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Structure of a 1:2 Sandwich Complex of Proflavine and Adenosine with an Unusual Puckering Disorder and a Site Shared by Sulfate and Water Molecules

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Abstract

The crystal structure of a 2:1 complex of adenosine and proflavine sulfate, $2C_{10}H_{14}N_5O_4^+ . C_{13}H_{12}N_3^+ . 1{\cdot}5SO_4^{2-} . 6{\cdot}5H_2O$, has been determined using 2897 three-dimensional X-ray intensities. The unit-cell constants are a = 15·116 (6), b = 6·715 (3), c = 22·384 (3) Å, β = 96·4 (2)°, space group $P2_1$. The structure is disordered, and has been refined to an R index of 0·14. The two independent adenosine molecules in the asymmetric unit are base paired through N(6) and N(7) atoms. The proflavine is sandwiched between the adenine–adenine pairs, translated along the b-axis direction. The complex exhibits extensive stacking and is additionally stabilized by hydrogen bonds involving the proflavine, adenosine and sulfate ions. One of the amino protons of the proflavine is engaged in a bifurcated hydrogen bond to the ribose hydroxyl oxygen atom O(2′)*A* and the furanose ring oxygen atom O(4′)*A* of an adjacent adenosine. The fully occupied sulfate group forms a hydrogen-bonded bridge to the quaternary nitrogen atom N(10) of proflavine and the amino group of adenine *B* stacked over it. The second sulfate group shares its site with three water molecules. A novel feature of this structure is the puckering disorder exhibited by ribose *B* which is interpreted as a 4:1 mixture of two conformers ^0E [O(4′)-*endo*] and ^2E [C(2′)-*endo*]. The torsion angle about the exocyclic bond C(4′)–C(5′) is *trans* for the ^0E conformer and *gauche*$^+$ for the ^2E conformer. The correlated conformational changes of the C(4′)–C(5′) bond and of the puckering parameters are indicative of the concerted changes inducible in nucleic acids. The ordered ribose of adenosine *A* exhibits a flattened symmetrical twist conformation, $^3_2T[P$ = 179 (5)°, τ_m = 24 (1)°], with an intramolecular hydrogen bond of 2·63 (2) Å between the ribose hydroxyls O(2′)···O(3′).

Introduction

With a view to understand the molecular mechanism of drug action on nucleic acids, X-ray structural investigations of drug complexes with self-complementary dinucleoside monophosphates have been carried out in several laboratories (Sobell, Tsai, Jain & Gilbert, 1977; Neidle, 1979; Berman, Neidle & Stodola, 1978). Work from this laboratory has shown that the non-self-complementary dinucleoside monophosphate cytidylyl(3′-5′)adenosine (CpA) forms a parallel chain dimer duplex with a proflavine molecule intercalated between the cytosine–cytosine and adenine–adenine self pairs (Westhof & Sundaralingam, 1980*a*; Westhof, Rao & Sundaralingam, 1980). Besides this example of an intercalated complex, another non-self-complementary dinucleoside monophosphate formed a sandwiched complex with proflavine (Neidle, Taylor, Sanderson, Shieh & Berman, 1978). We report here the crystal structure of a 2:1 complex between adenosine and proflavine wherein the drug is sandwiched between the adjacent adenine–adenine (A–A) self-pairs with the sugars in parallel orientation. An interesting observation in this structure is that the ribose of one of the adenosine molecules is disordered both in the ring pucker and the exocyclic C(4′)–C(5′) bond conformation (Sundaralingam, Westhof & Swaminathan, 1980).

Methods

Crystals of the adenosine–proflavine complex were grown by evaporation of an ethanol–water solution of a 2:1 mixture of adenosine and proflavine hemisulfate. The crystals are needle-shaped with a deep-red color indicating the presence of proflavine. There are two molecules of adenosine, one molecule of proflavine and 1·5 SO_4^{2-} ions in the asymmetric part of the unit cell. The intensity data were collected on a Picker FACS-1 diffractometer employing the θ–2θ scan technique [λ(Cu $K\alpha$) = 1·5418 Å] to 2θ = 127° using a crystal of dimensions 0·2 × 0·1 × 0·5 mm. Three standard reflections were collected at intervals of 3 h to monitor crystal decay and orientation and these indicated no significant change in their intensities. Out of 4053 reflections scanned, 2897 reflections had intensities $I \geq 1{\cdot}5\sigma(I)$. The data were corrected for Lorentz and

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polarization effects and differential absorption (φ curve).

Structure determination and refinement

The structure was determined by a combination of Patterson and direct methods using normalized structure amplitudes computed using molecular scattering factors (Main, 1975; Swaminathan, 1975). Initial attempts to solve the structure using the three-phase semi-invariant technique developed specially for the monoclinic space group $P2_1$ (Hauptman & Potter, 1979) revealed fragments of the base and proflavine rings, oriented perpendicular to the b axis of the unit cell. Tangent refinement (Karle, 1968) based on this partial structure information did not lead to complete structural solution. An analysis of the $\sum_2$ and the three-phase semi-invariant triples indicated that the phases of some reflections with very strong $|E|$ values could not be determined unequivocally. Further attempts employing direct methods of phasing without the inclusion of these reflections revealed fragments of the structure at different positions with similar orientation. Harker vectors calculated with one of these fragments fitted very well with the observed Harker-section peaks. The positions of nonhydrogen atoms of the molecular structure consisting of two adenosine molecules, one proflavine, and a sulfate group, were determined by the cyclic tangent-refinement technique based on this partial structural information. A structure factor calculation with these atoms gave an R value of 0·33 and block-diagonal least-squares refinement reduced the R to 0·27.

Disorder of the sugar of adenosine *B* and the second sulfate group: stoichiometry of the complex

A difference Fourier map computed at this point revealed the presence of five water molecules and a partially occupied sulfate group. The map also showed residual peaks of 1·0 to 2·0 e Å^{-3} around the O(3′), C(3′), C(4′), C(5′) and O(5′) atoms of the ribose of adenosine B (Fig. 1*a*,*b*), indicating a disorder of the sugar ring and of the hydroxymethyl group. In the next cycle of refinement, the water molecules and the partially occupied sulfate group were included (R = 0·20). With occupancy factors of 0·5 assigned to the atoms of this sulfate group and 0·8 for the sugar atoms O(3′), C(3′), C(4′), C(5′) and O(5′) of adenosine B, the R value dropped to 0·18. A difference Fourier map revealed a cluster of three peaks around the sulfate group with peak heights varying from 1·0 to 2·5 e Å^{-3}. The possibility that these peaks could be disordered sites for the O atoms of the sulfate was initially considered in the refinement and later rejected since there was no additional peak for the S atom. Therefore, the three peaks were assigned to water molecules with occupancy factors of 0·5 sharing the site with the sulfate group (Fig. 1*a*). The residual peaks around the disordered ribose atoms were assigned occupancy factors of 0·2 (Fig. 1*b*). This lowered the R value to 0·168. At this point, the partially occupied sulfate and ribose groups were subjected to the constrained–restrained least-squares refinement using the computer program of Hendrickson & Konnert (1979), modified by Rao (1979). The sulfate-group geometry was refined against its ideal geometry and the riboses were refined against idealized geometries for ^0E pucker (0·78 occupancy) and ^2E pucker (0·22 occupancy) which were calculated from the equations developed by Westhof & Sundaralingam (1980*b*) using the ring-closure algorithm of Merritt & Sundaralingam (1981). The R dropped to 0·15. During the constrained refinement the rest of the structure was kept fixed. Difference Fourier maps revealed the positions of the H atoms on the proflavine and the adenine bases. It was found that the proflavine is protonated at N(10) and that the adenine bases are protonated at N(1). Thus, the stoichiometry of the complex is $PFH^+ + 2AH^+ + 1{\cdot}5SO_4^{2-} + 6{\cdot}5H_2O$, confirming the presence of the partially occupied sulfate. In the final cycles of refinement, the partially occupied sulfate, water and ribose atoms were kept fixed, and the remaining structure was refined with anisotropic thermal parameters for the fully occupied non-hydrogen atoms. After inclusion of the H atoms, the final R value was 0·14.

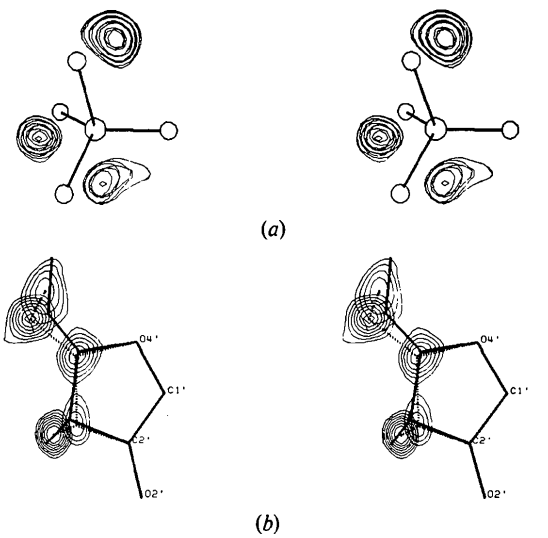

Fig. 1. Stereoviews of (*a*) the partial sulfate with the electron densities of the partial water molecules and (*b*) the disordered ribose of adenosine B with the electron densities of the minor occupied sites O(3′), C(3′), C(4′), C(5′) and O(5′). Contours are drawn at arbitrary intervals.

Table 1. *Fractional positional parameters and equivalent isotropic temperature factors of the nonhydrogen atoms*

	Occupancy*	x	y	z	B_{eq} (Å^2)†
Adenosine (molecule *A*)					
O(5')*A*		0·3743 (9)	0·0378 (25)	0·7724 (5)	7·0 (5)
C(5')*A*		0·3366 (14)	-0·0976 (37)	0·8109 (9)	6·8 (7)
C(4')*A*		0·3575 (8)	-0·0207 (27)	0·8798 (6)	3·7 (4)
O(4')*A*		0·4507 (6)	-0·0158 (20)	0·8950 (5)	5·2 (3)
C(1')*A*		0·4832 (7)	0·1791 (28)	0·9037 (5)	3·6 (4)
C(2')*A*		0·4075 (9)	0·3146 (31)	0·8848 (7)	4·2 (5)
O(2')*A*		0·4105 (7)	0·4919 (20)	0·9155 (6)	5·4 (4)
C(3')*A*		0·3245 (8)	0·1910 (25)	0·8863 (6)	3·7 (4)
O(3')*A*		0·2991 (6)	0·2200 (25)	0·9462 (4)	6·0 (4)
N(1)*A*		0·8193 (7)	0·1851 (31)	0·9276 (6)	6·0 (5)
C(2)*A*		0·7598 (9)	0·1735 (36)	0·9700 (7)	5·4 (5)
N(3)*A*		0·6743 (7)	0·1644 (30)	0·9606 (6)	5·3 (4)
C(4)*A*		0·6481 (7)	0·1911 (23)	0·9007 (7)	3·7 (4)
C(5)*A*		0·6992 (8)	0·2150 (25)	0·8536 (6)	3·3 (4)
C(6)*A*		0·7920 (7)	0·2153 (25)	0·8694 (7)	3·7 (4)
N(6)*A*		0·8517 (7)	0·2243 (22)	0·8292 (5)	4·0 (3)
N(7)*A*		0·6495 (7)	0·2306 (21)	0·8008 (5)	4·0 (3)
C(8)*A*		0·5693 (7)	0·2224 (26)	0·8141 (6)	3·7 (4)
N(9)*A*		0·5605 (6)	0·1974 (22)	0·8731 (5)	3·8 (3)
Adenosine (molecule *B*)					
O(5')*B*	0·78	1·1799 (10)	-0·1538 (35)	0·6282 (12)	10·3 (9)
C(5')*B*	0·78	1·1874 (12)	0·0297 (54)	0·6362 (17)	9·0 (12)
C(4')*B*	0·78	1·1325 (11)	0·1461 (41)	0·5875 (9)	5·4 (6)
O(4')*B*		1·0443 (6)	0·1145 (17)	0·6004 (5)	5·2 (3)
C(1')*B*		0·9941 (10)	0·2896 (25)	0·5846 (8)	4·7 (4)
C(2')*B*		1·0564 (11)	0·4562 (34)	0·6035 (10)	6·8 (6)
O(2')*B*		1·0286 (8)	0·6512 (36)	0·5812 (9)	11·7 (7)
C(3')*B*	0·78	1·1491 (11)	0·3770 (38)	0·6021 (7)	4·4 (6)
O(3')*B*	0·78	1·1797 (18)	0·4743 (47)	0·5547 (11)	14·2 (11)
N(1)*B*		0·6582 (7)	0·3418 (22)	0·5697 (5)	4·2 (3)
C(2)*B*		0·7171 (8)	0·3341 (25)	0·5305 (6)	3·7 (4)
N(3)*B*		0·7991 (7)	0·3263 (22)	0·5352 (6)	4·5 (6)
C(4)*B*		0·8331 (7)	0·3012 (22)	0·5963 (6)	3·1 (3)
C(5)*B*		0·7799 (7)	0·2921 (22)	0·6436 (6)	3·4 (3)
C(6)*B*		0·6890 (8)	0·3137 (25)	0·6325 (7)	4·2 (4)
N(6)*B*		0·6309 (6)	0·3139 (21)	0·6715 (5)	3·9 (3)
N(7)*B*		0·8336 (6)	0·2736 (21)	0·6955 (5)	3·7 (3)
C(8)*B*		0·9135 (8)	0·2650 (28)	0·6805 (7)	4·8 (5)
N(9)*B*		0·9139 (7)	0·2898 (21)	0·6158 (5)	4·0 (3)
C(3'')	0·22	1·1419 (42)	0·3726 (56)	0·5814 (33)	4·4 (14)
C(4'')*B*	0·22	1·1356 (39)	0·1524 (57)	0·5974 (30)	4·6 (13)
O(3'')*B*	0·22	1·1529 (19)	0·4516 (57)	0·5256 (15)	9·7 (7)
C(5'')*B*	0·22	1·2128 (28)	0·0406 (84)	0·6237 (22)	3·2 (9)
O(5'')*B*	0·22	1·2098 (42)	0·0314 (89)	0·6818 (32)	12·7 (19)
Proflavine					
C(1)*P*		0·1514 (9)	0·2874 (32)	0·3452 (7)	4·8 (5)
C(2)*P*		0·1851 (10)	0·3152 (30)	0·4032 (7)	5·0 (5)
C(3)*P*		0·2798 (9)	0·3215 (28)	0·4201 (8)	4·5 (5)
N(3)*P*		0·3118 (7)	0·3446 (27)	0·4776 (6)	5·2 (4)
C(4)*P*		0·3353 (9)	0·3067 (25)	0·3717 (6)	3·6 (4)
C(5)*P*		0·3794 (8)	0·2241 (32)	0·1649 (8)	5·1 (5)
C(6)*P*		0·3495 (8)	0·1959 (31)	0·1045 (6)	4·6 (5)
N(6)*P*		0·4032 (7)	0·1803 (27)	0·0636 (5)	4·7 (4)
C(7)*P*		0·2256 (10)	0·1849 (22)	0·0893 (8)	4·3 (4)
C(8)*P*		0·1978 (9)	0·2061 (30)	0·1316 (8)	5·1 (5)
C(9)*P*		0·1728 (8)	0·2427 (27)	0·2379 (7)	4·4 (4)
N(10)*P*		0·3533 (7)	0·2691 (19)	0·2689 (5)	3·2 (3)
C(11)*P*		0·2074 (8)	0·2756 (26)	0·2963 (7)	3·9 (4)
C(12)*P*		0·3000 (8)	0·2868 (22)	0·3148 (7)	3·7 (4)
C(13)*P*		0·3243 (10)	0·2464 (27)	0·2090 (9)	4·8 (5)
C(14)*P*		0·2318 (9)	0·2376 (26)	0·1944 (7)	3·9 (4)
Sulfates and water molecules					
S(1)		0·5842 (2)	0·1404 (7)	0·3390 (2)	3·8 (1)
O1(S1)		0·6779 (6)	0·1419 (21)	0·3265 (5)	5·1 (3)
O2(S1)		0·5339 (6)	0·2916 (23)	0·2976 (6)	5·8 (4)
O3(S1)		0·5797 (9)	0·2191 (26)	0·4017 (5)	7·3 (5)
O4(S1)		0·5460 (8)	-0·0493 (23)	0·3281 (6)	6·6 (4)
S2	0·54	1·0666 (8)	0·1918 (54)	0·9211 (10)	16·6 (12)
O1(S2)	0·54	1·0379 (14)	0·1761 (90)	0·8544 (14)	22·8 (21)
O2(S2)	0·54	1·1377 (15)	0·0689 (93)	0·9441 (14)	20·1 (22)
O3(S2)	0·54	0·9937 (20)	0·1185 (95)	0·9518 (17)	36·1 (23)
O4(S2)	0·54	1·0565 (29)	0·4101 (99)	0·9360 (25)	27·6 (25)
O*W*(1)		0·4997 (6)	-0·0005 (19)	0·4836 (5)	4·7 (3)
O*W*(2)		0·5544 (11)	0·6434 (24)	0·4101 (6)	7·6 (5)
O*W*(3)		0·7294 (11)	-0·1180 (28)	0·2494 (6)	8·5 (6)
O*W*(4)		1·0687 (10)	0·8302 (44)	0·7447 (11)	13·0 (9)
O*W*(5)		1·1027 (12)	0·4494 (66)	0·7722 (9)	16·5 (15)
O*W*(6)	0·46	0·9817 (25)	0·2071 (81)	0·9906 (18)	13·1 (12)
O*W*(7)	0·46	1·0666 (22)	0·0214 (60)	0·8981 (16)	12·0 (11)
O*W*(8)	0·46	1·1255 (13)	0·2881 (86)	0·9306 (15)	14·6 (13)

* The partial occupancy factors of the disordered atoms are given alongside the atom numbers.

† $B_{eq} = \frac{4}{3}\sum_{ij}\beta_{ij}\mathbf{a}_i.\mathbf{a}_j$ where a_i's are cell constants of the unit cell.

All of the calculations were performed on an in-house PDP 11/35 computer using programs developed in these laboratories (Rao, McAlister & Merritt, 1979). The scattering factors for C, N, O, S were from Cromer & Waber (1965) and for H from Stewart, Davidson & Simpson (1965).

Results

Fractional positional parameters and the equivalent isotropic temperature factors of the nonhydrogen atoms are listed in Table 1.* An *ORTEP* diagram of the ordered and major occupied atoms of the components of the complex is shown in Fig. 2. The bond lengths (mean e.s.d. 0·018 Å) and bond angles (mean e.s.d. 1·8°) are given in Fig. 3. An edge-on view of the molecular complex is shown in Fig. 4.

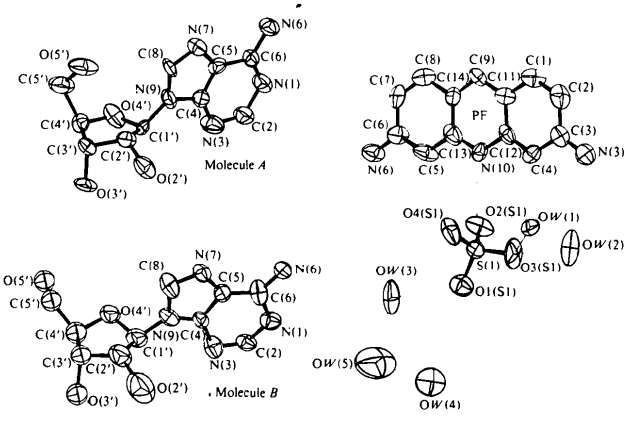

Fig. 2. *ORTEP* drawings (Johnson, 1965) of the adenosines, proflavine, sulfates and waters of the complex.

* Lists of structure factors, thermal parameters for nonhydrogen atoms and positional parameters for hydrogen atoms have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 36363 (10 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

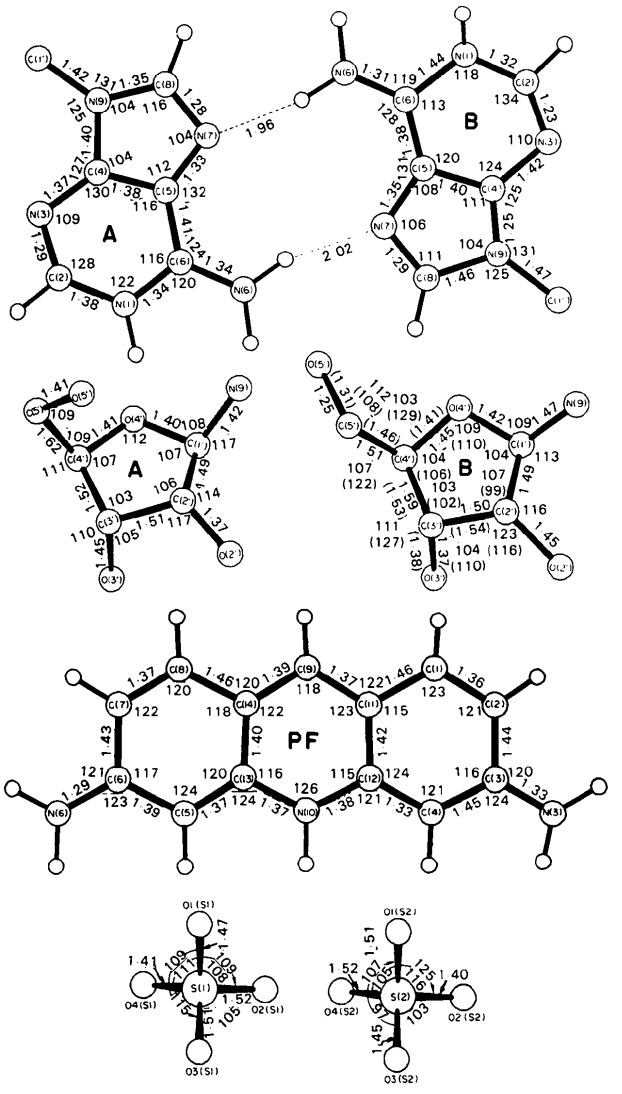

Fig. 3. Bond lengths (Å) and bond angles (°) of the components of the complex. Values within parentheses correspond to the minor sites.

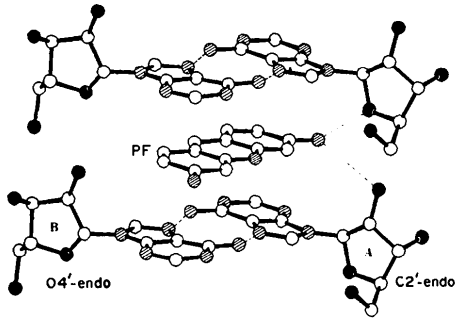

Fig. 4. Edge-on view of the adenosine–proflavine complex. The sandwiched proflavine is involved in a bifurcated hydrogen bond to the ring oxygen atom O(4') and the hydroxyl oxygen atom O(2') of the ordered adenosines.

Discussion

Molecular conformation and ribose disorder

Important conformational parameters of the complex are listed in Table 2. The orientations of the bases are *anti* in both adenosines. The sugar ring of molecule *A* is ordered and it exhibits a symmetrical twist pucker with an amplitude of pucker [24 (1)°] considerably smaller than the average value (39°) observed in the crystal structures of nucleosides and nucleotides (Altona & Sundaralingam, 1972). The low amplitude of pucker seems to have promoted an intramolecular hydrogen bond between the vicinal hydroxyl oxygen atoms O(2') and O(3'). The exocyclic C(4')–C(5') bond conformation is *gauche*$^+$.

The sugar of molecule *B* is disordered. The disorder involves both the ring and the exocyclic C(4')–C(5') bond. The major site (78%) has the O(4')-*endo* (^0E) pucker while the minor site (22%) a C(2')-*endo* (^2E) pucker. The major rotamer around the exocyclic C(4')–C(5') bond is *trans*, while the minor rotamer is at the outer limit of the usual *gauche*$^+$ range. Conformational disorder about the C(4')–C(5') bond has been observed quite frequently in nucleoside crystal structures (Sundaralingam, Rao & Abola, 1971; Suck, Saenger & Zechmeister, 1972), but pseudorotational disorder had not been seen until recently. After our work was reported (Sundaralingam *et al.*, 1980; Swaminathan, Westhof & Sundaralingam, 1980) we learned of two other recently determined structures with furanoid-ring disorder; one of them is an intercalated complex of the deoxydinucleoside monophosphate [d(CpG)] with proflavine (Neidle, Berman & Shieh, 1980) and the other is adenosine 5'-diphosphoric acid trihydrate (Hosur & Viswamitra, 1979). In both these cases the major puckered states are ^2E and ^3E and neither showed disorder of the C(4')–C(5') bond. Although, on the average, *gauche*$^+$ is the preferred conformer about the C(4')–C(5') bond, some ribose puckers are correlated with other con-

Table 2. *Conformational parameters* (°)

			Molecule *B*	
		Molecule *A*	Site 1	Site 2
Glycosyl	χ	78 (2)	53 (2)	
Ribose pucker		^2_3T	^0E	^2E
Endocyclic torsion angles of the ribose	τ_0	−8 (2)	−39 (2)	−30 (4)
	τ_1	20 (2)	26 (2)	43 (4)
	τ_2	−24 (2)	−6 (3)	−40 (7)
	τ_3	19 (2)	−17 (3)	24 (8)
	τ_4	−7 (2)	35 (2)	3 (7)
Pseudorotation parameters				
phase angle	P	179 (5)	99 (2)	158 (8)
amplitude of pucker	τ_m	24 (1)	39 (3)	44 (5)
C(3')–C(4')–C(5')–O(5')	ψ	56 (2)	181 (3)	99 (8)

formations. For example, the C(3′)-*endo* disfavors the *gauche*$^-$ orientation, while the O(4′)-*endo* favors the *trans* rotamer (Sundaralingam, 1973).

The protonated proflavine and adenine bases

The angle between least-squares planes passing through the two halves of the N(10)-protonated proflavine molecule is 0·6 (3)°. Thus there is no significant buckling of the proflavine molecule as observed for the intercalated proflavine in the CpA:PF complex (Westhof, Rao & Sundaralingam, 1980). Both adenine bases are protonated at the usual site N(1) and are paired through N(6) and N(7) atoms (Fig. 5). This type of self-pairing involving N(6) and N(7) atoms is quite common in crystal structures of di- and oligonucleotides: UpA (Rubin, Brennan & Sundaralingam, 1972), ApApA (Suck, Manor & Saenger, 1976) where the adenine N(1) site is protonated. But, this is the first observation of such a pairing in an adenosine nucleoside, where the base is also protonated at N(1). When the base is not protonated two other types of adenine–adenine self pairs are usually seen: one involving the Watson–Crick sites N(1) and N(6) and the other involving sites N(1), N(6), and N(6), N(7) (Prusiner & Sundaralingam, 1976). The Watson–Crick sites N(1) and N(6) of molecule *A* form a pair of hydrogen bonds with the partially occupied sulfate O atoms. The fully occupied sulfate anion is directly hydrogen bonded to N(6) of molecule *B* and to N(1) *via* a water molecule.

Molecular complex: packing, stacking and hydrogen bonding

The adenine and proflavine rings lie in alternate planes in the crystal, essentially perpendicular to the unique *b* axis. The A–A pairs in adjacent unit cells along **b** sandwich the proflavine, thus forming infinite vertical columns of alternately stacked A–A pairs and proflavines that form a molecular wall in the (202) planes. The water molecules and the sulfate ions lie in the watertight space in the (101) planes between the walls of the adenine and proflavine rings.

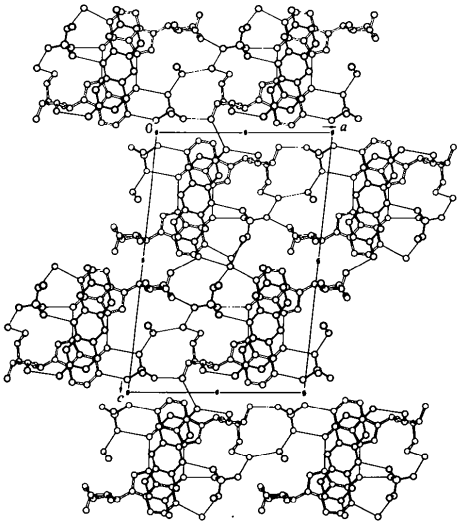

Fig. 5. Molecular packing of the adenosine–proflavine complex viewed along the *b* axis.

The proflavine molecule stacks over the A–A pair at a distance of 3·36 Å. The stacking pattern (Fig. 5) found here is very similar to that of the nonintercalated proflavine in the CpA:PF structure (Westhof & Sundaralingam, 1980*a*). The angle between the least-squares planes passing through the bases is 5·2 (3)°, while the angle between the planes through the stacked proflavine and the paired bases is only 0·3 (3)°. The C(1′)···C(1′) distance in the *trans* adenosine base pair is 11·10 (4) Å.

Hydrogen bonding (*see Table* 3)

The exocyclic amino nitrogen N(3) is hydrogen bonded to O(3′) of the disordered ribose (adenosine *B*) in the neighboring column and to O*W*(1). One of the protons of the amino nitrogen N(6)*P* is hydrogen bonded to O(3′) of the ordered adenosine *A* in a neighboring column, while the other is involved in a bifurcated hydrogen bond to O(4′) and O(2′) atoms of the ordered adenosine molecules sandwiching it (Fig. 4). This type of hydrogen-bond interaction between the drug molecule and the sugars of the stacking nucleosides has not been observed in the other known complexes. However, a very similar situation occurs in the crystal structure of $tRNA^{Phe}$ where the amino group of guanine G57, which is sandwiched between G18 and G19, is hydrogen bonded to O(2′) of G18 and O(4′) of G19 (Jack, 1979). Interestingly, the riboses of both G18 and G19 adopt a C(2′)-*endo* conformation and the attached bases have high-*anti* glycosyl torsion angles (Sundaralingam, 1980), as is the case in the present structure.

Hydrogen-bond interaction involving the furanose-ring O has now been observed in several structures, often with the glycosyl torsion angle in the high-*anti* range (90 to 120°) (Sprang, Scheller, Rohrer & Sundaralingam, 1978). The high χ range reduces steric hindrance to the ring O(4′) atom and facilitates hydrogen-bond formation to it. In the present structure, the ring oxygen O(4′)*A* is directly hydrogen bonded to the amino nitrogen N(6)*P* of the proflavine. In the CpA:PF complex (Westhof, Rao & Sundaralingam, 1980), the quaternary nitrogen N(10) of the intercalated proflavine is bridged through a water molecule to the ring oxygen O(4′) of the adenosine residue whose glycosyl torsion angle is 84°.

The O(2′) hydroxyl oxygen of the ordered adenosine *A* accepts a hydrogen bond from the N(6) atom of the proflavine and appears to be also involved in an intramolecular hydrogen bond to the O(3′) atom of the

same ribose ring. Such an intramolecular hydrogen bond is seldom observed in a nucleoside or a nucleotide (Rohrer & Sundaralingam, 1970; Schwalbe & Saenger, 1973*a*; Wang, Dammann, Barrio & Paul, 1974; Narayanan & Berman, 1975). The intramolecular hydrogen bond between the vicinal hydroxyls induces a sugar pucker in the broad C(2′)-*endo* range, $P = 90$ to 200°, a range not favored by ribonucleic acids which generally adopt C(3′)-*endo* puckers. Except in one case (Narayanan & Berman, 1975), the O(2′) hydroxyl is the donor atom and O(3′) is the acceptor.

The oxygen atoms O2(S1) and O4(S1) of the fully occupied sulfate group are hydrogen bonded respectively to N(10) of the proflavine and the amino nitrogen N(6) of the stacked adenosine *B*, thus bridging the proflavine and the stacked A–A base pair. The sulfate atom S(1) lies at 2·29 (8) Å above the A–A pair and 1·09 (8) Å below the proflavine ring. The remaining O atoms are hydrogen bonded to water molecules and to the hydroxyl oxygen atom O(5′) of adenosine molecules in neighboring columns. The two oxygen atoms O1(S2) and O3(S2) of the partially occupied

Table 3. *Hydrogen bonds and short contacts less than* 3·40 Å *in APF*

D–H···*A*	Symmetry code*	Translation for *A* (*x*,*y*,*z*)	*D*···*A* (Å)	*D*–H† (Å)	H···*A* (Å)	*D*–H···*A* (°)
O(5′)*A*···O2(S1)	2	(1,−1,1)	2·76 (2)			
O(2′)*A*···O(3′)	1	(0,0,0)	2·63 (2)			
O(3′)*A*···O2(S2)	1	(0,0,0)	2·64 (1)			
N(1)*A*–H(N1)···O3(S2)	1	(0,0,0)	2·67 (1)	1·02	1·68	161
N(6)*A*–H2(N6)*A*···N(7)*B*	1	(0,0,0)	2·99 (2)	1·0	2·02	163
N(6)*A*–H1(N6)*A*···O1(S2)†	1	(0,0,0)	2·83 (1)	1·03	1·81	169
O(5′)*B*···O1(S1)	2	(2,−1,1)	2·66 (2)			
O(3′)*B*···O(5′)*B*	1	(0,1,0)	2·99 (4)			
O(3′)*B*···N(3)*B*	2	(2,0,1)	3·14 (3)			
O(2′)*B*···O(5′)*B*	1	(0,1,0)	2·74 (3)			
O(2′)*B*···O(3′)*B*	1	(0,0,0)	2·70 (3)			
N(1)*B*–H(N1)*B*···O*W*(1)	2	(1,0,1)	2·76 (2)	0·90	1·90	157
N(6)*B*–H1(N6)*B*···N(7)*A*	1	(0,0,0)	2·93 (2)	0·99	1·96	167
N(6)*B*–H2(N6)*B*···O4(S1)	2	(1,0,1)	2·83 (2)	1·05	1·92	143
O(5″)*B*···O1(S1)	2	(2,−1,1)	3·14 (8)			
O(3″)*B*···N(3)*B*	2	(2,0,1)	2·99 (4)			
N(3)*P*–H1(N3)*P*···O(3′)*B*	1	(−1,0,0)	2·91 (3)	1·01	1·96	157
N(3)*P*–H1(N3)*P*···O(3″)*B*‡	1	(−1,0,0)	2·83 (3)	1·01	1·97	142
N(3)*P*–H2(N3)*P*···O*W*(1)	2	(1,0,1)	3·07 (2)	1·02	2·12	154
N(6)*P*–H1(N6)*P*···O(3′)*A*	1	(0,0,−1)	2·92 (2)	1·03	1·95	156
N(6)*P*–H2(N6)*P*···O(4′)*A*	2	(1,0,1)	3·07 (2)	0·94	2·41	128
N(6)*P*–H2(N6)*P*···O(2′)*A*	2	(1,−1,1)	3·08 (2)	0·94	2·33	136
N(10)*P*–H(N10)*P*···O2(S1)	1	(0,0,0)	2·74 (1)	1·03	1·71	178
O*W*(1)···O3(S1)	1	(0,0,0)	2·74 (2)			
O*W*(1)···O*W*(2)	1	(0,−1,0)	3·07 (2)			
O*W*(1)···O*W*(2)	2	(1,−1,1)	2·77 (2)			
O*W*(2)···O4(S1)	1	(0,1,0)	2·76 (2)			
O*W*(2)···O3(S1)	1	(0,0,0)	2·88 (2)			
O*W*(3)···O1(S1)	1	(0,0,0)	2·63 (2)			
O*W*(3)···O(5′)*A*	2	(1,−1,1)	2·81 (2)			
O*W*(3)···O*W*(5)	2	(2,−1,1)	2·67 (3)			
O*W*(3)···O(5″)*B*‡	2	(2,−1,1)	2·91 (8)			
O*W*(4)···O*W*(5)	1	(0,0,0)	2·67 (5)			
O*W*(4)···O(5′)*B*	1	(0,1,0)	3·26 (3)			
O*W*(4)···O(5″)*B*‡	1	(0,1,0)	3·00 (7)			
O*W*(5)···O1(S2)	1	(0,0,0)	2·85 (3)			
O*W*(5)···O*W*(4)	1	(0,0,0)	2·67 (5)			
O*W*(6)···N(1)*A*	1	(−1,0,0)	2·70 (4)			
O*W*(6)···O*W*(7)‡	1	(0,0,0)	2·84 (6)			
O*W*(6)···O*W*(8)‡	1	(0,0,0)	2·73 (5)			
O*W*(7)···O*W*(8)‡	1	(0,0,0)	2·09 (6)§			
O*W*(8)···O(3′)*A*	1	(0,0,0)	2·65 (2)			

* Symmetry codes are as follows: (1) x,y,z; (2) $-x, \frac{1}{2}+y, -z$.

† Bond lengths and angles involving the H atoms are given only for those determined from the difference Fourier map. Since the H atoms were not refined the standard deviations are not given.

‡ Partially occupied (see Table 1).

§ The short contact here is attributed to the disorder of the atomic sites.

sulfate group form a pair of hydrogen bonds to N(1) and N(6) atoms of the adenosine *A* (Fig. 6) in a way reminiscent of the phosphate–base hydrogen-bond interactions in nucleotide crystal structures (see also Prusiner & Sundaralingam, 1972; Chwang & Sundaralingam, 1974). A third oxygen atom O2(S2) is hydrogen bonded to the hydroxyl oxygen O(3′) of the neighboring adenosine *B*. The three water molecules, replacing the partially occupied sulfate, are linked to each other by hydrogen bonds to form a water triangle which in turn is hydrogen bonded to N(1), N(6) and O(3′) atoms of adenosine *A*.

The sulfate–water channel in the (101) plane is shown in Fig. 7. The ordered sulfate groups, related by the screw axis at $(\frac{1}{2},\frac{1}{2})$, are shown in the center and they form an interesting network with two of the five fully occupied water molecules. These water molecules and

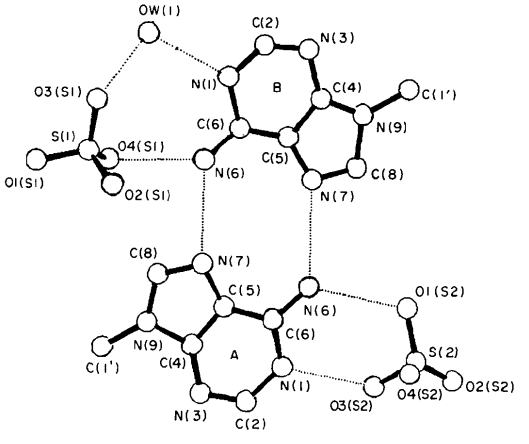

Fig. 6. The A–A base pair [with the base N(1) sites protonated] and the hydrogen bonding to the sulfate anions.

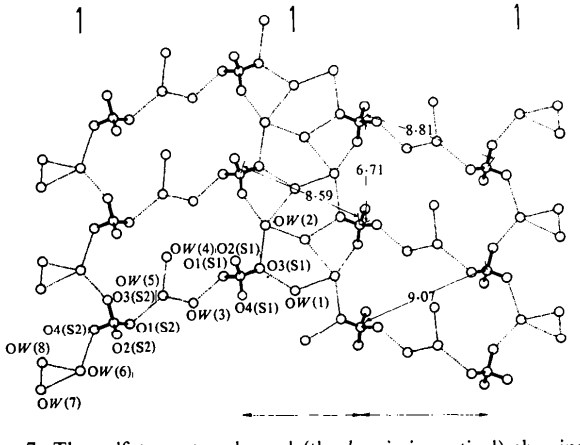

Fig. 7. The sulfate–water channel (the *b* axis is vertical) showing the ordered sulfate–water–sulfate interactions at the center of the picture, and the partial sulfate–water interactions on either ends of the picture. Note that, in the latter, the sulfate and the three waters (the water triangle) share the same site.

the sulfate oxygen atoms form two kinds of hydrogen-bonded five-membered rings. In one of them, one O atom of the sulfate participates, and in the other, two O atoms of the same sulfate participate. The partial sulfate groups related by the 2_1 symmetry axis at (1,0) are bridged by a partial water. The water molecules *OW*(3), *OW*(4), *OW*(5) lie between the rows of ordered and partial sulfate groups with the oxygen atom O1(S1) of the ordered sulfate hydrogen bonded to *OW*(3), and O1(*S*2) of the partial sulfate group hydrogen bonded to *OW*(5). It may be pointed out that five-membered hydrogen-bonded rings involving only water molecules have recently been seen in the structure of a drug–dinucleoside monophosphate complex (Neidle, Berman & Shieh, 1980).

It is interesting that the sulfate–sulfate separation (Fig. 7) along the *b* axis is of the order of the interphosphate separation in a polynucleotide chain, suggesting that a similar bridging network by water molecules could occur between phosphate groups in the grooves of nucleic acids or between polynucleotide chains.

Conclusion

In the solid state, usually one puckered state of the five-membered sugar ring of the nucleic acid monomers is observed; however, in solution there is an equilibrium between different puckered states, mainly involving ^2E and ^3E. When there are two independent molecules in the asymmetric part of the unit cell (which are usually related by pseudosymmetry) it is common to have similar conformations for the molecules and sugars that fall in the same puckering domain (Rahman & Wilson, 1972; Prusiner & Sundaralingam, 1976; Hogle, Sundaralingam & Lin, 1980). However, occasionally different puckers and conformations for the two molecules (in ψ and/or χ) in the crystal have been observed (Rahman & Wilson, 1970; Schwalbe & Saenger, 1973*b*; Thewalt, Bugg & Marsh, 1970; Munns, Tollin, Wilson & Young, 1970). In crystal structures of di- and oligonucleotides, cases of similar sugar puckers and mixed sugar puckers in the same molecule are known. The former appear to be preponderant in the uncomplexed systems, while the latter in the drug complexes.

The present structure of an adenosine–proflavine complex has revealed a two-state ribose-puckering disorder, $^0E \rightleftharpoons {}^2E$, occurring in concert with a rotational disorder about the exocyclic C(4′)–C(5′) bond, *trans* $\rightleftharpoons$ *gauche*$^+$. Such correlated changes in the pucker and the exocyclic-bond conformation have not been previously observed. It is interesting that the sugar of only one of the adenosine molecules is disordered while the other adenosine sugar is not, despite the fact that the bases of the two molecules are paired. The role of the co-crystallized proflavine in promoting the disorder is

not clear, although it is seen that the disordered ribose is hydrogen bonded to the proflavine and the ordered water, and the ordered ribose is hydrogen bonded to the partial water–sulfate sites. This is in contrast to the observation in the intercalated proflavine–d(CpG) complex (Neidle *et al.*, 1980), where the disorder of the deoxyribose is associated with the disordered water structure.

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References

ALTONA, C. & SUNDARALINGAM, M. (1972). *J. Am. Chem. Soc.* **94**, 8205–8212.

BERMAN, H. M., NEIDLE, S. & STODOLA, R. K. (1978). *Proc. Natl Acad. Sci. USA*, **75**, 828–832.

CHWANG, A. K. & SUNDARALINGAM, M. (1974). *Acta Cryst.* B**30**, 1233–1240.

CROMER, D. T. & WABER, J. T. (1965). *Acta Cryst.* **18**, 104–109.

HAUPTMAN, H. & POTTER, S. (1979). *Acta Cryst.* A**35**, 371–381.

HENDRICKSON, W. A. & KONNERT, J. (1979). *Biomolecular Structure, Conformation, Function and Evolution.* Vol. I, edited by R. SRINIVASAN, pp. 43–57. Oxford: Pergamon Press.

HOGLE, J., SUNDARALINGAM, M. & LIN, G. H.-Y. (1980). *Acta Cryst.* B**36**, 564–570.

HOSUR, M. V. & VISWAMITRA, M. A. (1979). *Curr. Sci.* **48**, 1027–1028.

JACK, A. (1979). *MTP International Review of Science: Biochemistry, Series 2A*. Vol. 24, pp. 211–256. London: Butterworths.

JOHNSON, C. K. (1965). *ORTEP*. Report ORNL-3794. Oak Ridge National Laboratory, Tennessee.

KARLE, J. (1968). *Acta Cryst.* B**24**, 182–186.

MAIN, P. (1975). International Summer School on Crystallographic Computing, Prague. Paper No. 48.

MERRITT, E. A. & SUNDARALINGAM, M. (1981). To be published.

MUNNS, A. R. I., TOLLIN, P., WILSON, H. R. & YOUNG, D. W. (1970). *Acta Cryst.* B**26**, 1114–1117.

NARAYANAN, P. & BERMAN, H. M. (1975). *Carbohydr. Res.* **44**, 169–180.

NEIDLE, S. (1979). *Prog. Med. Chem.* **16**, 151–221.

NEIDLE, S., BERMAN, H. M. & SHIEH, H.-S. (1980). *Nature (London)*, **288**, 129–133.

NEIDLE, S., TAYLOR, G., SANDERSON, M., SHIEH, H.-S. & BERMAN, H. M. (1978). *Nucleic Acids Res.* **5**, 4417–4422.

PRUSINER, P. & SUNDARALINGAM, M. (1972). *Acta Cryst.* B**28**, 2142–2148.

PRUSINER, P. & SUNDARALINGAM, M. (1976). *Acta Cryst.* B**32**, 161–169.

RAHMAN, A. & WILSON, H. R. (1970). *Acta Cryst.* B**26**, 1765–1775.

RAHMAN, A. & WILSON, H. R. (1972). *Acta Cryst.* B**28**, 2260–2270.

RAO, S. T. (1979). Unpublished results.

RAO, S. T., MCALISTER, J. & MERRITT, E. A. (1979). Unpublished results.

ROHRER, D. C. & SUNDARALINGAM, M. (1970). *J. Am. Chem. Soc.* **92**, 4956–4962.

RUBIN, J., BRENNAN, T. & SUNDARALINGAM, M. (1972). *Biochemistry*, **11**, 3112–3129.

SCHWALBE, C. H. & SAENGER, W. (1973*a*). *Acta Cryst.* B**29**, 61–69.

SCHWALBE, C. H. & SAENGER, W. (1973*b*). *J. Mol. Biol.* **75**, 129–143.

SOBELL, H. M., TSAI, C.-C., JAIN, S. C. & GILBERT, S. G. (1977). *J. Mol. Biol.* **114**, 333–365.

SPRANG, S., SCHELLER, R., ROHRER, D. & SUNDARALINGAM, M. (1978). *J. Am. Chem. Soc.* **100**, 2867–2872.

STEWART, R. F., DAVIDSON, E. R. & SIMPSON, W. T. (1965). *J. Chem. Phys.* **42**, 3175–3187.

SUCK, D., MANOR, P. C. & SAENGER, W. (1976). *Acta Cryst.* B**32**, 1727–1737.

SUCK, D., SAENGER, W. & ZECHMEISTER, K. (1972). *Acta Cryst.* B**28**, 596–605.

SUNDARALINGAM, M. (1973). *Conformation of Biological Molecules and Polymers.* Vol. V, edited by E. D. BERGMANN & B. PULLMAN, pp. 417–455. New York: Academic Press.

SUNDARALINGAM, M. (1980). *Biomolecular Structure, Conformation, Function and Evolution.* Vol. I, edited by R. SRINIVASAN, pp. 259–283. Oxford: Pergamon Press.

SUNDARALINGAM, M., RAO, S. T. & ABOLA, J. (1971). *J. Am. Chem. Soc.* **93**, 7055–7062.

SUNDARALINGAM, M., WESTHOF, E. & SWAMINATHAN, P. (1980). Collected Abstracts. The Seventh Aharon Katzir-Katchalsky Conference on Structural Aspects of Recognition and Assembly in Biological Macromolecules, Nof Ginossar, Israel, February 24–29, p. 94.

SWAMINATHAN, P. (1975). *Acta Cryst.* B**31**, 1608–1613.

SWAMINATHAN, P., WESTHOF, E. & SUNDARALINGAM, M. (1980). Am. Crystallogr. Assoc. Summer Meet., Calgary, Alberta. Abstract I5, p. 30.

THEWALT, U., BUGG, C. E. & MARSH, R. E. (1970). *Acta Cryst.* B**26**, 1089–1101.

WANG, A. H. J., DAMMANN, L. G., BARRIO, J. R. & PAUL, I. C. (1974). *J. Am. Chem. Soc.* **96**, 1205–1213.

WESTHOF, E., RAO, S. T. & SUNDARALINGAM, M. (1980). *J. Mol. Biol.* **142**, 331–361.

WESTHOF, E. & SUNDARALINGAM, M. (1980*a*). *Proc. Natl Acad. Sci. USA*, **77**, 1852–1856.

WESTHOF, E. & SUNDARALINGAM, M. (1980*b*). *J. Am. Chem. Soc.* **102**, 1493–1500.