Conformational Flexibility in Single-Stranded Oligonucleotides: Crystal Structure of a Hydrated Calcium Salt of Adenylyl-(3′-5′)-adenosine†

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ABSTRACT: The crystal and molecular structure of a hydrated calcium salt of adenylyl-(3′-5′)-adenosine(ApA) was determined from X-ray diffraction data collected on an automated diffractometer. Crystals of the salt are orthorhombic, space group $P2_12_12$, with a = 30.614 (3), b = 17.894 (2), and c = 5.373 (1) Å. The structure was solved by a combination of Patterson and direct methods and refined by least squares. The final value of the R index is 0.08. The 5′-terminal adenosine residue has a C(2′)-endo ribose and assumes a syn conformation, which is stabilized by an O(5′)-H···N(3) hydrogen bond within the nucleoside. The 3′-terminal nucleoside has a C(3′)-endo ribose and is in the anti conformation. Both ω and ω', the torsion angles within the phosphodiester group, are ~60°. Adenine bases from adjacent anions are joined by pairs of N(6)-H···N(1) hydrogen bonds and are stacked with symmetry-related bases. The calcium ion is bound to the dinucleoside phosphate by a direct interaction with the phosphate group and by outer-sphere, ligand-mediated interactions with O(2′) of the 5′-terminal nucleoside and N(7) of the 3′-terminal nucleoside. This tridentate interaction of the ApA anion with the calcium coordination sphere probably enhances the stability of the observed ApA conformation. When combined with other crystallographic studies of ApA conformations, the crystal structure of this calcium salt provides additional evidence that dinucleoside phosphates have considerable conformational flexibility.

Most types of ribonucleic acid (RNA) are composed of double-helical domains, among which are interspersed single-stranded, nonhelical segments. The recent structure determinations of yeast phenylalanine transfer RNA (Quigley et al., 1975; Sussman & Kim, 1976; Ladner et al., 1975; Stout et al., 1976) illustrate this structural organization. The helical regions of RNA molecules are thought to be reasonably well characterized, primarily as the result of X-ray diffraction studies of tRNA, of other polynucleosides (Arnott et al., 1972), and of oligonucleotide models that form double-helical structures (Seeman et al., 1976; Rosenberg et al., 1976; Hingerty et al., 1976). Less is known about the structures that are assumed by nonhelical segments of nucleic acids. Although crystallographic studies of tRNA have provided some excellent examples of conformations in single-stranded regions (Kim & Sussman, 1976; Jack et al., 1976a,b; Sundaralingam et al., 1976; Quigley & Rich, 1976), most of the data concerning possible conformations of these regions have come from crystallographic investigations of simple nucleosides and nucleotides.

Crystallographic studies have shown that nucleosides can assume a broad range of conformations but that this range is restricted when a phosphate group is added to the O(5′) position. The resulting 5′-nucleotides tend to assume solid-state conformations in which the glycosidic torsion angle is in the anti region, the torsion angles around the C(4′)–C(5′) bond are gauche, and the ribose conformation is either C(2′)- or C(3′)-endo. In addition, crystallographic studies of a variety of 3′- and 5′-nucleotides have shown that the main-chain conformations around the C(5′)–O(5′) and C(3′)–O(3′) bonds tend to be trans. These findings have led to the hypothesis that 5′-nucleotide subunits in polynucleotides may be considered as relatively inflexible units and that conformational flexibility within oligo- and polynucleotides is accordingly limited mainly to rotation about the phosphodiester bonds (Yathindra & Sundaralingam, 1973).

Efforts have been made to use conformational data from studies of simple nucleotides and oligonucleotides to aid in the prediction of the structures that might be expected for oligo- and polynucleotides (Sundaralingam, 1973; Kim et al., 1973). These predictions are fairly accurate in reproducing the structures of double-stranded, helical segments of nucleic acids when base-stacking and base-pairing constraints are imposed. However, it appears that the conformations of single-stranded regions are less predictable. The fundamental problem is that a single dinucleoside phosphate within an oligo- or polynucleotide has six main-chain dihedral angles that are subject to variation. Two of these, the two within the phosphodiester group, have relatively greater freedom of rotation than the others. Although conformations of the remaining four torsion angles may be limited to certain ranges, it is clear that these ranges are generally broad (20–60°) and that some of the torsion angles can occupy more than one range. While certain of the torsion angles appear to be highly correlated with others, the number of conformational degrees of freedom associated with the main chain in oligo- and polynucleotides remains large enough to impair efforts to predict the structures of single-strand regions.

In this paper we wish to further emphasize the flexibility of simple oligonucleotides as evidenced by limited crystallographic data now available for oligonucleotides composed of adenosine residues. We describe the crystal structure of the hydrated calcium salt of adenylyl-(3′-5′)-adenosine (ApA) and compare it with the crystal structures of adenylyl-(3′-5′)-adenylyl-(3′-5′)-adenosine (ApA^+pA^+; Suck et al., 1976) and of a complex of proflavin and adenylyl-(3′-5′)-adenosine (A^+pA; Neidle et al., 1978). These three crystal structures provide four examples of solid-state conformations of closely related ApA dinucleoside phosphates. The range of ApA conformations in this set provides evidence for significant conformational flexibility in single-stranded oligonucleotides.

Experimental Procedures

Clear, needle-like crystals of hydrated $Ca(ApA)_2$ were obtained by slowly cooling a hot aqueous solution, adjusted to pH 6, which contained a 2:1 molar ratio of $CaBr_2$ and the

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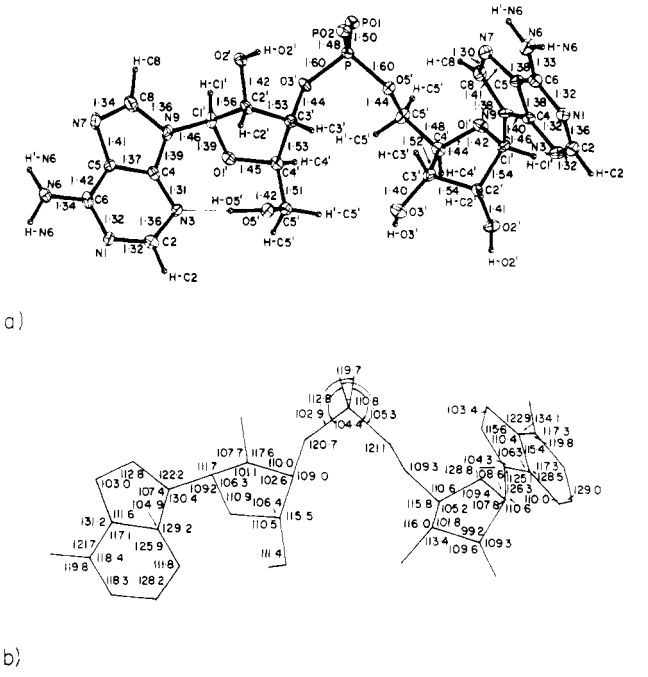
Table I: Crystal Data

stoichiometry	$Ca(C_{20}H_{24}O_{10}N_{10}P)_2 \cdot (4 + x)\ H_2O$
Z	2
space group	$P2_12_12$
a	30.614 (3) Å
b	17.894 (2) Å
c	5.373 (1) Å
ρ (calcd)	1.714 g cm^{-3}

dinucleoside phosphate. Oscillation and Weissenberg photographs showed the crystals to be orthorhombic, and space group $P2_12_12$ was indicated by the systematic absence of reflections $h00$ with h odd and $0k0$ with k odd. A tiny needle fragment with approximate dimensions of 0.2 × 0.02 × 0.01 mm was aligned on a Picker FACS-1 diffractometer with its c axis slightly inclined to the ϕ axis of the diffractometer. Approximate cell parameters for use in collection of intensity data were calculated by a least-squares analysis of the angular settings for several medium-angle reflections (Cu Kα,λ = 1.5418 Å). Intensity data were collected by use of a θ–2θ scanning technique. The scanning speed was 0.5°/min, and the background was counted for 40 s at each terminus of the scans. Scan ranges were calculated with a base width of 1.2° augmented to account for wavelength dispersion. Measurements were made for each of the 1833 independent reflections with $2\theta < 100°$. Three reference reflections, which were monitored periodically, showed no significant intensity fluctuations during the collection of intensity data. More precise values for the unit cell parameters were determined immediately after data collection by a least-square analysis of 2θ values for 19 medium-angle reflections (Cu Kα, λ = 1.5418 Å; T = 22 ± 2 °C). These cell parameters, which were used for all subsequent calculations, are not significantly different from those obtained prior to collecting intensity data; they are listed in Table I together with other crystal data.

Reflections with scan counts below background levels were given their calculated negative intensity values and were retained in all subsequent calculations. The intensites were assigned $\sigma^2(I)$ according to the statistics of the scan and background counts plus an additional term $(0.03S)^2$, S being the scan count. Intensities and their variances were corrected for Lorentz and polarization effects; absorption corrections were not applied (μ = 15.7 cm^{-1}).

The trial structure was obtained by a combination of Patterson methods and direct methods with the aid of the computer program MULTAN 78 (Main et al., 1978). Examination of a three-dimensional Patterson map revealed the location of the calcium ion, which occupies a special position of 2-fold symmetry, and the phosphorus atom of the phosphate group. A three-dimensional Fourier synthesis calculated with phases based on the calcium and phosphorus positions permitted the location of the four phosphate oxygen atoms. The coordinates for these six atoms were used in the calculation of normalized structure factors by MULTAN 78. The E map computed from the phase set with the highest combined figure of merit contained peaks corresponding to one complete nucleoside and three atoms of the ribose of the second nucleoside. A Fourier synthesis phased on these atoms revealed the positions of the remaining nonhydrogen atoms of the dinucleoside phosphate and of two water molecules. Four water positions with partial occupancy were subsequently added to the model on the basis of difference Fourier maps and successive least-squares refinement cycles. These partially occupied water positions were assigned occupancies of 0.5 based on peak heights in the difference maps; occupancy values were held constant during the refinement.



(a)

(b)

FIGURE 1: Bond distances (Å) and angles (deg) in the ApA anion. Esd's are 0.01–0.02 Å and 1° for distances and angles, respectively. This drawing and those in Figures 2–4 were made with the aid of the computer program ORTEP (Johnson, 1965). Thermal ellipsoids are drawn at the 20% probability level.

The trial structure was refined by use of a modified version of the full-matrix least-squares program ORFLS (Busing et al., 1962; Busing, 1971). The quantity minimized was $\sum w(F_o^2 - F_c^2/k^2)^2$, where k is a scale factor and the weight w is equal to $1/\sigma^2(F_o^2)$. Scattering factors and anomalous dispersion corrections were from Tables 2.2A, 2.2C, and 2.3.1 of the *International Tables for X-ray Crystallography* (1974). Hydrogen atoms for the dinucleoside phosphate and the two water molecules at full occupancy were located in difference Fourier maps during the later stages of refinement. The hydrogen atoms were assigned the isotropic temperature factors of the atoms to which they are bonded and were included in the calculation of structure factors but not in the least-squares refinement. The final R index $(\sum||F_o| - |F_c||/\sum|F_o|)$ is 0.080, and the goodness-of-fit $([\sum w(F_o^2 - F_c^2)^2/(m - s)]^{1/2}$, where m is the number of reflections used and s is the number of parameters refined) is 1.33. Considering only those 1499 reflections with $F_o^2 > \sigma(F_o^2)$, the R index is 0.066 and the goodness of fit is 1.43. All parameter shifts during the last cycle of refinement were <0.3 σ. A final difference Fourier map showed no peaks or troughs that exceeded 0.6 e Å^{-3}. [Tables of thermal parameters and a list of structure factors are available (see paragraph at end of paper regarding supplementary material).]

Results

Atomic coordinates with estimated standard deviations are listed in Tables II and III. Errors in atomic positions are about 0.003 Å for Ca^{2+} and P and 0.01 Å for C, N, and O atoms. The conformation of the dinucleoside phosphate is shown in Figure 1. Included in this figure are the bond lengths and angles involving only nonhydrogen atoms. Pertinent torsion angles are listed in Table IV. The ApA anion assumes an extended destacked conformation in this crystal structure. The values of the torsion angles ω and ω' correspond to those of the antiparallel conformation A_1 of Kim et al. (1973). Similar conformations about the phosphodiester group have been observed previously in other crystal structures, notably

Table II: Non-Hydrogen-Atom Coordinates[a]

atom	X	Y	Z
Ca	10000	10000	9703 (7)
P	9376 (1)	9128 (2)	4583 (7)
O(1)	9444 (3)	9578 (4)	225 (1)
O(2)	9608 (3)	9340 (4)	690 (2)
	5′-Terminal Adenosine		
N(1)	6900 (3)	8691 (6)	1439 (2)
C(2)	7149 (5)	8284 (7)	1291 (3)
N(3)	7439 (3)	8523 (6)	1116 (2)
C(4)	7457 (4)	9254 (8)	1106 (3)
C(5)	7218 (4)	9739 (7)	1249 (3)
C(6)	6941 (4)	9423 (9)	1432 (3)
N(6)	6708 (3)	9850 (6)	1589 (2)
N(7)	7316 (4)	10490 (6)	1192 (2)
C(8)	7602 (4)	10429 (8)	1004 (4)
N(9)	7711 (3)	9709 (6)	954 (2)
C(1′)	8036 (4)	9513 (7)	765 (2)
O(1′)	7885 (2)	8905 (5)	628 (2)
C(2′)	8477 (4)	9265 (7)	885 (2)
O(2′)	8725 (2)	9916 (4)	933 (2)
C(3′)	8650 (4)	8724 (7)	687 (3)
O(3′)	8855 (2)	9134 (4)	490 (2)
C(4′)	8232 (4)	8366 (7)	588 (2)
C(5′)	8113 (4)	7623 (8)	703 (3)
O(5′)	8112 (3)	7663 (4)	966 (2)
	3′-Terminal Adenosine		
N(1)	11169 (3)	5879 (6)	1028 (2)
C(2)	10953 (5)	5425 (8)	865 (3)
N(3)	10693 (3)	5608 (5)	681 (2)
C(4)	10646 (4)	6343 (7)	671 (3)
C(5)	10845 (4)	6879 (7)	816 (3)
C(6)	11115 (4)	6609 (8)	1002 (3)
N(6)	11333 (4)	7054 (6)	1158 (2)
N(7)	10703 (3)	7600 (6)	746 (2)
C(8)	10429 (4)	7468 (7)	566 (3)
N(9)	10379 (3)	6728 (6)	502 (2)
C(1′)	10072 (4)	6393 (6)	325 (2)
O(1′)	9902 (3)	6966 (5)	171 (2)
C(2′)	9686 (4)	6030 (6)	464 (3)
O(2′)	9499 (3)	5473 (4)	312 (2)
C(3′)	9377 (4)	6711 (6)	477 (3)
O(3′)	8944 (3)	6520 (4)	536 (2)
C(4′)	9446 (4)	7067 (7)	224 (2)
C(5′)	9323 (4)	7866 (7)	203 (2)
O(5′)	9495 (2)	8268 (4)	413 (1)
	Water		
W(1)	9580 (3)	11091 (4)	1054 (2)
W(2)	8391 (3)	6292 (5)	1128 (2)
W(3)	8578 (7)	11291 (13)	728 (5)
W(4)	8693 (6)	11249 (11)	1214 (4)
W(5)	7063 (8)	6480 (13)	495 (6)
W(6)	6083 (6)	6059 (9)	511 (5)

[a] All X and Y parameters have been multiplied by 10^4; all Z parameters have been multiplied by 10^3 except those of the Ca and P atoms, which are multiplied by 10^4. Atom positions W(3)–W(6) are assigned occupancies of 0.5.

Table III: Hydrogen Coordinates ($\times 10^3$)

atom	X	Y	Z
	5′-Terminal Adenosine		
H(C2)	711	775	1326
H1(N6)	649	960	1697
H2(N6)	672	1035	1584
H(C8)	777	1087	920
H(C1′)	811	992	648
H(C2′)	842	902	1046
H(O2′)	897	979	1038
H(C3′)	882	835	774
H(C4′)	828	828	407
H1(C5′)	784	746	639
H2(C5′)	832	724	646
H(O5′)	785	800	1050
	3′-Terminal Adenosine		
H(C2)	1099	490	901
H1(N6)	1153	685	1287
H2(N6)	1132	758	1150
H(C8)	1028	785	484
H(C1′)	1020	601	221
H(C2′)	978	583	628
H(O2′)	943	510	437
H(C3′)	947	708	610
H(O3′)	876	644	397
H(C4′)	926	679	100
H1(C5′)	941	807	44
H2(C5′)	899	791	196
	Water		
H1(W1)	930	1120	1130
H2(W1)	950	1140	900
H1(W2)	820	670	1070
H2(W2)	830	590	1020

Table IV: Torsion Angles (Degrees)[a]

		5′	3′
χ	O(1′)–C(1′)–N(9)–C(8)	−138, syn	16, anti
ρ	C(5′)–C(4′)–C(3′)–O(3′)	147	77
ψ	O(5′)–C(5′)–C(4′)–C(3′)	52	46
ϕ_{oo}	O(5′)–C(5′)–C(4′)–O(1′)	−69	−73
τ_0	C(4′)–O(1′)–C(1′)–C(2′)	−16	8
τ_1	O(1′)–C(1′)–C(2′)–C(3′)	32	−30
τ_2	C(1′)–C(2′)–C(3′)–C(4′)	−34	39
τ_3	C(2′)–C(3′)–C(4′)–O(1′)	27	−37
τ_4	C(3′)–C(4′)–O(1′)–C(1′)	−7	19
ϕ'	C(4′)–C(3′)–O(3′)–P	−141	
ω'	C(3′)–O(3′)–P–O(5′)	61	
ω	O(3′)–P–O(5′)–C(5′)		61
ϕ	P–O(5′)–C(5′)–C(4′)		−174
τ_m		−34	39
P		172	9

[a] Esd's are ~1°.

those of uridylyl-(3′–5′)-adenosine (Sussman et al., 1972) and ApA^+pA^+ (Suck et al., 1976). Quite recently, this conformation has been identified as one of two alternating phosphodiester conformations in a new family of left-handed double-helical structures discovered by crystal structure determinations of self-complementary deoxyoligonucleotides [Wang et al. (1980) and references cited therein] and fiber diffraction studies of synthetic DNA polynucleotide duplexes [Leslie et al. (1980) and references cited therein].

In hydrated $Ca(ApA)_2$, the two sugar rings show different conformations: the 5′-terminal ribose is C(2′)-endo, and the 3′-terminal ribose is C(3′)-endo. In the C(2′)-endo ribose, C(2′) is displaced by 0.56 (1) Å from the least-squares plane through C(1′), O(1′), C(3′), and C(4′), none of which deviate by more than 0.04 (1) Å from the plane. In the C(3′)-endo ribose, C(3′) is 0.62 (1) Å out of the least-squares plane through the other four atoms, and none of the other four atoms deviate by more than 0.04 (1) Å from this plane. The pseudorotation parameters τ_m and P (Altona & Sundaralingam, 1972) for the two ribose rings are included in Table IV.

The two nucleosides of the ApA anion display different conformations about the glycosidic linkages. The 3′-terminal adenosine residue is in the more common anti conformation (Donohue & Trueblood, 1960). The 5′-terminal adenosine residue assumes a syn conformation; as is often found for purine nucleosides that display syn conformations, an O(5′)-H···N(3) hydrogen bond is formed within the nucleoside (Rao & Sundaralingam, 1970). Both nucleosides display gauche–gauche conformations about the C(4′)–C(5′) bonds. The purine rings are essentially planar, with no ring–atom deviations that are >0.04 (1) Å, although deviations by substituent atoms N(6) and C(1′) reach 0.07–0.15 Å.

Distances and angles in the dinucleoside phosphate are for the most part similar to those in other adenosine and adenylate

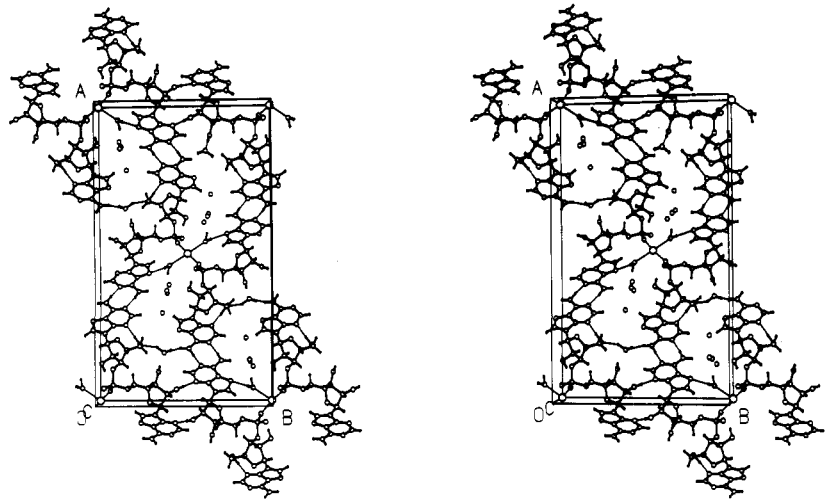


FIGURE 2: Stereo representation of the three-dimensional crystal structure. Interactions within a sheet of ApA anions parallel to the *ab* plane are emphasized.

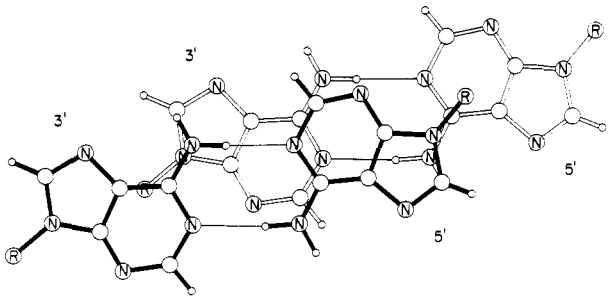


FIGURE 3: Base pairing and base stacking.

crystal structures, but a comparison of analogous distances within the molecule reveals considerable variation, as much as 0.03–0.04 Å between bond distances and 3° in bond angles. Some of these variations, such as the differences in bond angles at N(9) for the syn 5′-terminal nucleoside and the anti 3′-terminal nucleoside, may reflect real differences in molecular environment. Many of the variations are, however, probably attributable to experimental errors.

Hydrogen-bond distances and angles are given in Table V, and the crystal packing scheme is depicted in Figure 2. The ApA anions are assembled in columns parallel to the *c* axis. Within these columns adenines are stacked, and columns adjacent in *a* are joined together by pairs of N(6)-H···N(1) hydrogen bonds between neighboring adenines; these stacking and hydrogen-bonding interactions are depicted in Figure 3. The stacking interactions are between the bases of ApA anions that are related by the *c* translation. The interplanar spacings between stacked purine rings are 3.4–3.7 Å. A major feature of the stacking pattern is the interaction between amino groups and imidazole rings; similar stacking interactions are found in other crystal structures of adenine nucleosides (Bugg, 1972). In addition to the stacking contacts that are shown in Figure 3, the ribose O(1′) atoms form close contacts (3.1–3.5 Å) with the atoms in adjacent bases; these O(1′)–purine stacking interactions are typical of those found in crystal structures of nucleosides and nucleotides (Bugg et al., 1971).

As shown in Figure 3, the adenine bases form hydrogen-bonded pairs. This base pairing joins 3′- and 5′-terminal adenosines on molecules related by the 2-fold screw axes that run parallel to *a*. The hydrogen bonds join an N(6) donor and N(1) acceptor on a 3′-terminal adenosine to an N(1) acceptor and N(6) donor on a 5′-terminal adenosine. Similar pairings between adenine residues have been observed previously [Voet & Rich (1970) and references cited therein]. In this structure, adenine pairing produces wavy ribbons of ApA anions extending parallel to *a*. As seen in Figure 2, these ribbons are linked to ribbons adjacent in *b* by calcium coordination and by hydrogen bonding, either directly or via water molecules. The result is a pleated-sheet structure perpendicular to *c*. Sheets adjacent in *c* are linked by base-stacking interactions, calcium coordination, and additional hydrogen bonds.

The calcium coordination pattern is illustrated in Figure 4. The calcium ion is coordinated to six oxygen atoms that are arranged in a distorted octahedron. Because the calcium ions are positioned on 2-fold crystallographic axes, only three kinds of ligand atoms are used for calcium binding. These are the phosphate oxygen atoms O(1) and O(2) and the water molecule W(1). Calcium–oxygen distances are 2.258 (3) Å to

Table V: Hydrogen Bond Distances and Angles[a]

donor, D	hydrogen, H	acceptor, A	symmetry code[b]	D···A (A)	H···A (A)	D-H···A (deg)
O(5′)	H(O5′)	N(3)		2.70	1.61	169
N(6)	H1(N6)	N(1)*	a	2.94	1.97	166
N(6)	H2(N6)	W(2)	b	3.01	2.31	135
N(6)*	H1(N6)*	N(1)	c	3.08	2.08	173
N(6)*	H2(N6)*	W(4)	d	3.05	2.12	168
O(2′)	H(O2′)	O(1)	e	2.77	1.80	178
O(2′)*	H(O2′)*	N(3)*	f	2.83	1.87	179
O(3′)*	H(O3′)*	W(2)	g	2.80	1.85	180
W(1)	H1(W1)	N(7)*	d	3.00	2.07	149
W(1)	H2(W1)	W(4)		2.86	1.92	165
W(1)	H2(W1)	W(6)	h	3.10	2.27	142
W(2)	H1(W2)	O(5′)		2.74	1.83	152
W(2)	H2(W2)	N(7)	h	3.11	2.32	140
W(3)		O(2′)		2.73		
W(3)		N(7)*	d	2.97		
W(3)		W(4)		2.64		
W(3)		W(4)	g	2.78		
W(4)		O(2′)		2.82		
W(5)		W(4)	h	2.80		
W(5)		N(7)	h	3.10		
W(5)		W(6)		3.09		
W(6)		O(2′)	i	3.20		
W(6)		N(7)*	j	3.00		

[a] Atoms belonging to the 3′-terminal adenosine are indicated with an asterisk. Note that water molecules W(3)–W(6) are of fractional occupancy. Esd's are 0.01 A for D···A distances, ~0.10 A for H···A distances, and 4–6° for D-H···A angles. [b] (a) $x - 1/2$, $3/2 - y$, $3 - z$; (b) $3/2 - x$, $1/2 + y$, $3 - z$; (c) $1/2 + x$, $3/2 - y$, $3 - z$; (d) $2 - x$, $2 - y$, z; (e) x, y, $1 + z$; (f) $2 - x$, $1 - y$, z; (g) x, y, $z - 1$; (h) $3/2 - x$, $y - 1/2$, $2 - z$; (i) $3/2 - x$, $y - 1/2$, $1 - z$; (j) $x - 1/2$, $3/2 - y$, $1 - z$.

Table VI: Comparison of Corresponding Torsion Angles in ApA Segments from the Crystal Structures of Hydrated $Ca(ApA)_2$, a Proflavin–A^+pA Complex, and the Trinucleoside Diphosphate ApA^+pA^+ (ApA^+ and A^+pA^+) and from 11-fold RNA[a]

	(a) ApA	(b) A^+pA	(c) A^+pA^+	(d) ApA^+	(e) RNA
χ'	−138	−119	28	8	14
ϕ'	−141	−88	−151	−137	−152
ω'	61	−70	77	−77	−74
5′-terminal ribose	C(2′)-endo	C(2′)-endo	C(3′)-endo	C(3′)-endo	C(3′)-endo
ω	61	−67	93	−63	−62
ϕ	−174	175	−172	161	180
ψ	46	168	56	53	47
χ	16	71	27	28	14
3′-terminal ribose	C(3′)-endo	C(3′)-endo	C(3′)-endo	C(3′)-endo	C(3′)-endo

[a] The torsion angles are defined in Table IV.

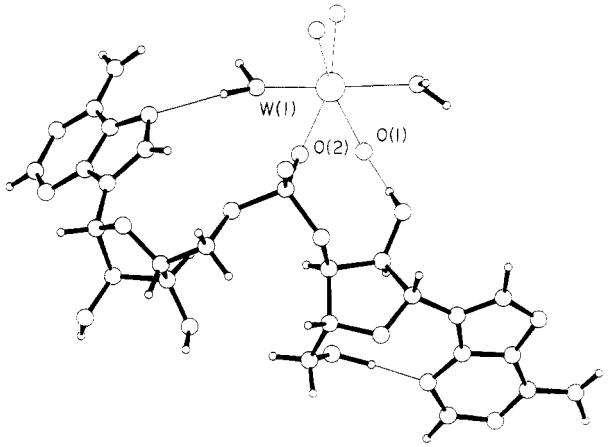


FIGURE 4: The tridentate interaction between the ApA anion and the Ca^{2+} ion.

O(2), 2.310 (3) Å to O(1), and 2.381 (3) Å to W(1). The phosphate oxygen atoms in the calcium coordination shell are all from different crystallographically related ApA anions. Nevertheless, there is a strong interaction between calcium and ApA ions that may be described as a tridentate attachment of the anion to the calcium coordination sphere. This interaction, shown in Figure 4, involves direct binding between calcium and ApA ions via O(2) and indirect binding conducted through other ligands in the coordination polyhedron. The two indirect bonds are (1) a water-mediated interaction linking N(7) of the 3′-terminal adenosine to the calcium ion by a hydrogen bond in which W(1) is the donor and (2) a linkage via O(1) of an anion adjacent in *c* that is the result of a hydrogen bond to O(1) from O(2′) of the 5′-terminal adenosine.

The role of water in the hydrogen-bonding scheme is complex, and it is further complicated by partial occupancy and disorder. The disordered water molecules, W(3)–W(6), are involved in hydration of O(2′) of the 5′-terminal adenosine, N(7) of the 3′-terminal adenosine, and, to a lesser extent, N(7) of the 5′-terminal adenosine. Several strategies to accomplish this hydration are apparently employed. The result is columns of partially occupied water sites that run parallel to *c*. Although not all of these sites can be occupied simultaneously, it is clear that in these regions of the structure the interactions between ApA anions are conducted through water molecules.

Discussion

Most of the oligonucleotides that have been studied crystallographically are self-complementary. In crystals, they generally form base-paired structures with conformations closely resembling those expected in double helices. ApA is incapable of forming a conventional double-stranded structure, and, in the absence of constraints that might be imposed by complementary base pairing, it is free to display any conformation that may be accessible to single-stranded oligonucleotides. Furthermore, NMR studies suggest that, under solvent conditions in which base stacking is not favored, mono- and oligonucleotides of adenosine are not conformationally more restricted than the nucleoside (Evans & Sarma, 1976). It is of some interest to compare the conformation of the ApA anion in this structure with those found for ApA dinucleoside phosphate units in other crystal structures. The values of several torsion angles describing the ApA conformation in hydrated $Ca(ApA)_2$ are compared in Table VI with corresponding values from the crystal structures of the proflavin–A^+pA complex (Neidle et al., 1978), the dinucleoside phosphate segments in the crystal structure of ApA^+pA^+ (Suck et al., 1976), and the ApA units from 11-fold, double-helical RNA (Arnott et al., 1972). The nucleoside subunits in these structures display several common features; for example, the 3′-terminal ribose conformations in all are C(3′)-endo, the 5′-terminal ribose conformations are either C(2′)-endo or C(3′)-endo, and the ϕ[P–O(5′)–C(5′)–C(4′)] torsion angles are all near 180°. The ApA^+ conformation in ApA^+pA^+ is closely related to that found within double-helical RNA. In only one example, from the proflavin–A^+pA structure, does the conformation of the 3′-terminal nucleoside differ strikingly from the preferred conformation in the solid state. Only two distinctly different conformations about the phosphodiester linkage are represented. Nevertheless, in most cases, corresponding torsion angles in these five examples display considerable variation, and four very different overall conformations result.

A more revealing summary of the conformational flexibility within these ApA units is depicted in the stereoscopic drawings of Figure 5. To emphasize the relationships among the ApA oligonucleotides, we show all of them as viewed perpendicular to the C(4′)–O(1′)–C(1′) planes of 3′-terminal ribose sugars, with the C(4′)–O(1′) vectors oriented in the horizontal direction. When viewed in this manner, it is clear that these dinucleoside phosphates cover a wide range of conformations. The similarity between the ApA^+ conformation and that of double-helical RNA can be readily seen by comparing parts d and e of Figure 5, but this conformation is quite different from those depicted in parts a–c of Figure 5.

There are reasons to expect that the conformations of oligonucleotide units within polynucleotides may be more restricted than within a simple dinucleoside phosphate (Sundaralingam & Westhof, 1979). However, the observed ApA structures in crystallographic studies of nucleic acid components confirm that dinucleoside phosphates not involved in base-paired double-helical structures can exhibit a number of very different conformations that have profound implications

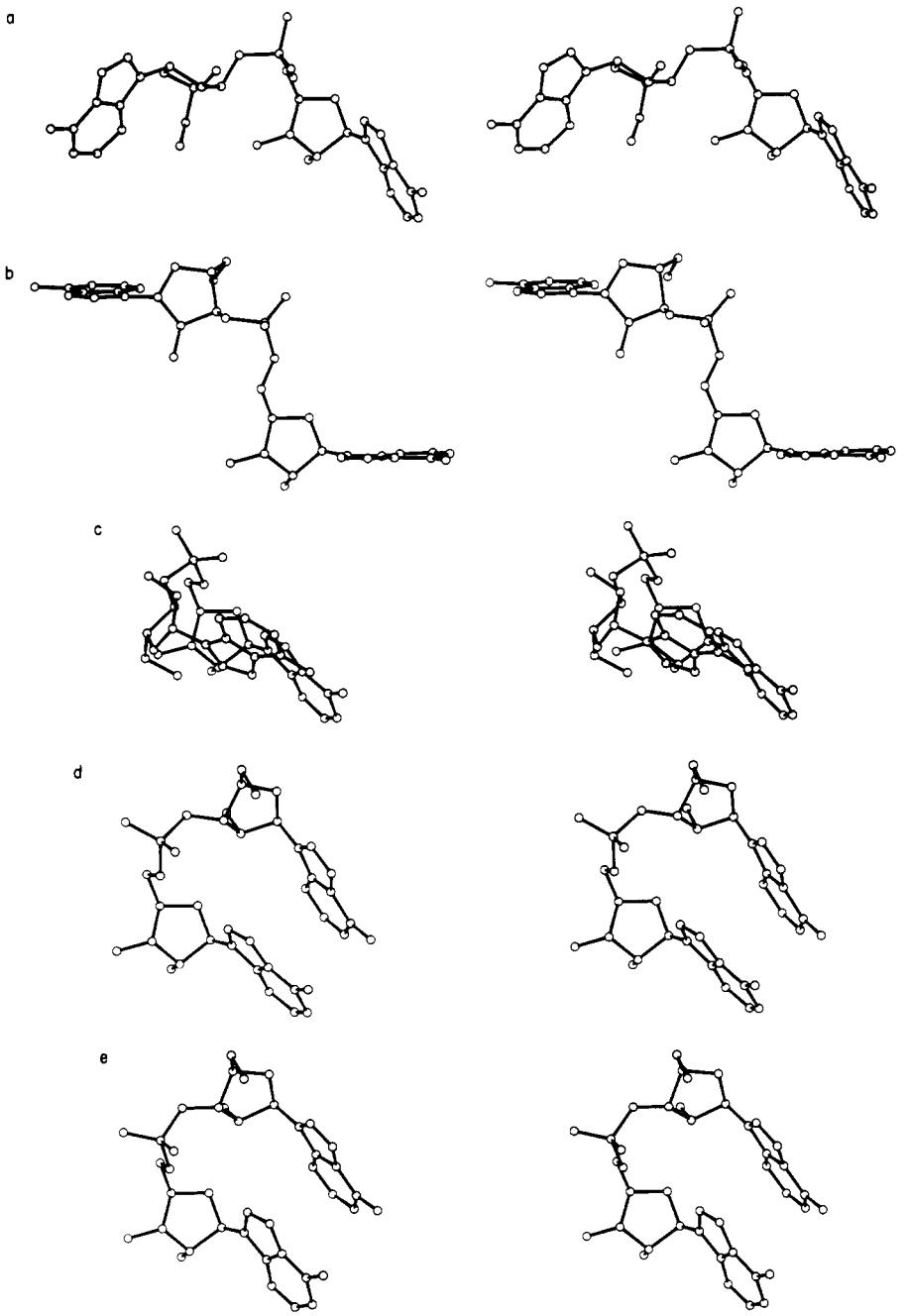


FIGURE 5: Stereodrawings showing the conformations of the ApA units in (a) the crystal structure of hydrated Ca(ApA)$_2$, (b) the crystal structure of a proflavin–A$^+$pA complex, (c) the crystal structure of ApA$^+$pA$^+$ (A$^+$pA$^+$ segment), (d) the crystal structure of ApA$^+$pA$^+$ (ApA$^+$ segment), and (e) 11-fold RNA. The 3′-terminal ribose unit is shown in the same orientation in all examples.

for the course of the main chain. A number of examples of single-stranded regions that illustrate conformational flexibility are found in the structure of tRNA. The actual conformation that is assumed by a dinucleoside phosphate unit in a single-stranded region can be expected to be highly dependent upon environmental factors. These factors might include interactions with metal ions as in the crystal structure of hydrated Ca(ApA)$_2$, the formation of suitable hydrogen bonds between various segments of a polynucleotide, intrastrand and interstrand base-stacking interactions, and intermolecular interactions between nucleic acids and proteins or other types of molecules. Considering the evidence that nucleosides, nucleotides, oligonucleotides, and polynucleotides have all demonstrated sizable ranges of values for the torsion angles that define the course of the sugar–phosphate chain, it appears that the problems of predicting the structures of single-stranded regions within nucleic acids remain formidable.

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Supplementary Material Available

A listing of thermal parameters and of observed and calculated structure factor amplitudes (14 pages). Ordering information is given on any current masthead page.

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