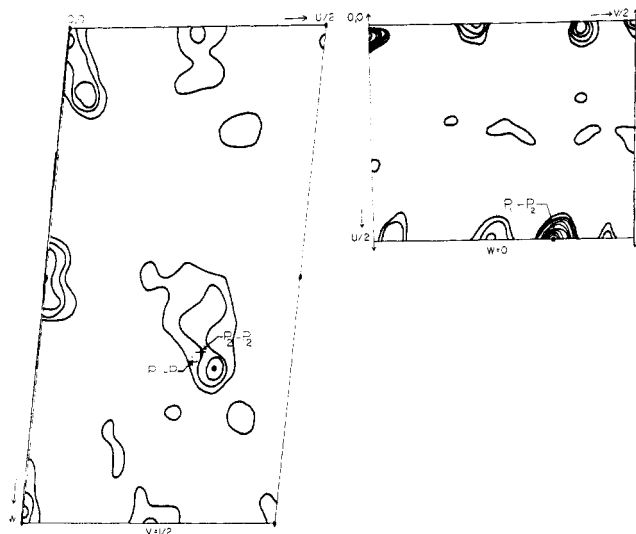


FIGURE 2: The Patterson-Harker section at  $v = 1/2$ , and the section at  $w = 0$  showing the P-P vectors. The positions marked with solid circles were assumed as representing the P-P vectors in the initial work, while the crosses indicate the P-P vectors generated from the final structure. Notice that the final P positions are considerably shifted from the initial position.

successfully in the structure determination of puromycin (Sundaralingam and Arora, 1969). The Harker peak at  $u = 0.35$ ,  $w = 0.70$  in the Patterson function using the high-angle data became even more pronounced while all of the remaining peaks in the Harker section were greatly diminished. This suggested that the peak at  $u = 0.35$ ,  $w = 0.70$  was due to the accidental overlap of the Harker vectors from the two independent phosphorus atoms of UpA1 and UpA2. This would occur in the special cases where the two phosphorus atom coordinates were related by either of the following relationships:  $\Delta x = 0$ ,  $\Delta z = 0$ ;  $\Delta x = 0.5$ ,  $\Delta z = 0$ ; or  $\Delta x = 0$ ,  $\Delta z = 0.5$ . The first phosphorus atom could immediately be assigned coordinates  $x = 0.175$ ,  $z = 0.35$ . The origin along the  $b$  axis is arbitrary in this space group, and a value of  $y = 0.25$  was assigned to the atom. Determination of the relative position of the second phosphorus atom required the location of the P(1)-P(2) cross vector in the three-dimensional Patterson function. The Patterson section of  $z = 0$  gave a very large peak at  $u = 0.5$  and  $v = 0.35$  (Figure 2b), which was compatible with the observed Harker overlap discussed above. This permitted the determination of the coordinates of the second phosphorus atom which were  $x = 0.675$ ,  $y = 0.90$ ,  $z = 0.35$ .

The two phosphorus positions were used to determine the phases of those reflections with  $E \geq 1.5$ . These phases were then refined by the application of the tangent formula (Karle and Hauptman, 1956). The  $E$  map computed using the refined phases clearly revealed the positions of the two independent adenine bases, the attached ribose C(1') atoms and the phosphate oxygen atoms. The adenine bases were self-paired through the interbase hydrogen bonds N(6)-H...N(7). However, it was not immediately apparent if the Watson and Crick sites of the bases were involved in the complementary pairing. The tangent refinement was repeated using the 30 atomic positions located at this stage as the phasing model. The resultant  $E$  map clearly revealed one of the uracil bases and parts of the other uracil base and riboses. It was clear at this point that the Watson and Crick complementary

pairing was absent in UpA. A total of 54 atoms were used in subsequent difference-Fourier calculations which revealed the remaining nonhydrogen atoms including the water oxygen.

**Structure Refinement.** Initially, the structure was refined by the block diagonal least-squares method with all 79 non-hydrogen atoms having isotropic thermal parameters. This was followed by full-matrix least-squares refinement of the positional and isotropic thermal parameters of one UpA molecule at a time resulting in an  $R$  value of 0.12. The non-hydrogen atoms were then subjected to two cycles of full-matrix least-squares refinement with anisotropic thermal parameters for the atoms; the  $R$  value dropped to 0.063. The refinement was carried out in four overlapping blocks containing the parameters of one nucleotide unit in each block because of the limitations in computer storage space. At this stage, a difference-Fourier map was computed which revealed the positions of 46 of the 50 hydrogen atoms. The hydrogen atom positional and isotropic thermal parameters were subjected to two cycles of refinement, which was followed by two cycles of refinement of the heavy-atom parameters; the  $R$  was 0.043. Eight of the refined hydrogen atoms were removed because they had refined to unreasonable positions. A difference-Fourier map, however, revealed the correct location of these eight hydrogen atoms in addition to the remaining four not found in the earlier difference map. Although one of the water and several of the hydroxyl hydrogen atoms appeared in the difference electron-density maps somewhat smeared and diffuse due to thermal motion, the hydrogen electron-density peak heights ( $0.4$ – $0.6$  e  $\text{\AA}^{-3}$ ) were, in general, about three times as large as background peaks.

Up to this stage weights based on counting statistics were used in the refinement (Stout and Jensen, 1968). However, an error plot of  $|\Delta F|$  vs.  $(|F_o| - |F_c|)$  over different ranges of  $|F_o|$  indicated that the curve could be approximated better by the following Hughes (1941) type of weighting scheme

$$\text{if } |F_o| \leq 10.0, \frac{1}{\sqrt{w}} = 5.0 - 0.35|F_o|$$

$$\text{if } |F_o| > 10.0 \text{ and } < 45.0, \frac{1}{\sqrt{w}} = 1.5$$

$$\text{if } |F_o| \geq 45.0, \frac{1}{\sqrt{w}} = 0.375|F_o|$$

The above weighting scheme was used in the subsequent refinements; eight intense reflections apparently suffering from secondary extinction were given zero weight. In the final refinement the shifts in all of the atomic parameters were less than two-tenths the esd's. The final  $R$  values are 0.040 for the 3179 observed reflections, and 0.060 for all 4024 observed and unobserved reflections. The corresponding weighted  $R$  values are 0.050 and 0.052, respectively.

Atomic scattering factors used throughout the refinement are from Cromer and Waber (1965) (CNOP) and from Stewart *et al.* (1965) (H).

## Results

The final positional and thermal parameters for the non-hydrogen atoms and hydrogen atoms including their estimated standard deviations are listed in Tables II and III, respectively. The thermal vibration ellipsoids of the heavy atoms are represented by the drawings in Figure 3. The atoms

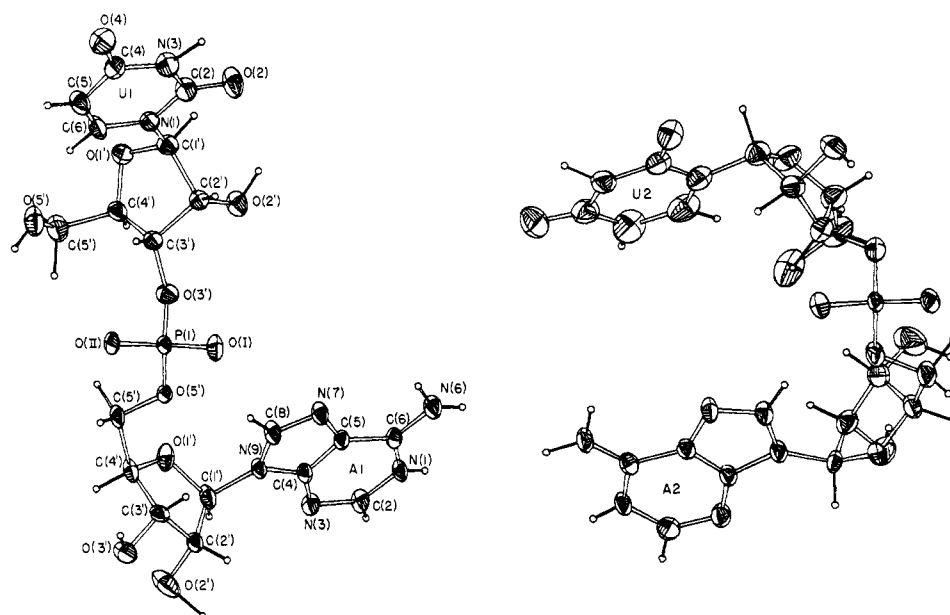


FIGURE 3: The thermal vibration ellipsoids for the nonhydrogen atoms in (a) UpA1 and (b) UpA2. The ellipsoids were scaled to include 40% probability surfaces. The drawings were made using the ORTEP program (Johnson, 1965). The atom numbering is also indicated (also, see text).

have been labeled according to the conventional numbering scheme used for nucleosides. Throughout the text the letters U, A, and R have been appended to the atom labels to designate uracil or adenine base and ribose sugars, and the numbers 1 and 2 for UpA molecule 1 and molecule 2. The bond distances and angles involving the nonhydrogen atoms of the four bases, four riboses and two phosphodiester residues are shown in Figure 4. The average estimated standard deviations in bond lengths and angles for the various residues are given in Table IV. Bond distances and bond angles involving the hydrogen atoms fall in the usual range for X-ray determination and are summarized in Table V.

## Discussion

**Zwitterionic Character of UpA.** The location of the hydrogen atoms attached to the N(1) position of the adenine bases unequivocally establish that the adenine residues in both UpA molecules are protonated by the phosphate protons. Therefore, both UpA molecules exist as zwitterions containing cationic adenine bases and anionic phosphodiester groups. All of the known crystal structures of adenine and cytosine mononucleotides also exist as zwitterions with protonation at N(1) of adenine (Sundaralingam, 1966; Sundaralingam and Jensen, 1965b) and N(3) of cytosine (Sundaralingam and Jensen, 1965a). Since the present study also indicates that adenine is protonated (pH 3.2), it is reasonable to expect cytosine-containing dinucleosides to be also present as zwitterions at low pH.

**Molecular Dimensions of UpA.** The molecular dimensions of UpA are shown in Figure 4.

**Adenine Cations.** It has been shown earlier (Sundaralingam and Jensen, 1965b; Sundaralingam, 1966) that a protonated adenine ring shows marked differences in bond distances and angles compared to the neutral species. In particular, the bond length of N(1)-C(6) increases while C(6)-N(6) decreases, and concomitantly bond angles N(1)-C(2)-N(3)

and N(1)-C(6)-C(5) decrease while C(2)-N(1)-C(6) increases. Such changes are observed in the adenine residues of both UpA molecules (Figure 4a). The bond distances and bond angles involving the nonhydrogen atoms of the adenine bases of UpA molecules 1 and 2 (A1 and A2) are generally in agreement with each other and compare favorably to those of other N(1) protonated adenine compounds (Rao and Sundaralingam, 1970; Voet and Rich, 1970).

**Uracil Bases.** The bond distances and bond angles involving the nonhydrogen atoms of the uracil bases of UpA1 and UpA2 (U1 and U2) are shown in Figure 4a. As with A1 and A2 the dimensions of U1 and U2 generally agree with each other and with those of other uracil compounds (Voet and Rich, 1970). It appears that in both U1 and U2 the C(4)-O(4) bond distance is slightly longer than the C(2)-O(2) bond. At the same time the exocyclic bond angles involving the carbonyl group at C(4) are more asymmetric than those involving the carbonyl group at C(2). The same trend is found in other uracil compounds and probably reflects the differences in the local environments of C(2) and C(4) rather than differences in hydrogen bonding to the carbonyl oxygen atoms (Shefter *et al.*, 1969).

**Riboses.** The bond distances and bond angles involving the nonhydrogen atoms of the four sugar rings are shown in Figure 4b. The dimensions of the riboses attached to the adenine base of molecules 1 and 2 (RA1 and RA2) are essentially the same, as are those of the riboses attached to U1 and U2 (RU1 and RU2), and are in agreement with the values found earlier (Sundaralingam and Jensen, 1965b). All four sugars exhibit C(3')-endo, C(2')-exo ring puckering. In each case the two atoms that show the largest deviations from the furanose ring plane are involved in the smallest two endocyclic angles, the smaller of which being involved with atom C(2') that shows the second largest puckering from the five-atom plane. There are also cases known where the smallest endocyclic angle is associated with the atom that shows the largest puckering from the five-atom plane (Sundaralingam,

TABLE II  
POSITIONAL AND THERMAL PARAMETERS OF NONHYDROGEN ATOMS IN UPAl

ATOM	X	Y	Z	$\beta_{11}$	$\beta_{22}$	$\beta_{33}$	$\beta_{12}$	$\beta_{13}$	$\beta_{23}$
N(1)A	3539( 3)	10747( 3)	7046( 3)	32( 2)	22( 3)	47( 4)	-2( 2)	-5( 3)	9( 3)
C(2)A	3952( 3)	10585( 4)	8106( 5)	36( 3)	18( 4)	66( 6)	-5( 3)	-4( 4)	2( 4)
N(3)A	4084( 3)	9644( 3)	8644( 4)	35( 2)	21( 3)	57( 5)	-1( 3)	-7( 3)	-7( 3)
C(4)A	3730( 3)	8836( 4)	7973( 4)	35( 3)	26( 4)	43( 5)	4( 3)	3( 3)	8( 4)
C(5)A	3308( 3)	8905( 4)	6880( 4)	28( 2)	25( 4)	43( 5)	5( 3)	-3( 3)	-1( 4)
C(6)A	3194( 3)	9946( 4)	6348( 4)	32( 3)	19( 4)	51( 5)	0( 3)	5( 3)	8( 4)
N(6)A	2805( 3)	10115( 3)	5312( 4)	44( 3)	28( 4)	59( 5)	2( 3)	-5( 3)	3( 4)
N(7)A	3046( 2)	7900( 3)	6474( 3)	34( 2)	25( 4)	51( 5)	-3( 2)	-9( 3)	3( 3)
C(8)A	3281( 3)	7258( 4)	7337( 4)	42( 3)	16( 4)	54( 6)	-4( 3)	-9( 4)	4( 4)
N(9)A	3722( 2)	7753( 3)	8286( 3)	36( 2)	21( 3)	40( 4)	1( 2)	-4( 3)	4( 3)
N(1)U	3985( 3)	2011( 3)	2372( 3)	38( 2)	26( 3)	45( 4)	0( 3)	2( 3)	-7( 3)
C(2)U	3521( 3)	2044( 4)	1320( 5)	37( 3)	33( 5)	59( 6)	-5( 3)	-6( 3)	-7( 4)
O(2)U	3385( 3)	2874( 3)	742( 4)	62( 3)	31( 3)	82( 5)	-4( 3)	-21( 3)	3( 3)
N(3)U	3192( 3)	1070( 4)	914( 4)	44( 3)	39( 4)	61( 5)	-5( 3)	-7( 3)	-11( 4)
C(4)U	3333( 4)	77( 4)	1462( 5)	46( 3)	39( 5)	63( 6)	9( 4)	3( 4)	-5( 4)
O(4)U	3035( 3)	-743( 3)	972( 4)	53( 3)	35( 3)	87( 5)	-9( 3)	4( 3)	-17( 3)
C(5)U	3811( 4)	121( 4)	2567( 5)	52( 4)	33( 5)	53( 6)	-3( 3)	0( 4)	7( 4)
C(6)U	4123( 4)	1056( 4)	2979( 4)	49( 4)	37( 5)	44( 5)	3( 3)	-9( 4)	8( 4)
C(1')A	4100( 4)	7268( 4)	9385( 4)	48( 4)	27( 4)	48( 6)	1( 3)	-12( 4)	7( 4)
C(2')A	3529( 4)	6902( 4)	10261( 4)	53( 4)	26( 4)	41( 5)	11( 4)	0( 4)	2( 4)
C(3')A	3418( 3)	5709( 4)	9963( 4)	38( 3)	37( 5)	31( 5)	2( 3)	2( 3)	-7( 4)
C(4')A	4193( 3)	5369( 4)	9540( 4)	32( 3)	26( 4)	47( 5)	-3( 3)	-16( 3)	7( 4)
C(5')A	4189( 3)	4508( 4)	8586( 4)	44( 3)	21( 4)	50( 5)	6( 3)	-5( 4)	9( 4)
O(1')A	4536( 2)	6345( 3)	9068( 3)	32( 2)	28( 3)	88( 5)	-2( 2)	-3( 3)	13( 3)
O(2')A	3875( 4)	7038( 4)	11438( 3)	107( 5)	53( 4)	46( 4)	-15( 4)	-9( 4)	2( 4)
O(3')A	3214( 3)	5143( 3)	10990( 3)	57( 3)	34( 7)	52( 7)	-8( 4)	6( 4)	-1( 6)
O(5')A	3638( 2)	4802( 3)	7590( 3)	46( 2)	20( 3)	37( 4)	11( 2)	1( 2)	0( 3)
C(1')U	4391( 3)	3037( 4)	2780( 4)	30( 3)	39( 4)	49( 5)	-5( 3)	2( 3)	-6( 4)
C(2')U	3870( 3)	3782( 4)	3480( 4)	28( 2)	26( 4)	44( 5)	-6( 2)	-2( 3)	-8( 3)
C(3')U	4135( 3)	3433( 4)	4742( 4)	28( 2)	31( 4)	42( 5)	-3( 3)	1( 3)	-6( 3)
C(4')U	5022( 3)	3227( 4)	4703( 4)	29( 2)	40( 4)	39( 5)	0( 3)	-2( 3)	-2( 4)
C(5')U	5395( 3)	2447( 5)	5644( 5)	40( 3)	54( 5)	71( 6)	2( 4)	-8( 4)	5( 5)
O(1')U	5067( 2)	2792( 3)	3535( 3)	29( 2)	52( 3)	50( 3)	2( 2)	0( 2)	-4( 3)
O(2')U	4087( 2)	4883( 3)	3283( 3)	45( 2)	32( 3)	67( 4)	-7( 2)	-4( 2)	3( 3)
O(3')U	4034( 2)	4210( 3)	5657( 3)	40( 2)	33( 3)	51( 3)	-7( 2)	8( 2)	-9( 2)
O(5')U	4962( 2)	1454( 3)	5585( 3)	45( 2)	45( 3)	90( 5)	5( 2)	-11( 3)	14( 3)
P(1)	3355( 1)	4003( 1)	6532( 1)	31( 1)	15( 1)	38( 1)	1( 1)	0( 1)	2( 1)
O(II)	2593( 2)	4431( 3)	5956( 3)	33( 2)	33( 3)	76( 4)	3( 2)	-11( 2)	9( 3)
O(III)	3377( 2)	2852( 2)	6961( 3)	45( 2)	18( 3)	60( 4)	6( 2)	4( 2)	11( 3)

POSITIONAL AND ANISOTROPIC THERMAL PARAMETERS ARE  $\times 10^4$   
ANISOTROPIC TEMPERATURE FACTOR IS OF THE FORM  $\exp(-(\beta_{11}h^2 + \dots 2\beta_{12}hk + \dots))$   
STANDARD DEVIATIONS REFER TO THE LEAST SIGNIFICANT DIGITS

1965; Sundaralingam and Jensen, 1965b). In each sugar the O(1')-C(4') distance is longer than the O(1')-C(1') distance as noted previously (Sundaralingam, 1965).

There are, however, some differences in bond distances and bond angles between the two types of sugars, RA1 and RA2, on one hand, and RU1 and RU2, on the other. The most significant differences involve the glycosyl bond distances and the exocyclic angles about C(3'). The purine N(9)-C(1') glycosyl bond lengths (1.461 and 1.464 Å) are shorter than the pyrimidine N(1)-C(1') glycosyl bond lengths (1.492 and 1.494 Å) as has been observed previously (Lin *et al.*, 1971). In both RA1 and RA2 the angle O(3')-C(3')-C(4') is markedly greater than angle O(3')-C(3')-C(2'), whereas in RU1 and RU2 the reverse is true. This change is attributable to the presence of the phosphate group at C(3') in the RU moieties. In addition, the C(3')-O(3') bond distances of the hydroxyl groups in RA1 and RA2 are shorter than those of RU1 and RU2 which carry the phosphate group. In general, the C-O(H) bonds are lengthened upon phosphorylation (Sundaralingam and Jensen, 1965b). The C(2')-O(2') bond lengths also increase in the RU residues with the presence of the adjacent 3'-phosphate groups. Similar though less signifi-

cant differences occur between the RA and RU moieties at the 5' side, *viz.*, the angles about C(4') and the C(4')-C(5') and C(5')-O(5') bond distances.

There appears to be additional trends in the differences between the adenine and uracil riboses in UpA: the adenine ribose rings have smaller C(1')-O(1')-C(4') bond angles, larger O(1')-C(4')-C(3') angles, shorter C(1')-C(2') and C(3')-C(4') bond distances and longer O(1')-C(1') and O(1')-C(4') bond distances than the corresponding values in the uracil sugars. Some of these latter differences are within three esd's and thus they do not pass the usual criteria of significance. Nevertheless, the trends may be real, reflecting the combined effects of pyrimidine and phosphate substitution at C(1') and O(3') *vs.* purine and phosphate substitutions at C(1') and O(5').

**Phosphodiester Groups.** The phosphodiester groups are negatively charged since the only phosphate proton has been transferred to the N(1) sites of adjacent adenine bases. It is believed that the phosphodiester groups of the nucleic acids also exist as anions under physiological pH's, with the important difference that in the complementary double-helical nucleic acids N(1) of adenine is not protonated. The crystal

TABLE II (Continued)

## POSITIONAL AND THERMAL PARAMETERS OF NONHYDROGEN ATOMS IN UP2

ATOM	X	Y	Z	$\beta_{11}$	$\beta_{22}$	$\beta_{33}$	$\beta_{12}$	$\beta_{13}$	$\beta_{23}$
N(1)A	1791( 3)	5686( 3)	2834( 4)	37( 2)	21( 3)	61( 5)	2( 2)	-9( 3)	0( 3)
C(2)A	1336( 4)	5852( 4)	1780( 5)	46( 3)	37( 5)	65( 6)	-6( 3)	0( 4)	-8( 4)
N(3)A	1110( 3)	6784( 3)	1342( 4)	42( 3)	38( 4)	59( 5)	-1( 3)	-7( 3)	4( 3)
C(4)A	1420( 3)	7609( 4)	2026( 4)	28( 2)	28( 4)	52( 5)	1( 3)	-5( 3)	-1( 4)
C(5)A	1906( 3)	7535( 4)	3078( 4)	28( 2)	29( 4)	51( 5)	1( 3)	-3( 3)	-2( 4)
C(6)A	2113( 3)	6502( 4)	3537( 4)	24( 2)	31( 4)	64( 6)	-1( 3)	4( 3)	3( 4)
N(6)A	2525( 3)	6312( 3)	4555( 4)	39( 2)	28( 3)	64( 5)	2( 3)	-7( 3)	8( 3)
N(7)A	2100( 3)	8556( 3)	3537( 4)	34( 2)	33( 3)	63( 5)	3( 2)	-18( 3)	6( 3)
C(8)A	1708( 3)	9211( 4)	2759( 4)	34( 3)	26( 4)	58( 5)	-4( 3)	-14( 3)	-1( 4)
N(9)A	1286( 3)	8695( 3)	1832( 3)	34( 2)	26( 3)	54( 4)	1( 2)	-10( 3)	0( 3)
N(1)U	1147( 3)	10594( 4)	7547( 4)	38( 2)	64( 5)	59( 5)	-8( 3)	3( 3)	-1( 4)
C(2)U	1774( 4)	10499( 5)	8404( 5)	38( 3)	62( 6)	57( 5)	-6( 3)	8( 3)	-3( 5)
O(2)U	2130( 2)	11297( 3)	8824( 4)	42( 2)	51( 4)	82( 4)	-14( 3)	-5( 3)	5( 4)
N(3)U	1990( 3)	9472( 4)	8766( 4)	39( 2)	52( 4)	71( 5)	-1( 3)	17( 3)	-1( 4)
C(4)U	1605( 4)	8531( 5)	8379( 5)	54( 4)	60( 6)	72( 6)	-9( 4)	6( 4)	-10( 5)
O(4)U	1834( 3)	7664( 4)	8847( 5)	78( 4)	54( 4)	136( 7)	0( 4)	6( 4)	-2( 5)
C(5)U	977( 5)	8668( 6)	7485( 7)	76( 6)	63( 7)	112( 9)	-32( 5)	1( 6)	-2( 7)
C(6)U	745( 4)	9687( 6)	7121( 6)	52( 4)	94( 7)	78( 7)	-22( 5)	-5( 4)	-9( 6)
C(1')A	733( 3)	9161( 4)	884( 4)	38( 3)	26( 4)	37( 4)	-4( 3)	-8( 3)	8( 3)
C(2')A	-89( 3)	9311( 5)	1274( 4)	33( 3)	59( 5)	52( 5)	-2( 3)	-9( 3)	3( 4)
C(3')A	-39( 3)	10476( 5)	1745( 5)	29( 3)	67( 6)	65( 6)	12( 3)	-3( 3)	-2( 5)
C(4')A	476( 3)	11025( 4)	904( 5)	38( 3)	34( 4)	63( 5)	7( 3)	-15( 3)	-4( 4)
C(5')A	953( 4)	11991( 4)	1390( 5)	58( 4)	31( 4)	67( 6)	3( 4)	-15( 4)	2( 4)
O(1')A	1023( 2)	10197( 3)	594( 3)	41( 2)	27( 3)	65( 4)	3( 2)	9( 2)	9( 3)
O(2')A	-629( 2)	9217( 4)	241( 3)	38( 2)	83( 4)	70( 4)	-1( 3)	-18( 2)	-1( 4)
O(3')A	-780( 3)	10949( 5)	1834( 5)	46( 3)	133( 3)	140( 4)	4( 3)	-2( 3)	-23( 3)
O(5')A	1433( 2)	11675( 3)	2454( 3)	48( 2)	27( 3)	65( 4)	0( 2)	-16( 2)	-2( 3)
C(1')U	860( 3)	11711( 5)	7233( 5)	35( 3)	51( 5)	66( 5)	-4( 3)	3( 3)	-3( 4)
C(2')U	1367( 3)	12267( 4)	6353( 5)	34( 3)	46( 4)	68( 5)	-6( 3)	11( 3)	-4( 4)
C(3')U	929( 3)	11942( 5)	5165( 5)	27( 2)	64( 5)	60( 5)	-2( 3)	2( 3)	9( 4)
C(4')U	66( 3)	11959( 6)	5423( 5)	32( 3)	86( 6)	70( 6)	7( 4)	6( 3)	2( 5)
C(5')U	-495( 4)	11197( 8)	4659( 6)	29( 3)	142( 10)	83( 7)	-1( 5)	0( 4)	7( 7)
O(1')U	90( 2)	11643( 4)	5657( 3)	32( 2)	103( 5)	61( 4)	1( 3)	11( 2)	6( 3)
O(2')U	1316( 2)	13401( 3)	6554( 4)	45( 2)	51( 3)	95( 5)	10( 2)	13( 3)	-5( 3)
O(3')U	1028( 2)	12695( 3)	4212( 3)	35( 2)	60( 3)	65( 4)	12( 2)	9( 2)	15( 3)
O(5')U	-184( 3)	10138( 5)	4651( 4)	63( 3)	101( 5)	112( 6)	-34( 4)	-30( 3)	17( 5)
P(2)	1762( 1)	12555( 1)	3434( 1)	27( 1)	21( 1)	43( 1)	2( 1)	-1( 1)	3( 1)
O(II)	1878( 2)	13592( 3)	2842( 3)	40( 2)	23( 3)	69( 4)	-3( 2)	0( 2)	5( 3)
O(III)	2433( 2)	12003( 3)	4153( 3)	29( 2)	39( 3)	78( 4)	0( 2)	1( 2)	10( 3)
O(IV)	2101( 3)	3807( 4)	9051( 4)	48( 2)	54( 3)	117( 5)	-3( 2)	4( 2)	-8( 3)

structure of UpA provides invaluable information on the molecular dimensions of the 3',5'-phosphodiester group under two different local crystal environments and for two conformations.

The bond distances and angles involving the two phosphodiester groups are shown in Figure 4c. The geometry around the phosphorus atoms is a highly distorted tetrahedron. As is normally the case those oxygen atoms involved in the phosphodiester linkages show much longer P-O distances than the anionic P-O bonds (Sundaralingam and Jensen, 1965b; Sundaralingam, 1969; Shefter *et al.*, 1969). At the same time, the valence angles involving the ester oxygen atoms O(3')-P-O(5') are much smaller than those involving the anionic oxygen atoms O(I)-P-O(II). In UpA1 the two ester P-O bonds are significantly different; the shorter value of 1.583 Å is associated with the (-)gauche conformation about the O(5')-P bond, while the longer bond of 1.610 Å with the trans conformation about the O(3')-P bond. In addition, the valence angle C(5')-O(5')-P(1) is 5° larger than the valence angle P(1)-O(3')-C(3'). The differences are mainly attributable to the different conformations about the P-O ester bonds and to a lesser extent on the different linkages, 5' vs. 3'.

In UpA2 the conformations about the P-O ester bonds are the same ((+)gauche) resulting in similar bond distances (1.603, 1.607 Å) for the P-O ester bonds. Thus, the linkage differences (5' vs. 3') probably contribute to the small differences in the valence angles at O(3') and O(5'). In both molecules the anionic P-O bond distances are different, being particularly significant in UpA2.

**Molecular Conformation.** Stereoscopic views of the molecular conformation of UpA1, UpA2, and the corresponding dinucleoside portion of RNA-11 are shown in Figure 5. A comparison of these conformations is given below.

**Base Planarity.** The least-squares planes and atomic displacements for the pyrimidine and purine bases are given in Table VI. The displacements of atoms from the least-squares planes calculated for the nine-membered purine systems in A1 and A2 indicate that the adenine bases are not strictly planar. The dihedral angle between the pyrimidine ring and the imidazole ring is 2.2° in A1 and 2.1° in A2. Such noncoplanarity in purine ring systems is not uncommon. In A2 the substituent atoms N(6) and C(1') show large displacements from the base plane in the same direction. The six-membered pyrimidine rings in U1 and U2 are essen-

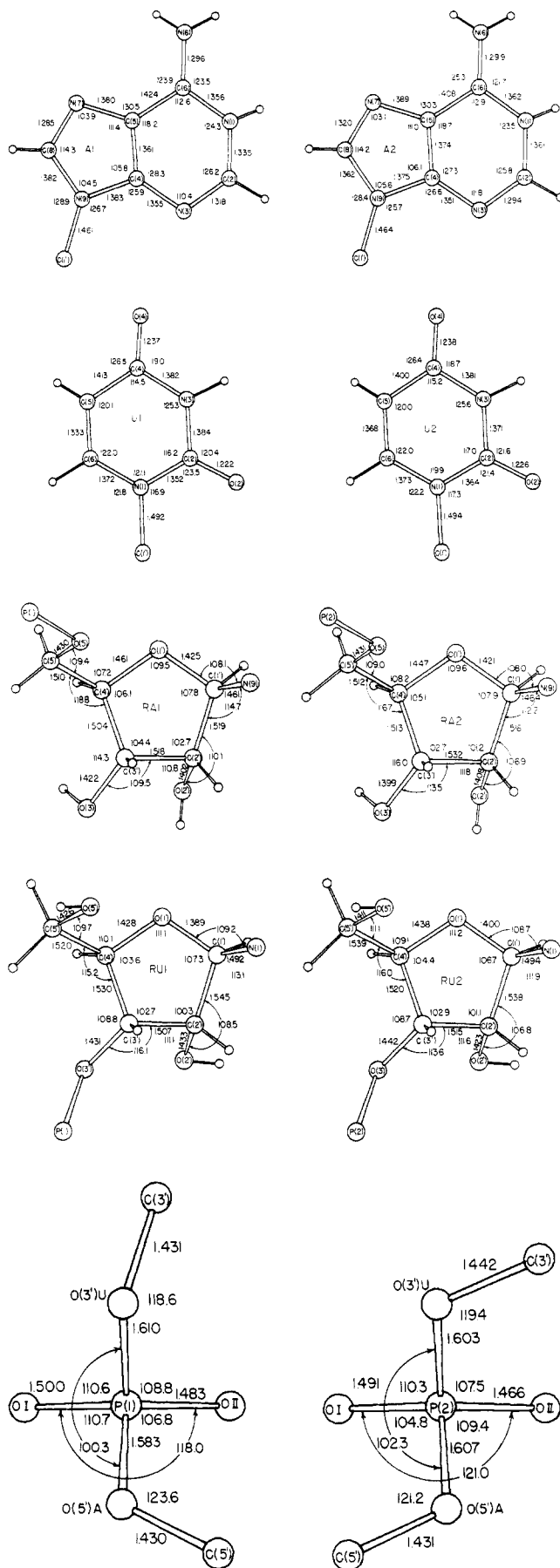


FIGURE 4: The bond distances and bond angles in UpA: (a, upper) adenine and uracil bases, (b, middle) adenosine and uridine riboses, and (c, lower) phosphodiester groups.

Table III

Positional and Thermal Parameters of Hydrogen Atoms in UpA<sup>a</sup>

ATOM	X	Y	Z	B
UPA1				
H(1)A	356	1133	683	4.4
H(2)A	426	1119	848	4.5
H(1')A	276	951	500	7.7
H(2')A	276	1073	504	7.3
H(8)A	323	657	723	7.4
H(3)U	279	117	21	4.2
H(5)U	388	-47	291	6.1
H(6)U	449	105	377	4.3
H(1')A	445	784	986	4.1
H(2')A	304	733	1022	4.8
H(3')A	296	567	929	5.4
H(4')A	454	512	1030	4.8
H(5')A	481	433	827	5.8
H(2')U	408	406	883	5.8
H(02')A	350	783	1167	9.4
H(03')A	302	466	1064	9.4
H(1')U	449	342	200	3.2
H(2')U	333	359	313	4.6
H(3')U	382	277	483	4.6
H(4')U	540	392	500	4.5
H(5')U	601	234	571	4.7
H(2')U	518	264	656	7.3
H(02')U	392	483	237	7.0
H(05')U	490	127	630	7.0
UPA2				
H(1)A	196	508	296	4.5
H(2)A	118	524	128	9.8
H(1')A	255	682	503	7.0
H(2')A	265	557	482	8.6
H(8)A	167	989	302	6.0
H(3)U	233	942	762	4.6
H(5)U	64	806	716	9.9
H(6)U	26	980	645	4.6
H(1')A	81	868	13	4.8
H(2')A	-11	886	200	4.7
H(3')A	27	1040	259	4.8
H(4')A	16	1141	4	5.9
H(5')A	51	1257	162	6.0
H(2')U	112	1225	84	6.1
H(02')A	-128	912	42	5.6
H(03')A	-89	1136	107	5.6
H(1')U	85	1197	816	5.8
H(2')U	196	1176	644	2.3
H(3')U	109	1108	487	4.0
H(4')U	-19	1264	535	5.0
H(5')U	-54	1167	397	6.9
H(2')U	-99	1121	492	6.9
H(02')U	167	1375	608	7.5
H(05')U	-50	961	454	7.5
H(1'w)	183	379	925	6.0
H(2'w)	250	317	925	6.0

<sup>a</sup> All hydrogen atom positions were obtained from difference Fourier maps and most were refined (see text). Those not refined are marked by (-). Positional parameters of hydrogen atoms are  $\times 10^3$ .

tially planar (Table VI). In each case the O(4) carbonyl oxygen atom is displaced more than O(2). The C(1') substituents show the largest displacements which are on the same side of the plane as those of the O(4) atoms.

**Ribose Puckering.** The displacements of atoms from the least-squares planes of the five-membered sugar rings are given in Table VII. In all four cases the C(3') atoms are displaced most followed by the C(2') atoms. The C(3') atoms are displaced 0.456 (RA1), 0.584 (RU1), 0.555 (RA2), and 0.555 Å (RU2) from the best four-atom planes on the same side as C(5'), and the C(2')'s 0.453, 0.540, 0.519, and 0.536 Å, respectively, from the next best four-atom planes on the opposite side as C(5'). Hence the puckering of the four ribose rings may be described as a twist conformation  $^3T_2$  (C(3')-

TABLE IV: A Summary of the Estimated Standard Derivatives in the Base, Ribose, and Phosphate of UpA1 and UpA2.<sup>a</sup>

		$\sigma$ (Å)		$\sigma$ (deg)
		Range	Av	
Base	A1	0.006-0.008	0.006	0.4
	U1	0.006-0.008	0.007	0.4
	A2	0.006-0.007	0.006	0.4
	U2	0.007-0.011	0.008	0.4
Ribose	RA1	0.006-0.008	0.007	0.4
	RU1	0.006-0.007	0.007	0.4
	RA2	0.006-0.008	0.007	0.4
	RU2	0.006-0.010	0.007	0.4
Phosphate	P1		0.004	0.2
	P2		0.004	0.2

<sup>a</sup> A summary of bond distances and angles involving hydrogen atoms is presented in Table V.

TABLE V: Bond Distances and Angles Involving the Hydrogen Atom.<sup>a</sup>

Bond Distances	Mean	Range	No. of Values
C <sub>sp<sup>2</sup></sub> -H	0.95	0.86-1.06	8
C <sub>sp<sup>3</sup></sub> -H	1.07	0.65-1.18	24
N-H	0.89	0.77-1.08	8
O-H	0.97	0.76-1.21	8
O <sub>w</sub> -H	1.01	0.97-1.05	2
Angle			
C <sub>sp<sup>2</sup></sub> -C <sub>sp<sup>2</sup></sub> -H	120	114-125	6
N-C <sub>sp<sup>2</sup></sub> -H	119	114-130	10
C <sub>sp<sup>3</sup></sub> -C <sub>sp<sup>3</sup></sub> -H	108	95-124	36
O-C <sub>sp<sup>3</sup></sub> -H	111	104-119	8
N-C <sub>sp<sup>3</sup></sub> -H	103	95-109	4
C <sub>sp<sup>2</sup></sub> -N-H	117	105-123	12
C <sub>sp<sup>3</sup></sub> -O-H	105	93-118	8
H-O <sub>w</sub> -H	114		1

<sup>a</sup> The average esd's in the distances and angles are about 0.08 Å and 3°, respectively.

endo,C(2')-exo) which is one of the preferred modes of puckering of ribose rings (Sundaralingam, 1969). The torsion angles about the ribose ring bonds and other conformational angles involving the ribose are given in Table VIII. These angles can be reduced to the pseudorotation parameters, the phase angle of pseudorotation and the amplitude of puckering ( $P, \tau_m$ ): RA1 (1.9, 31.3°), RA2 (8.6, 36.9°), RU1 (11.4, 38.6°), and RU2 (5.6, 38.7°); all these values fall within the ranges found for the purine and pyrimidine nucleosides and nucleotides (Altona and Sundaralingam, 1972). The conformation about the C(4')-C(5') bond is gauche, gauche in all four sugars (Table VIII) (Sundaralingam, 1965, 1969; Shefter and Trueblood, 1965); this is one of the preferred conformations for the nucleosides and the only conformation found for the

TABLE VI: Deviations of Atoms from the Least-Squares Planes of the Bases.

Atoms	A1	A2	Atoms	U1	U2
N(1)	-0.025 <sup>a</sup>	-0.028 <sup>a</sup>	N(1)	0.005 <sup>a</sup>	0.007 <sup>a</sup>
C(2)	-0.007 <sup>a</sup>	0.017 <sup>a</sup>	C(2)	0.007 <sup>a</sup>	-0.006 <sup>a</sup>
N(3)	0.020 <sup>a</sup>	0.003 <sup>a</sup>	N(3)	-0.020 <sup>a</sup>	0.013 <sup>a</sup>
C(4)	0.015 <sup>a</sup>	0.017 <sup>a</sup>	C(4)	0.021 <sup>a</sup>	-0.019 <sup>a</sup>
C(5)	0.018 <sup>a</sup>	0.020 <sup>a</sup>	C(5)	-0.010 <sup>a</sup>	0.021 <sup>a</sup>
C(6)	-0.001 <sup>a</sup>	-0.010 <sup>a</sup>	C(6)	-0.003 <sup>a</sup>	-0.015 <sup>a</sup>
N(7)	0.024 <sup>a</sup>	0.019 <sup>a</sup>	O(2)	0.025	-0.025
C(8)	-0.033 <sup>a</sup>	-0.015 <sup>a</sup>	O(4)	0.063	-0.093
N(9)	0.012 <sup>a</sup>	-0.023 <sup>a</sup>	C(1')	0.133	-0.146
N(6)	0.002	-0.094	O(1')	0.075	-0.536
C(1')	-0.021	-0.185			
O(1')	0.972	0.457			
rms $\Delta$	0.020	0.018		0.013	0.015
$\sigma$ (rms $\Delta$ )	0.004	0.005		0.005	0.006

<sup>a</sup> Atoms included in calculation of plane. Equations of the planes: A1,  $0.888X - 0.128Y - 0.441Z = -0.541$ ; A2,  $0.860X + 0.019Y - 0.510Z = 0.878$ ; U1,  $0.857X - 0.156Y - 0.491Z = 3.862$ ; U2,  $0.693X - 0.067Y - 0.718Z = -6.200$ .

TABLE VII: Deviations of the Atoms (Å) from the Least-Squares Planes of the Riboses.

Atoms	RA1	RU1	RA2	RU2
O(1')	0.005 <sup>a</sup>	-0.041 <sup>a</sup>	-0.031 <sup>a</sup>	0.020 <sup>a</sup>
C(1')	0.108 <sup>a</sup>	-0.104 <sup>a</sup>	-0.109 <sup>a</sup>	0.118 <sup>a</sup>
C(2')	-0.178 <sup>a</sup>	0.209 <sup>a</sup>	0.203 <sup>a</sup>	-0.210 <sup>a</sup>
C(3')	0.184 <sup>a</sup>	-0.239 <sup>a</sup>	-0.225 <sup>a</sup>	0.226 <sup>a</sup>
C(4')	-0.119 <sup>a</sup>	0.175	0.161 <sup>a</sup>	-0.153 <sup>a</sup>
C(5')	0.737	-0.609	-0.634	0.686
rms $\Delta$	0.135	0.170	0.161	0.163
$\sigma$ (rms $\Delta$ )	0.005	0.005	0.005	0.006

<sup>a</sup> Atoms included in calculation of plane. Equation of the planes: RA1,  $-0.518X + 0.003Y - 0.855Z = -12.106$ ; RU2,  $0.422X + 0.897Y - 0.135Z = 6.055$ ; RA2,  $-0.478X + 0.035Y - 0.878Z = -0.908$ ; RU1,  $0.214X - 0.968Y - 0.133Z = -15.055$ .

TABLE VIII: Torsion Angles in the Riboses.

Torsion Angle	RA1 (deg)	RU1 (deg)	RA2 (deg)	RU2 (deg)
$\tau_0$	9.0	4.7	6.5	8.2
$\tau_1$	-24.9	-26.7	-26.6	-28.0
$\tau_2$	30.7	36.9	35.7	40.2
$\tau_3$	-26.3	-35.6	-33.1	-32.5
$\tau_4$	10.9	19.2	17.0	15.3
$\phi_{oo}$	-67.8	-62.7	-62.3	-67.5
$\phi_{oc}$	52.3	54.0	55.9	49.9



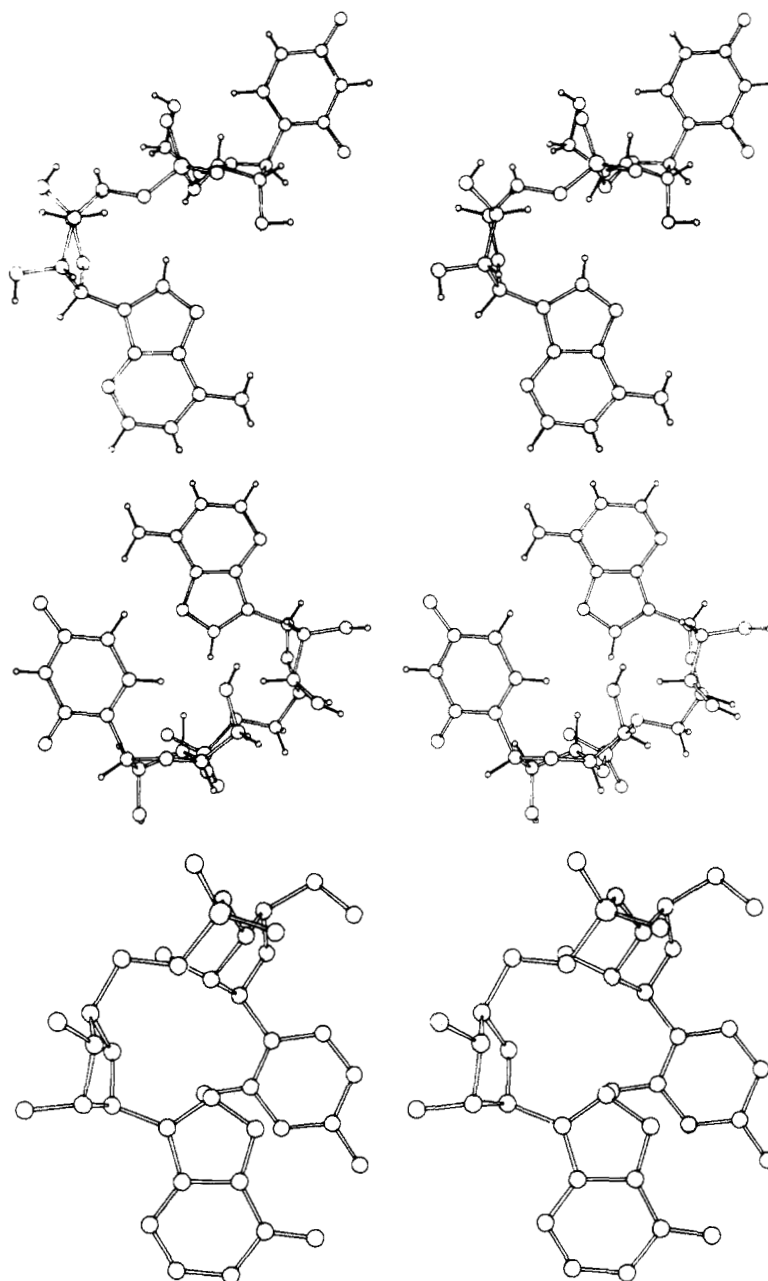


FIGURE 5: Stereoscopic views of the molecular conformations of (a, upper) UpA1, (b, middle) UpA2, and (c, lower) corresponding dinucleoside phosphate of RNA-11, all viewed normal to the adenine plane.

known 5'-nucleotides and polynucleotides (Sundaralingam, 1972a,b).

**Glycosyl Torsion Angles.** The torsion angles  $\chi_{CN}$  (Sundaralingam, 1969) about the glycosyl bond N(9)–C(1') in the adenosine portions of UpA1 and UpA2 are 48.7 and 35.5°, respectively, and correspond to the anti (Donohue and Trueblood, 1960) orientation of the sugar relative to the base (Table IX). These values lie within the range of values observed in  $\beta$ -purine nucleosides and nucleotides (Sundaralingam, 1969). The dihedral angle between the five-atom least-squares plane calculated for the ribose and the nine-atom least-squares plane calculated for the adenine base is 94.8° for RA1 and A1, and 87.8° for RA2 and A2. The  $\chi_{CN}$  torsion angles about the N(1)–C(1') bond in the two uridine moieties of UpA1 and UpA2 are 20.3 and 10.3°, respectively, and correspond to the preferred anti conforma-

tion exhibited by  $\beta$ -pyrimidine nucleosides and nucleotides having C(3')-endo ribose puckering (Sundaralingam, 1969). The dihedral angle between the pyrimidine base and the ribose is 73.3° for U1 and RU1, and 72.0° for U2 and RU2. It is of interest to note that on the average the glycosyl torsion angles of the two uridine moieties are 27° smaller than those of the adenosine moieties. This difference may be due to the difference in the bases and/or to the relative position of the bases with respect to the phosphodiester group, *i.e.*, on the 3' or 5' side.

**Conformational Rigidity of the Nucleotide.** Each UpA molecule may be considered as made up of two nucleotides, a 5'-AMP moiety and a 3'-UMP moiety, each sharing the same phosphate group. Accordingly they can be compared to the available data on these two classes of mononucleotides (Sundaralingam, 1969, 1972).

TABLE IX: A Comparison of Torsion Angles of UpA, Mononucleotides, and Polynucleotides.<sup>a</sup>

Bond	Conformation	UpA			5'-Nucleotides		3'-Nucleotides		Polynucleotides	
		UpA 1	UpA 2	Av	Range	Av	Range	Av	Range	Av
C(1')-N	C(3')-endo	48.7(A),20.0(U)	35.5(A),10.3(U)	28	12 to 44	30	4 to 28	16	-1 to 17	11
	C(2')-endo	-	-		34 to 46	41	39 to 45	42	73 to 81	77
P-O(5') <sup>c</sup>	g <sup>+</sup>	-	83.6		64 to 72	69		61	-	-
	t	-	-	b		-166		-127	-	-
	g <sup>-</sup>	-87.8	-		-52 to -80	-68	-65 to -75	-70	-42 to -48	66
O(5')-C(5')	one minimum	-168.1	-157.7	-163	149 to -136	-173	-		143 to -162	-179
C(5')-C(4')	gg	52.3(A),54.0(U)	55.9(A),49.9(U)	53	40 to 66	54	43 to 50	46	34 to 69	53
	gt	-	-		-			171	-	
	tg	-	-		-		-	-	-	
C(4')-C(3')	C(3')-endo	93.4(A),90.5(U)	82.7(A),86.8(U)	88	75 to 87	82	82 to 84	83	78 to 83	80
	C(2') endo	-	-		145 to 153	150	144 to 152	149	141 to 145	143
C(3')-O(3')	one minimum	-136.5	-158.0	-147	-		-91 to -123	-114	170 to -144	-161
O(3')-P <sup>c</sup>	g <sup>+</sup>	-	84.0		54 to 92	74		68	-	
	t	162.7	-	b		-153		171		-148
	g <sup>-</sup>	-	-			-69	-63 to -64	-64	-58 to -103	-69

<sup>a</sup> The torsion angles for the 3'- and 5'-nucleotides are from Sundaralingam(1969) and from the following structures which have been completed since: guanosine 5'-phosphate trihydrate(Murayama et al.,1969), deoxycytidine 5'-phosphate monohydrate(Viswamitra et al.,1971),adenosine triphosphate trihydrate(Kennard et al.,1971), rubidium adenosine 5'-di phosphate(F.M.Muller, private communication) and disodium uridine 3'-phosphate tetrahydrate(Viswamitra et al.,1972). The torsion angles for the polynucleotides are from Sundaralingam(1969) and Arnott(1970).<sup>b</sup>Whenever the conformations are separated by an energy barrier the average is not pertinent. <sup>c</sup>The P-O(5') and P-O(3') angles for the 5'- and 3'- nucleotides involve the acid proton attached to the phosphate group. The latter angles are given here to show that they are pertinent to the understanding of the actual phosphodiester conformations(Sundaralingam,1969, 1972).

**5'-AMP Moiety.** A comparison of the torsion angles of the 5'-AMP moieties of UpA1 and UpA2 indicate that they have similar conformations. The glycosyl torsion angles are in the anti range, the conformation about the C(4')-C(5') bonds are gauche,gauche and the sugars exhibit C(3')-endo,-C(2')-exo (<sup>3</sup>T<sub>2</sub>) puckering. Similarly, the known 5'-nucleotides show exclusive preference for the anti conformation about the glycosyl bond and the gg conformation about the C(4'),C(5') bond, together with either C(3')-endo or C(2')-endo sugar puckering (Table IX) (Sundaralingam, 1969; Rao and Sundaralingam, 1970; Sundaralingam and Abola, 1972c,d).

**3'-UMP Moiety.** The conformations of the two 3'-UMP residues of UpA are also very similar, with only small differences being observed in the various torsion angles. The largest difference (21°) occurs in the conformation about the C(3')-O(3') bonds and appears to be correlated with the difference in conformation about the ester P-O(3') bonds (see below). As in the 5'-AMP residues, the glycosyl torsion angles are anti, the conformations about C(4')-C(5') bonds are gg, and the sugars exhibit C(3')-endo,-C(2')-exo (<sup>3</sup>T<sub>2</sub>) puckering. A comparison with other 3'-nucleotides (Table IX) indicates that these nucleotides also prefer the anti glycosyl conformation, the gg conformation about C(4'),C(5') bond and either C(3')-endo or C(2')-endo sugar puckering. However, 3'-nucleotides appear to have more conformational flexibility about the C(4')-C(5') bond as evidenced by the gauche,trans conformation about C(4')-

C(5') bond as, for example, in 3'-AMP (Sundaralingam, 1966). Nucleosides have not been shown here. They are found to exhibit all three staggered conformations about the C(4')-C(5') bond, gg, gt and tg, with a preference for the gg conformation (Sundaralingam, 1965, 1969; Shefter and Trueblood, 1965). In addition, the nucleosides occur in anti and syn conformation about the glycosyl bond with the pyrimidines showing a strong preference for the anti conformation.

**Conformation of the Phosphodiester Groups.** It is through the phosphodiester group that the 3'-uracil and 5'-adenosine moieties are linked to form UpA. The torsion angles about the P-O(5') bonds in UpA1 and UpA2 are -87.8 and 83.6°, respectively, while those about the P-O(3') bonds are 162.7 and 84.0°, respectively (Table IX). Thus, the phosphodiester conformation is (-)gauche,trans in UpA1 and (+)gauche,-(+)gauche in UpA2. The important observation that the four nucleotide moieties of UpA have similar conformations has already been noted (Rubin et al., 1971; Seeman et al., 1971). The major differences, therefore, in the conformation of the two UpA molecules are in the torsion angles about both P-O ester bonds (Figure 6). Likewise UpA1 and UpA2 differ from helical polynucleotides principally in the phosphodiester conformation which is (-)gauche,(-)gauche in the latter. Although the conformation about the P-O(5') bond in UpA1 is (-)gauche as in the helical polynucleotides, the conformation about the P-O(3') bond is trans and differs by 86° from the average value of the corresponding torsion

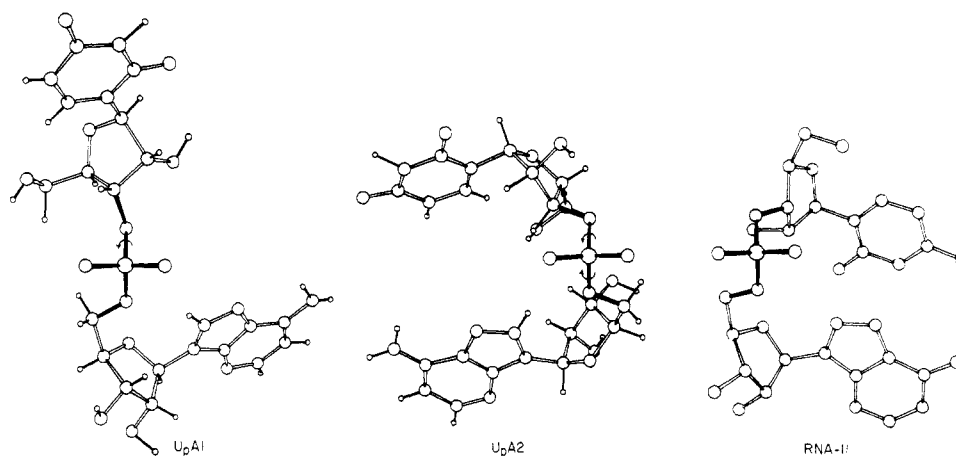


FIGURE 6: A comparison of the conformations of the phosphodiester group in (a, left) UpA1, (–) gauche, trans; (b, middle) UpA2, (+) gauche, (+) gauche, (c, right) RNA-11, (–) gauche, (–) gauche (see text). A view along the intersection of the O–P–O planes.

TABLE X: Hydrogen-Bond Distances and Angles.<sup>a</sup>

A–H···B	Symmetry Code for B	A			
		A–H	H···B	A···B	A–H–B (deg)
N(1)A1–H···O(II)1	I <sup>b</sup>	0.77	1.91	2.615	153
N(1)A2–H···O(I)2	II	0.80	1.85	2.590	152
N(6)A1–H(1)···N(7)A2	III	0.83	2.23	2.938	144
N(6)A2–H(1)···N(7)A1	III	0.82	2.20	2.983	159
N(6)A1–H(2)···O(II)2	III	0.82	1.91	2.713	165
N(6)A2–H(2)···O(I)1	III	0.98	1.90	2.803	151
N(3)U1–H···O(2)U2	IV	1.00	1.83	2.825	174
N(3)U2–H···O(4)U1	V	1.08	1.84	2.910	172
O(2')RA1–H···O(4)U1	V	1.21	2.05	3.107	144
O(2')RA2–H···O <sub>w</sub>	IV	1.17	1.60	2.742	165
O(3')RA1–H <sup>c</sup> ···O(2)U1	III	0.76	2.30	2.834	128
O(3')RA1–H <sup>c</sup> ···O <sub>w</sub>	III	0.76	2.49	3.194	155
O(3')RA2–H···O(4)U2	VI	1.00	2.27	2.826	114
O(2')RU1–H···O(3')RA1	IV	1.03	1.90	2.857	152
O(2')RU2–H···O(I)1	I	0.94	1.80	2.635	150
O(5')RU1–H···O(2')RU1	VII	0.85	2.43	2.750	103
O(5')RU2–H···O(2')RU2	VIII	0.85	2.29	3.096	157
C–H···O Hydrogen Bonds					
C(2)A2–H···O(2')A2	IX	0.96	2.25	3.181	163
C(8)A1–H···O(5')A1	d	0.86	2.31	3.100	152
C(8)A2–H···O(5')A2	d	0.89	2.32	3.092	145
C(6)U1–H···O(5')U1	d	1.03	2.17	3.162	160
C(6)U2–H···O(5')U2	d	1.06	2.13	3.101	161

<sup>a</sup> Symmetry code: I,  $x, 1 + y, z$ ; II,  $x, -1 + y, z$ ; III,  $x, y, z$ ; IV,  $x, -1 + y, -1 + z$ ; V,  $x, 1 + y, 1 + z$ ; VI,  $-x, 1/2 + y, 1 - z$ ; VII,  $1 - x, -1/2 + y, 1 - z$ ; VIII,  $-x, -1/2 + y, 1 - z$ ; IX,  $-x, -1/2 + y, -z$ . <sup>b</sup> Hydrogen-bonding scheme involving water molecule is shown in figure. <sup>c</sup> This hydrogen atom is involved in a possible bifurcated hydrogen bond with O(2)U1 and O<sub>w</sub>. <sup>d</sup> Intramolecular hydrogen bond.

angle of the polynucleotides (Table IX). On the other hand, the conformations about the P–O(5') and P–O(3') bonds in UpA2 are both (+)gauche and differ by 149 and 161°, respectively, from the corresponding average values of the helical polynucleotides.

We have seen that as we proceed from nucleosides to 3'-nucleotides to 5'-nucleotides the degree of conformational

freedom becomes more and more restricted. The structure analysis of UpA has provided additional support for our earlier findings that the preferred conformation of the 5'-nucleotides can be carried over to the di-, oligo-, and polynucleotides. The structure of UpA further demonstrates that the nucleotide unit can be regarded as conformationally "rigid" and that the principal flexibility in the polynucleo-

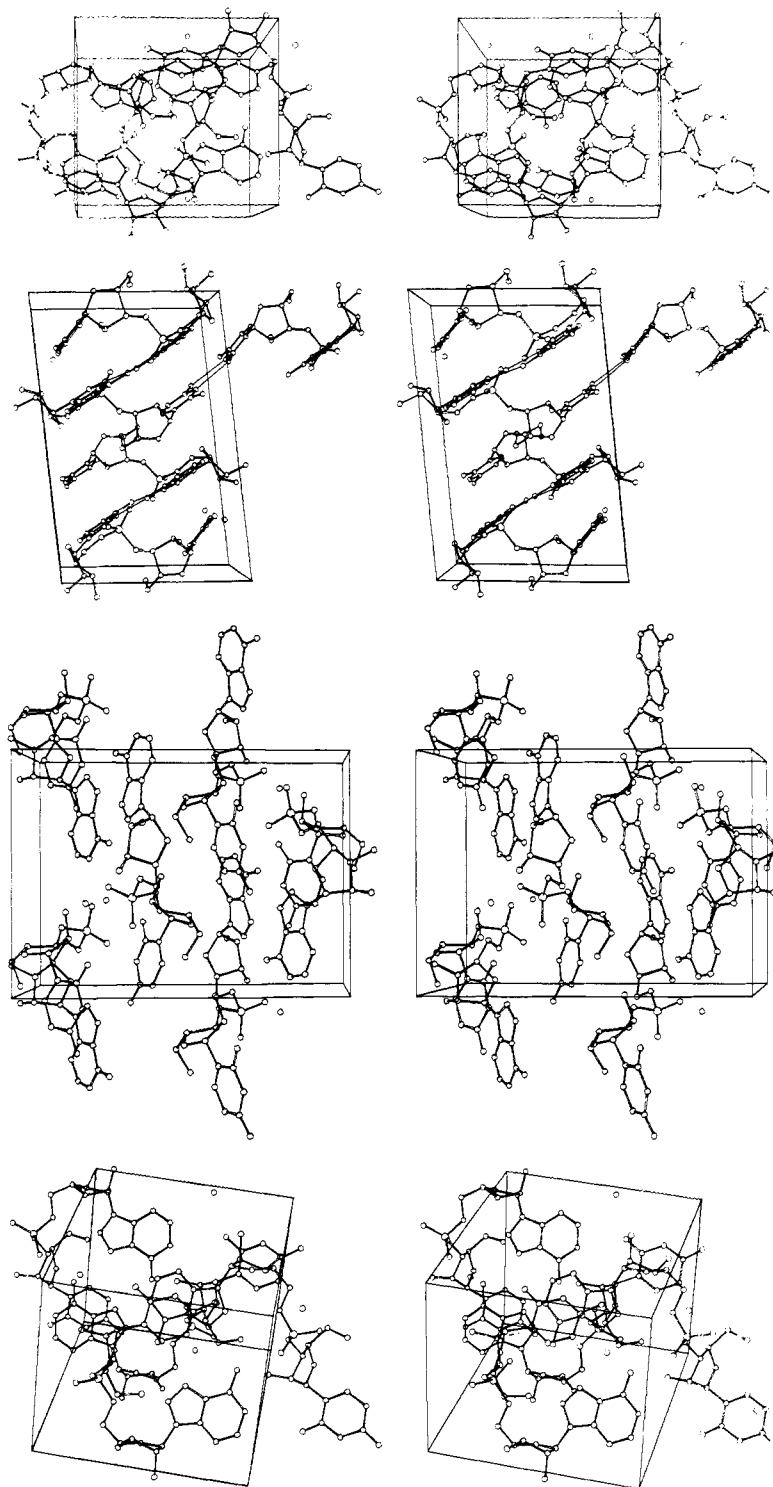


FIGURE 7: Stereoscopic views of the molecular packing as viewed along the (a, top)  $a$  axis, (b, upper middle)  $b$  axis, (c, lower middle)  $c$  axis, and (d, bottom) normal to the 402 crystal planes.

tide chain resides in the P-O ester bond<sup>1</sup> and the C(3')-C(4') bond of the sugar.

**Hydrogen Bonding.** The overall crystal structure is best described as made up of alternate sheets of self-paired uracil

<sup>1</sup> It is worthwhile to mention that similar rotations about the P-O ester bonds of the phospholipids would also be largely responsible for the conformational changes of the phospholipids of both biological membranes and phospholipid bilayers (Sundaralingam, 1971b).

and adenine bases lying in the  $\bar{4}02$  planes. This explains why the  $\bar{4}0,2$  reflection has the largest normalized structure amplitude,  $|E_0|$ . The intersheet separation is about 3.4 Å. Various views of the unit cell showing the molecular packing are given in Figure 7. Although we were looking with a watchful eye for the Watson-Crick A-U complementary pairing of the bases, it turned out that there was only self-pairing of the bases A-A and U-U. The protonation of the adenine base at N(1) does not allow the Watson-Crick A-U pairing scheme,

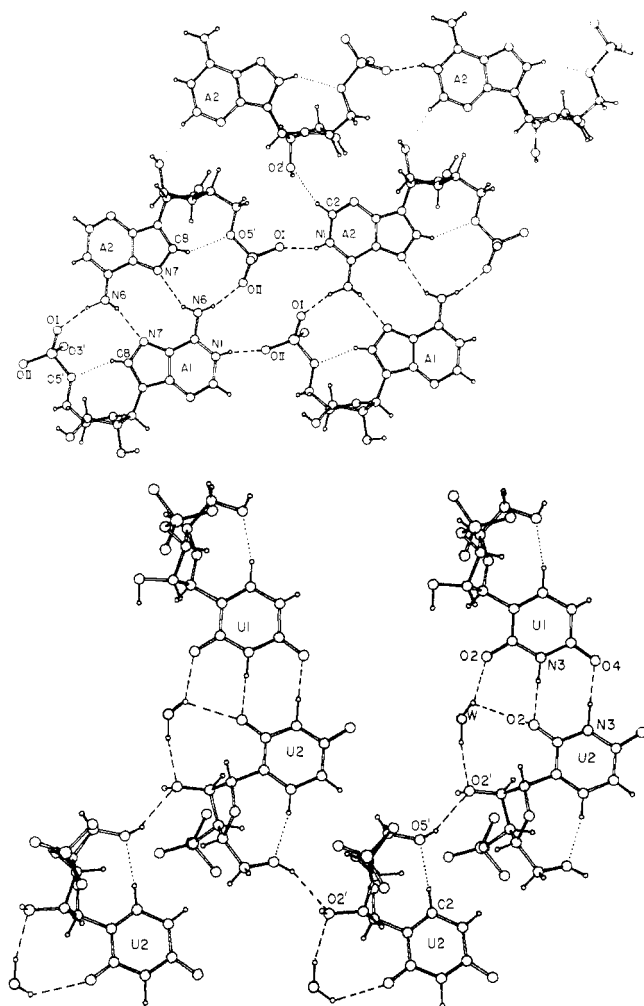


FIGURE 8: The intralayer hydrogen bonding in (a, upper) adenine sheets and (b, lower) uracil sheets. The intra- and intermolecular C-H...O hydrogen bonds are shown by dotted lines.

although the self-pairing of the adenine bases can occur even when N(1) is not protonated as, for example, in the adenine derivative-barbiturate structure (Kim and Rich, 1968). The distances and angles involving the hydrogen bonds in UpA are given in Table X. The distribution of the donor and acceptor hydrogen bonds for each atom of UpA1 and UpA2 is given in Table XI.

**Self-Pairing of the Adenines in the Adenine Sheets.** In the adenine planes each A1 base is paired with an A2 base by means of two N(6)-H...N(7) hydrogen bonds. The A1-A2 self-pair is further stabilized by two N(6)-H...O-P hydrogen bonds. The adenine pairs are interconnected by way of N(1)A1-H...O(II)-P(1) and N(1)A2-H...O(I)-P(2) hydrogen bonds and C(2)A2-H...O(2')A2 interactions resulting in infinite sheets of hydrogen-bonded adenosine residues (Figure 8). The former hydrogen bonds involving protonated N(1) atoms and phosphate oxygen atoms are characteristic of nucleotide structures and no doubt owe their strength to the ionic character of the donor and acceptor atoms (Sundaralingam, 1966; Sundaralingam and Abola, 1972c).

**A-A Self-Pair Has a Diad Axis.** The geometry of the A-A pair is such that the adenine ribophosphate portions are related by a pseudodiad axis with an average deviation of 0.03 Å from true diad symmetry. The adenosine self-pairing found in UpA is very similar to that proposed for poly(A)

TABLE XI: A Comparison of the Distribution of Donor and Acceptor Hydrogen Bonds in UpA1 and UpA2.<sup>a</sup>

Atom	No. of Donor H Bonds		No. of Acceptor H Bonds		Total No. of H Bonds	
	UpA1	UpA2	UpA1	UpA2	UpA1	UpA2
N(1)A	1	1	0	0	1	1
N(3)A	0	0	0	0	0	0
N(6)A	2	2	0	0	2	2
N(7)A	0	0	1	1	1	1
N(3)U	1	1	0	0	1	1
O(I)	0	0	2	1	2	1
O(II)	0	0	1	1	1	1
O(2)U	0	0	2 <sup>b</sup>	2 <sup>b</sup>	2	2
O(4)U	0	0	2	1	2	1
O(2')RA	1	1	0	1	1	2
O(3')RA	2 <sup>b</sup>	1	1	0	3	1
O(5')RA	0	0	1	1	1	1
O(2')RU	1	1	1	2	2	3
O(3')RU	0	0	0	0	0	0
O(5')RU	1	1	1	1	2	2
C(2)A	0	1	0	0	0	1
C(8)A	1	1	0	0	1	1
C(6)U	1	1	0	0	1	1
Total	11	11	12	11	23	22

<sup>a</sup> The water molecule is apparently involved in five hydrogen bonds, three donor and two acceptor (see Figure 10).

<sup>b</sup> Including bifurcated hydrogen bonds (see Figure 10).

(Rich *et al.*, 1961). In UpA, however, each C(8) hydrogen atom of the adenine bases is involved in an intramolecular hydrogen bond with the O(5') phosphate oxygen atom; the C(8)...O(5') distances are 3.11 and 3.09 Å, the H...O(5') distances 2.33 and 2.33 Å and the C(8)-H...O(5') angles 152 and 144° in UpA1 and UpA2, respectively. The C(8)...O(5') distance in the poly(A) model is 3.50 Å, indicating that no interaction is present. The presence of the C(8)-H...O(5') intramolecular bond in UpA draws the phosphorus atoms of each pair closer to each other than in the poly(A) model (11.35 *vs.* 11.89 Å). This also results in the adenine bases being inclined at 9.5° with respect to each other as opposed to 20° in the poly(A) model. The inclusion of C(8)-H...O(5') intramolecular hydrogen bonds in a poly(A) model would result in a more compact helical structure. The molecular parameters of the adenosine pairs observed in UpA would provide a more refined model for poly(A). Details of this model will be published elsewhere.

**Self-Pairing of the Uracils in the Uracil Sheets.** The uracil bases also exhibit self-pairing with N(3)-H of U1 hydrogen bonded to O(2) of U2 and N(3)-H of U2 to O(4) of U1 (Figure 8). However, they do not possess a diad axis; instead the uracil moieties are related in an antiparallel fashion with paired bases inclined 16.9° with respect to each other. The U1-U2 pairs are interconnected by way of hydrogen bonds between uridine ribose hydroxyl groups: O(5')RU1-H...O(2')RU1 and O(5')RU2-H...O(2')RU2. The uracil self-pairing found in UpA has not been observed in any previous structure. Although the C(6) hydrogen atoms are involved in intramolecular hydrogen bonds with O(5') atoms, there is no additional hydrogen-bond stabilization

TABLE XII: Inter Base Contacts and the Inter Ribose-Base Contacts Less Than 3.42 Å.

Base-Base		Ribose-Base	
Contacts	Distances (Å)	Contacts	Distances (Å)
U1...A2(I) <sup>a</sup>		RA1...U1(III)	
O(4)U1...C(8)A2	3.087	O(1')RA1...N(1)U1	3.201
O(4)U1...N(9)A2	3.244	C(6)U1	3.412
C(4)U1...C(8)A2	3.384		
		RU1...A1(IV)	
U2...A1(II)		O(1')RU1...N(9)A1	3.301
O(2)U2...N(1)A1	3.337	C(4)A1	3.074
O(2)U2...C(2)A1	3.404	C(8)A1	3.149
N(3)U2...C(4)A1	3.245	C(5)A1	3.163
N(3)U2...C(5)A1	3.283	N(7)A1	3.213
O(4)U2...C(8)A1	3.161		
O(4)U2...N(9)A1	3.348	RA2...U2(V)	
		O(1')RA2...N(3)U2	2.899
		C(2)U2	2.919
		O(2)U2	3.171
		RU2...A2(VI)	
		O(1')RU2...N(3)A2	3.185
		C(4)A2	3.288
		C(2)A2	3.313

<sup>a</sup> Symmetry code: I,  $x, -1 + y, z$ ; II,  $x, y, z$ ; III,  $1 - x, 1/2 + y, 1 - z$ ; IV,  $1 - x, -1/2 + y, 1 - z$ ; V,  $x, y, -1 + z$ ; VI,  $-x, 1/2 + y, 1 - z$ .

through the phosphate groups as in the adenosine self-pairs, perhaps explaining why poly(U) does not form stable double-helical fibers as does poly(A).

**Location and Role of the Water in the Intra- and Interlayer Hydrogen Bonding.** The adenine and uracil layers are cross-linked *via* hydrogen bonds involving ribose O(2') and O(3') hydroxyl hydrogens: O(3')RA1-H...O(2)U1, O(2')RA1-H...O(4)U1, O(2')RU1-H...O(3')RA1, and O(3')RA2-H...O(4)U2. Since the adenine bases and the uracil bases form alternating sheets, it would have been envisaged that the smaller size of the uracil *vs.* the adenine base will create pockets in the uracil sheets. Indeed the water of crystallization was found lodged in the uracil sheets. The water serves to interconnect the adenine and uracil layers. Each water molecule lies in a cavity surrounded by five oxygen atoms with which it makes close contacts (Figure 9). The water donates one of its hydrogen atoms to O(2') of RU2 and accepts one from O(2') of RA2. The other water hydrogen atom is apparently involved in a bifurcated hydrogen bond with the O(2) carbonyl oxygen atoms of U1 and U2. There appears to be a second bifurcated hydrogen bond between the hydrogen atom of O(3')RA1 and the oxygen atoms of O(2)U1 and water.

**C-H...O Interactions.** The C-H...O hydrogen bonding in UpA deserves special mention because of the recurrence and possible importance of such interactions in the chemistry of the genetic material. There are several C-H...O hydrogen bonds observed in UpA, one intermolecular hydrogen bond between C(2)A2-H and O(2')A2, and four intramolecular interactions, each of the latter involving base carbon atoms, C(8) in adenosines and C(6) in uracils, and ribose O(5')

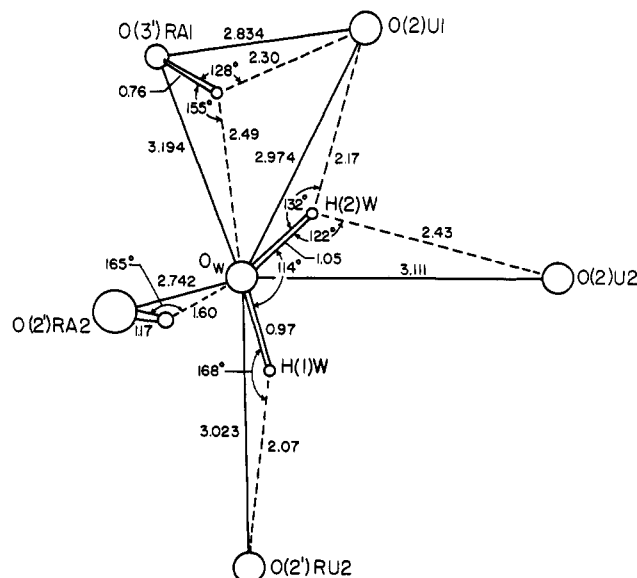


FIGURE 9: A schematic drawing showing the pentacoordination about the water molecule. Oxygen atoms O(2)U1, O(2)U2, and O(2')RU2 are approximately in the same plane as the water molecule, whereas O(2')RA2 and O(3')RA1 atoms are above and below this plane, respectively. It appears that both H(2)W and the hydrogen of O(3')RA1 are involved in bifurcated hydrogen bonds; in each case one of the two hydrogen bonds is weaker than the other. The symmetry code of the atoms are: O(2)U1  $x, y, 1 + z$ ; O(2)U2 and O(2')RU2  $x, -1 + y, z$ ; O(2')RA2  $-x, -1/2 + y, 1 - z$ ; O(3')RA1  $x, y, z$ .

oxygen atoms. In the adenosine moiety of both UpA1 and UpA2, C(8) donates its hydrogen to the O(5')A of the phosphodiester linkage, while in uridine portions of UpA1 and UpA2, C(6) donates its hydrogen to O(5')U. These intramolecular hydrogen bonds exhibit approximately the same dimensions: C...O distances 3.1 Å, H...O distance of about 2.3 Å, and C-H...O angles ranging from 144 to 158 Å (Table IX). Apparently the presence of the electronegative nitrogen atoms N(7) and N(9) on either side of C(8)A and N(1) on one side of C(6)U enhances the electronegativities of these carbon atoms, thereby making them suitable for hydrogen bonding (Sutor, 1963; Sundaralingam, 1966). Such intramolecular C-H...O(5') hydrogen bonds in polynucleotide structures may provide additional stabilization to the preferred anti conformation of the base and the *gauche, gauche* conformation about C(5')-O(5') bond for the 5'-nucleotides (see also Sundaralingam, 1971a). It is conceivable that the C-H...O(5') interactions are enhanced in the 5'-phosphorylated nucleotides as compared to the nucleosides themselves. Although the intramolecular C-H...O hydrogen bonds are expected to be weak, they nevertheless have an overall stabilizing effect on polynucleotide stereochemistry.

**Base-Base and Ribose-Base Stacking Interactions.** Each of the four independent base residues in UpA is involved in two kinds of close interactions: base-base stacking and base-ribose O(1') interactions. The former interactions involve partial overlap of vertically stacked adenine and uracil bases (Figure 10). There are close contacts between the O(4) carbonyl oxygen of U1 and the imidazole ring of an adjacent A2 base and close contacts between the two carbonyl oxygens and the N(3) nitrogen of U2 and the purine ring of an adjacent A1 base (Table XII). The stacked bases are inclined to each other. The dihedral angles are: U1 and A2, 10.1° and U2 and A1, 19.9°. It is interesting to note that neither of the

TABLE XIII: Conformations of the Phosphodiester Group in Dinucleosides and Polynucleotides.<sup>a</sup>

Conformation about the Ester P-O Bond		Fiber Diffraction Investigations <sup>c</sup>							
		Single-Crystal Investigations	W & C-DNA						Poly-(rI·dC)
O(5')-P	P-O(3')	UpA <sup>b</sup>	2',5'-ApU <sup>d</sup> (Shefter <i>et al.</i> , 1969)	(Watson and Crick, 1953)	B-DNA (Langridge <i>et al.</i> , 1960)	A-DNA (Fuller <i>et al.</i> , 1965)	C-DNA (Marvin <i>et al.</i> , 1961)	RNA-11 (Arnott <i>et al.</i> , 1967)	Poly(A) (Rich <i>et al.</i> , 1961)
+g	+g	X2							
+g	t								
+g	-g	e							
t	+g								
t	t								
t	-g			X					
-g	+g	e							
-g	t	X1	X				X		
-g	-g				X	X		X	X

<sup>a</sup> See also Sundaralingam (1969). <sup>b</sup> The crosses indicate the observed conformation. X1 = UpA1, X2 = UpA2. <sup>c</sup> With the possible exception of (Watson and Crick)-DNA which was mainly obtained from model-building studies, all other polynucleotides shown were studied by a combination of model building and X-ray diffraction. <sup>d</sup> This is an unnatural linkage, but given here for comparison. <sup>e</sup> For steric reasons these conformations are disallowed.

N(6) amine nitrogen atoms of A1 or A2 is involved in stacking interactions in contrast to some other adenine nucleotides (Bugg *et al.*, 1971). The characteristically small base-stacking interactions in conjunction with the predominant base-ribose

interactions in protonated bases has been observed previously in a number of nucleotide and nucleoside crystal structures (Sundaralingam, 1968; Bugg *et al.*, 1971). Each ribose O(1') oxygen atom is involved in close contacts with atoms of a "complementary" base, that is, the O(1') atoms of RA1 and RA2 interact with U1 and U2 bases, respectively, while the O(1') atoms of RU1 and RU2 interact with A1 and A2 bases, respectively. A summary of these ribose O(1')-base contacts, many of which are close to or less than the sum of the van der Waals radii of the atoms involved, is given in Table XII. Overall, the base interactions in UpA are such that each base is involved in a stacking interaction with a base, on one side, and a ribose O(1') atom, on the other.

## Conclusion

The concept of a rigid nucleotide unit developed here and elsewhere (Sundaralingam, 1972b) makes a turning point in the history of nucleic acid structural research. This concept, which parallels the development of the rigid peptide unit (Pauling and Corey, 1953), is expected to have far-reaching consequences in understanding conformations of polynucleotides. The rigidity of the nucleotide unit combined with the rotational flexibility of the P-O bonds permits a specific and simple description of unwinding of double-helical nucleic acids, for example, during denaturation or replication. Furthermore, the tertiary folding of polynucleotide chains and the formation of loop regions in molecules such as tRNA or rRNA are also most probably achieved by rotations about the P-O ester bonds. A consideration of only the three possible staggered conformations about each P-O bond would provide theoretically nine possible conformations for the phosphodiester group (Table XIII). The (-)gauche, (-)gauche conformation is by far the most preferred, and it is found in practically all of the known double-helical polynucleotides (Sundaralingam, 1969). The only other conformation for the phosphodiester group that is

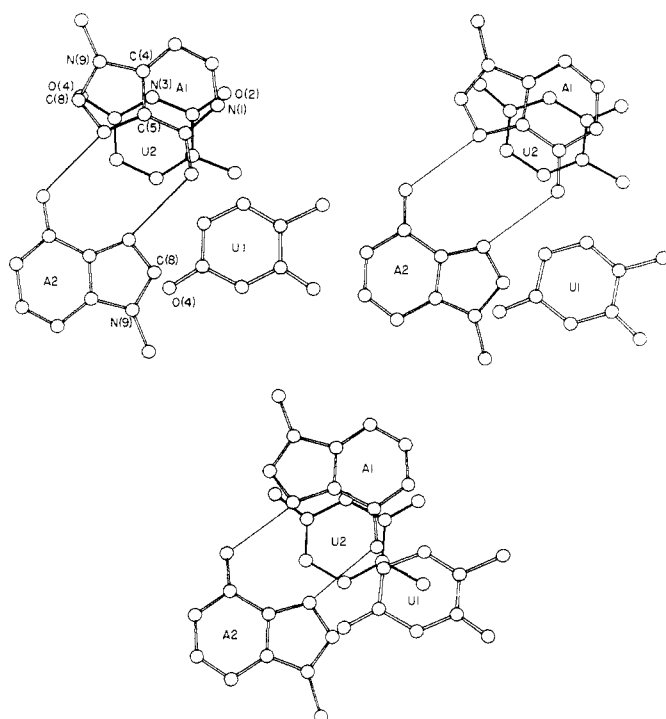


FIGURE 10: The base-base interactions as viewed normal to (a) the plane through the A1-A2 self-pair, (b) the U1 base, and (c) the U2 base. The U1 base lies above the A1-A2 base pair and U2 below. Note that there is essentially no base overlap between U1 and A2, whereas between A1 and U2 there is considerable overlap. Close contact distances are given in Table XI.

found in a helical polynucleotide is  $(-)$ gauche,trans, e.g., C-DNA (Table XIII). It is interesting that the latter conformation is similar to that of UpA1. In contrast, UpA2 exhibits the conformation  $(+)$ gauche, $(+)$ gauche which cannot form helical structures, but rather it would lend itself to the formation of abrupt turns in a polynucleotide chain. For example, the anticodon loops in tRNAs can be formed if the dinucleoside containing the "wobble" base (Crick, 1966) and the base adjacent to it on the 5' side occurs in the UpA2 conformation. Similarly, a stereochemically plausible anticodon loop can be constructed with the dinucleoside containing the anticodon base on the 3' side and the base adjacent to it occurring in the UpA2 conformation. The main difference between our model of the anticodon and that given by Fuller and Hodgson (1967) and Levitt (1969) is that in our model the other nucleotides in the anticodon loop occur in the preferred rigid conformation described above, while Fuller and Hodgson and Levitt have used less preferred conformations for some of the loop nucleotides (see also Sundaralingam, 1972a,b).

In addition to double-helical conformations the conformations of single-stranded regions of nucleic acids will also be of importance in understanding the complex protein-nucleic acid interactions involved in several biological processes. There are many nucleic acid enzyme mechanisms which involve the direct recognition and binding of single-stranded regions of nucleic acids by protein molecules. The present study has allowed the prediction of specific conformations for localized regions of single-stranded nucleic acids. Such regions would exhibit a unique disposition of stereospecific binding sites which could be recognized by the tertiary structure of the enzyme (Rubin *et al.*, 1971; Crick, 1971; Beddell and Sundaralingam, 1972).<sup>2</sup> For example, the open structure of UpA1 could fit into the active-site cleft of an enzyme exposing a maximum number of potential hydrogen-bonding sites on the base for interaction with the protein side chains and main chain. However, many of the base sites would not be available for hydrogen binding if the single-stranded regions of the nucleic acid existed in a helical conformation with stacked bases (Beddell and Sundaralingam, 1972).<sup>2</sup> Some evidence for the unstacked conformation of the single-stranded regions of the nucleic acids in nucleic acid-protein complexes comes from X-ray crystallographic studies of substrate binding to enzymes. For instance, the 5'-methylene analog of UpA is bound in the active site of ribonuclease in an open conformation (Richards, 1971) similar to the UpA1 structure.

## References

- Altona, C., and Sundaralingam, M. (1972), *J. Amer. Chem. Soc.* (in press).
- Arnott, S. (1970), *Progr. Biophys. Mol. Biol.* 21, 265.
- Arnott, S., Wilkins, M. H. F., Fuller, W., Langridge, R. (1967), *J. Mol. Biol.* 27, 535.
- Brown, D. M., and Todd, A. R. (1952), *J. Chem. Soc.*, 52.
- Bugg, C. E., Thomas, J., Sundaralingam, M., and Rao, S. T. (1971), *Biopolymers* 10, 175.
- Crick, F. H. C. (1966), *J. Mol. Biol.* 19, 548.
- Crick, F. H. C. (1971), *Nature (London)* 234, 25.
- Crick, F. H. C., and Watson, J. D. (1954), *Proc. Roy. Soc., Ser. A* 223, 80.
- Cromer, D. T., and Waber, J. T. (1965), *Acta Crystallogr.* 18, 104.
- Dekker, C. R., Michelson, A. M., and Todd, A. R. (1953), *J. Chem. Soc.*, 947.
- Donohue, J., and Trueblood, K. N. (1960), *J. Mol. Biol.* 2, 363.
- Fuller, W., and Hodgson, A. (1967), *Nature (London)* 215, 817.
- Fuller, W., Wilkins, M. H. F., Wilson, H. R., and Hamilton, L. D. (1965), *J. Mol. Biol.* 12, 60.
- Hughes, E. W. (1941), *J. Amer. Chem. Soc.* 63, 1737.
- Johnson, C. K. (1965), Oak Ridge National Laboratory Report, ORNL-3794, Oak Ridge, Tenn.
- Karle, J., and Hauptman, H. (1956), *Acta Crystallogr.* 2, 635.
- Kennard, O., Isaacs, N. W., Motherwell, W. D. S., Coppola, J. C., Wampler, D. L., Larson, G. C., Watson, D. G. (1971), *Proc. Roy. Soc., Ser. A* 325, 401.
- Kim, S. H., and Rich, A. (1968), *Proc. Nat. Acad. Sci. U. S.* 60, 402.
- Langridge, R., Marvin, D. A., Seeds, W. E., Wilson, H. R., Harper, C. W., Wilkins, M. H. F., and Hamilton, L. D. (1960), *J. Mol. Biol.* 2, 38.
- Levitt, M. (1969), *Nature (London)* 224, 759.
- Lin, G. H.-Y., Sundaralingam, M., and Arora, S. K. (1971), *J. Amer. Chem. Soc.* 93, 1235.
- Marvin, D. A., Spencer, M., Wilkins, M. H. F., and Hamilton, L. D. (1961), *J. Mol. Biol.* 3, 547.
- Murayama, W., Nagashima, N., and Shimizu, Y. (1969), *Acta Crystallogr., Sect. B* 25, 2236.
- O'Brien, E. J., and McEvan, A. W. (1970), *J. Mol. Biol.* 48, 243.
- Pauling, L., and Corey, R. B. (1953), *Proc. Roy. Soc., Ser. B* 141, 21.
- Rao, S. T., and Sundaralingam, M. (1970), *J. Amer. Chem. Soc.* 92, 4963.
- Rich, A., Davies, D. R., Crick, F. H. C., and Watson, J. D. (1961), *J. Mol. Biol.* 3, 71.
- Richards, F. M. (1971), *Cold Spring Harbor Symp. Quant. Biol.* 36.
- Rubin, J., Brennan, T., and Sundaralingam, M. (1971), *Science* 174, 1020.
- Seeman, N. C., Sussman, J. L., Berman, H. M., and Kim, S. H. (1971), *Nature (London) New Biol.* 223, 90.
- Shefter, E., Barlow, M., Sparks, R. A., and Trueblood, K. N. (1969), *Acta Crystallogr., Sect. B* 25, 895.
- Shefter, E., and Trueblood, K. N. (1965), *Acta Crystallogr.* 18, 1067.
- Stewart, R. F., Davidson, E. R., and Simpson, W. T. (1965), *J. Chem. Phys.* 42, 3175.
- Stout, G. H., and Jensen, L. H. (1968), X-Ray Structure Determination, New York, N. Y., The Macmillan Co., pp 454-458.
- Sundaralingam, M. (1965), *J. Amer. Chem. Soc.* 87, 599.
- Sundaralingam, M. (1966), *Acta Crystallogr.* 21, 495.
- Sundaralingam, M. (1968), *Biopolymers* 6, 189.
- Sundaralingam, M. (1969), *Biopolymers* 7, 821, and references therein.
- Sundaralingam, M. (1971a), *J. Amer. Chem. Soc.* 93, 6644.
- Sundaralingam, M. (1971b), *Anal. N. Y. Acad. Sci.* (in press).
- Sundaralingam, M. (1972a), in *Purines—Theory and Experiment*, Bergmann, E., and Pullman, B., Ed., Jerusalem, Israel Academy of Sciences and Humanities.
- Sundaralingam, M. (1972b), in *Conformations of Biological Molecules and Polymers*, Bergmann, E., and Pullman, B., Ed., Jerusalem, Israel Academy of Sciences and Humanities (in press).

<sup>2</sup> To be published.



- Sundaralingam, M., and Abola, J. (1972c), *Nature (London)* 235, 244.
- Sundaralingam, M., and Abola, J. (1972d), *J. Amer. Chem. Soc.* (in press).
- Sundaralingam, M., and Arora, S. K. (1969), *Proc. Nat. Acad. Sci. U. S.* 64, 1021.
- Sundaralingam, M., and Jensen, L. H. (1965a), *J. Mol. Biol.* 13, 914.
- Sundaralingam, M., and Jensen, L. H. (1965b), *J. Mol. Biol.* 13, 930.
- Sundaralingam, M., and Putkey, E. (1970), *Acta Crystallogr., Sect. B* 26, 790.
- Sundaralingam, M., Rao, S. T., and Abola, J. (1971), *J. Amer. Chem. Soc.* 93, 7055.
- Sutor, D. J. (1963), *J. Chem. Soc.*, 1105.
- Viswamitra, M. A., Reddy, B. S., James, M. N. G., and Williams, H. J. B. (1972), *Acta Crystallogr.* (in press).
- Viswamitra, M. A., Reddy, B. S., Lin, G. H.-Y., and Sundaralingam, M. (1971), *J. Amer. Chem. Soc.* 93, 4565.
- Voet, D., and Rich, A. (1970), *Progr. Nucl. Acid Res. Mol. Biol.* 10, 183.
- Watson, J. D., and Crick, F. H. C. (1953a), *Nature (London)* 171, 737.
- Watson, J. D., and Crick, F. H. C. (1953b), *Nature (London)* 171, 964.

## Purification of Hepatic Microsomal Membranes<sup>†</sup>

Robert R. Weihing,<sup>‡</sup> Vincent C. Manganiello,<sup>§</sup> Rosanna Chiu,<sup>‡</sup> and Alvah H. Phillips\*

**ABSTRACT:** A method is described for the isolation in high yields of apparently intact microsomal membranes. Solutions of high ionic strength are used to remove attached ribosomes and large amounts of protein, including serum protein, which may be adsorbed to the outer surface of the membranes. Subsequent extraction with dilute sodium deoxycholate tends to release preferentially small quantities of protein probably retained within the closed vesicles. Several lines of evidence

suggest that the membranes are relatively free of extraneous protein after these purification steps. The isolated membranes, in contrast to the originally heterogeneous vesicles from which they are derived, exhibit a highly uniform buoyant density. This observation supports earlier suggestions that the membranes of smooth and rough endoplasmic reticulum are structurally similar.

As attention focuses on the detailed molecular properties of biological membranes, it becomes increasingly important not only that membranes can be isolated in a highly purified state but also that their purification can be achieved without altering the properties responsible for their normal structural and functional role in the cell. In this regard none of the methods commonly used for the purification of hepatic microsomal membranes seem to be completely satisfactory.

Microsomal membranes that are free of attached ribosomes and other morphologically detectable cell components have been prepared by methods utilizing differential and density gradient centrifugation to isolate the preexisting smooth microsomal vesicles (Chauveau *et al.*, 1962; Dallner, 1963; Glauman and Dallner, 1968). Because of the mild conditions of isolation, these methods insure recoveries of relatively intact membranes but do not remove serum proteins appar-

ently contained within the closed vesicles (Peters, 1962) nor, with the conditions of low ionic strength normally employed, would they be expected to remove adsorbed cytoplasmic proteins.

The membranes of the rough as well as smooth microsomal vesicles have been purified by methods utilizing chemical, enzymatic, and mechanical treatment to remove the membrane-attached ribosomes and the contents of the vesicles. As discussed in a review by Dallner and Ernster (1968), several of these methods (*e.g.*, extraction with EDTA, exposure to hypotonic solutions, sonication) do not appear to be very effective. More recently treatment of microsomes with trypsin (Omura *et al.*, 1967), 2 M lithium chloride (Scott-Burden and Hawtrey, 1969), or 0.25 M Tris (pH 8) followed by sonication (Glaumann, 1970) have been shown to remove membrane attached ribosomes. These treatments, however, do not seem to completely release the contents from the vesicles, and Tris sonication also does not completely release RNA. On the other hand, treatment with 0.26% sodium deoxycholate seems to effectively release both ribosomes and contents (Ernster *et al.*, 1962), and for this reason has been widely adopted as a method for purifying the membranes (Omura *et al.*, 1967) and also for separating and identifying the contents of the vesicles (Redman, 1967). This treatment, however, also releases considerable quantities of phospholipid from the sedimenting vesicles and it, as well as trypsin digestion, also releases NADPH-cytochrome *c* reductase and cytochrome *b<sub>5</sub>* (Ernster *et al.*, 1962), two presumably membrane linked proteins (Omura *et al.*, 1967). These observations, as well as

<sup>†</sup> From the Department of Physiological Chemistry, The Johns Hopkins School of Medicine, Baltimore, Maryland, and from the Section of Biochemistry and Biophysics, University of Connecticut, Storrs, Connecticut. Received September 4, 1970. This investigation was supported by USPHS Research Grant GM-12474, by the University of Connecticut Research Foundation, and by Grant PRS-25 from the American Cancer Society. Part of this work has been published in a brief report (Phillips *et al.*, 1967).

<sup>‡</sup> Present address: Worcester Foundation for Experimental Biology, Shrewsbury, Mass. 01545.

<sup>§</sup> Present address: Section on Metabolism, Laboratory of Molecular Disease, National Heart and Lung Institute.

\* Requests for reprints should be addressed to: Box U-125, University of Connecticut, Storrs, Conn. 06268.