Crystal Structure of 2′-*O*-Succinyladenosine 3′,5′-(Cyclic)phosphate Monohydrate: A Model Compound to Study Protein–Nucleic Acid Interactions

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The crystal and molecular structure of 2′-*O*-succinyladenosine 3′,5′-(cyclic)phosphate monohydrate has been determined by X-ray diffraction techniques. The compound crystallizes in the orthorhombic space group $P2_12_12_1$; $Z=4$ with $a=5.633(1)$, $b=15.853(2)$, $c=20.607(3)$ Å. The final R value is 0.029. The adenine base is protonated at N(1). The base is in syn conformation ($\chi=-95.8°$) with respect to the sugar, which has a C(3′)-*endo*-C(4′)-*exo* pucker ($_4T^3$). A six-membered ring formed by phosphate group and ribose shows chair conformation. The succinic acid moiety, which resembles side chains of certain amino acids, is found to have a bent skeleton. The cyclic AMP, which is known to play diverse roles in many metabolic processes, forms the nucleotide portion of the title compound. The succinic acid moiety that is covalently linked to this nucleotide in the present compound, shows direct and water mediated interactions with phosphate and base nitrogens of the nucleotide respectively. Such compounds can therefore serve as models to study the protein-nucleic acid interactions, which play a central role in many of the biological processes.

Adenosine cyclic 3′,5′-phosphate (cAMP) is known to play a central role in various hormonal metabolic control mechanisms.[1,2] It is also known to influence the activity of certain enzymes.[3,4] In light of these observed biological properties a few crystal structure studies of cyclic nucleotides and their analogues have been carried out in the past,[5] to understand the conformational flexibility and interactions of the cyclic nucleotide in the presence of different chemical groups. We report in this context, the crystal structure of 2′-*O*-succinyladenosine 3′,5′-(cyclic)phosphate monohydrate. It has the cyclic nucleotide covalently linked to a succinic acid moiety that resembles the side chains of amino acids like glutamic acid, aspartic acid etc. It is of interest to study the interactions between the nucleotide and the succinic acid moiety in the title compound, as a model for amino acid side chain-nucleic acid interactions, which are important in determining the structures and functions of protein-nucleic acid assemblies. Structural studies on similar model compounds have been reported in literature.[6–10]

Experimental Studies and Structure Determination

Needle-shaped crystals were grown from an aqueous solution of the title compound which was purchased from Sigma Chemicals. Intensity data were collected on a CAD-4 diffractometer with Ni-filtered Cu$K\alpha$($\lambda=1.5418$ Å) radiation using a crystal of dimensions 0.04×0.06×0.64 mm. Cell parameters refined from 18 accurately measured reflections. The crystal data are given in Table 1.

Table 1. Crystal Data

Formula	$C_{14}H_{16}N_5O_9P_1\cdot H_2O$
F.W.	447.30
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
a/Å	5.633(1)
b/Å	15.853(2)
c/Å	20.607(3)
V/Å^3	1840.2
Z	4
D_x/g cm^{-3}	1.55

Intensities were measured using ω-2θ scans. Three strong reflections monitored periodically showed that the crystal was stable to X-rays. Absorption correction[11] applied: transmission factors varied from 91.9 to 98.1%. Lorentz and polarization corrections were also applied. 1784 reflections were measured in the range $0<\theta<65°$, of which 1656 reflections were considered to be observed with $|F_o|\geqslant 3\sigma(F)$.

The structure was solved by the direct methods using MULTAN11/82[12] and the calculations were performed with the Enraf-Nonius Structure Determination Package[13] on a PDP11-44 computer. An E-map computed with the best set of phases (CFOM=2.979) revealed the atoms of the six-membered ring formed by phosphate group and ribose sugar. A weighted Fourier synthesis at this stage gave rest of the structure. Of the 18 hydrogens, 17 were located from difference Fourier maps computed at various stages of full-matrix least-squares refinement on F's using SHELX 400[14] with the non-H atoms refined anisotropically and the H atoms refined isotropically. One of the water hydrogens which was not located from the difference maps, was fixed in the direction of the hydrogen bond initially, but was varied in the final stages of refinement. Final $R=0.029$, $R_w=0.032$; no. of parameters refined were 343. Individual weights $w\propto 1.0/[\sigma^2(F)+0.002\ F^2]$ were used. Maximum shift/error is 0.11; residual density in the final difference electron density map is within ± 0.20 e Å^{-3}. The atomic scattering factors are as supplied in SHELX 76.

Results and Discussion

The conformation of the molecule along with atomic numbering scheme is shown in Fig. 1. The

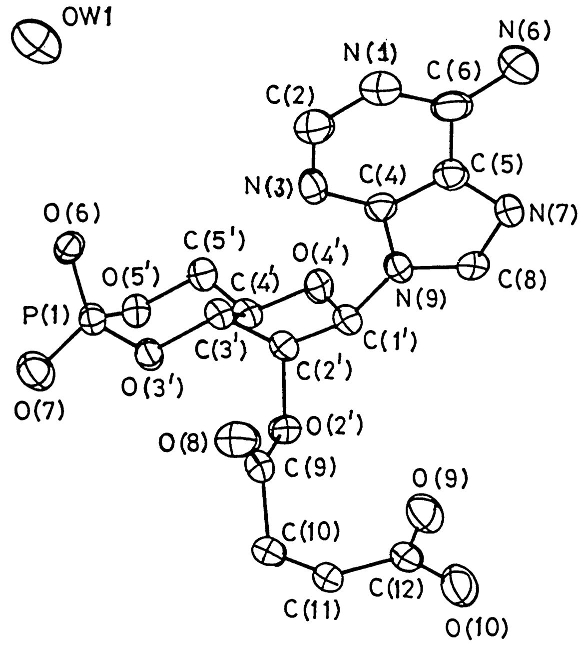

Fig. 1. A perspective view of the molecule with atomic numbering scheme and the thermal ellipsoids at 50% probability level.

Table 2. Final Fractional Coordinates and Equivalent Temperature Factors for Non-Hydrogens along with e.s.d.'s in Parentheses

$$U_{\mathrm{eq}}=(1/3)\sum_i\sum_j U_{ij}a_i^* a_j^* \mathbf{a}_i \cdot \mathbf{a}_j$$

Atom	$x^{a)}$	$y^{a)}$	$z^{a)}$	$U_{\mathrm{eq}}{}^{a)}/\text{Å}^2$
P(1)	4376(1)	2183(0)	7157(0)	283(2)
O(6)	3078(4)	1770(1)	7699(1)	380(7)
O(7)	4185(5)	3104(1)	7083(1)	444(8)
C(1′)	5841(6)	−185(2)	5762(1)	259(8)
C(2′)	3773(6)	457(2)	5791(1)	241(8)
O(2′)	4109(4)	1019(1)	5249(1)	271(6)
C(3′)	4291(5)	896(2)	6431(1)	244(8)
O(3′)	3548(4)	1758(1)	6480(1)	281(6)
C(4′)	6977(6)	833(2)	6474(2)	270(9)
O(4′)	7342(4)	−32(1)	6309(1)	327(6)
C(5′)	7811(6)	1067(2)	7138(2)	323(9)
O(5′)	7129(4)	1945(1)	7206(1)	343(7)
N(1)	114(5)	−2357(2)	6690(1)	377(9)
C(2)	280(7)	−1521(2)	6811(2)	398(11)
N(3)	1848(5)	−1009(2)	6563(1)	359(8)
C(4)	3350(6)	−1418(2)	6162(1)	268(8)
C(5)	3430(6)	−2272(2)	6022(1)	303(9)
C(6)	1665(6)	−2781(2)	6311(1)	334(9)
N(6)	1421(8)	−3604(2)	6225(2)	488(11)
N(7)	5235(5)	−2455(2)	5599(1)	348(8)
C(8)	6198(6)	−1718(2)	5478(1)	305(9)
N(9)	5123(5)	−1065(1)	5797(1)	267(7)
O(8)	157(4)	1170(2)	5153(1)	468(9)
O(9)	3730(5)	225(1)	3894(1)	541(9)
O(10)	6855(6)	620(2)	3345(1)	547(9)
C(9)	2120(6)	1314(2)	4959(1)	270(9)
C(10)	2672(7)	1857(2)	4382(2)	348(10)
C(11)	4945(7)	1663(2)	4022(2)	345(10)
C(12)	5091(7)	780(2)	3750(1)	386(10)
OW1	4632(7)	758(2)	9593(2)	681(12)

a) $\times 10^4$.

Table 3. Bond Lengths (l) and Bond Angles (Φ) Involving Non-Hydrogen Atoms along with the e.s.d.'s in Parentheses

Bond	l/Å	Bond	l/Å
P(1)-O(6)	1.487(2)	C(2)-N(3)	1.304(5)
P(1)-O(7)	1.472(2)	N(3)-C(4)	1.349(4)
P(1)-O(3′)	1.618(2)	C(4)-C(5)	1.385(4)
P(1)-O(5′)	1.599(2)	C(4)-N(9)	1.370(4)
C(1′)-O(4′)	1.430(3)	C(5)-C(6)	1.412(4)
C(1′)-C(2′)	1.548(5)	C(5)-N(7)	1.370(4)
C(1′)-N(9)	1.454(4)	C(6)-N(6)	1.324(5)
C(2′)-C(3′)	1.520(3)	N(7)-C(8)	1.312(4)
C(2′)-O(2′)	1.441(3)	C(8)-N(9)	1.368(4)
C(3′)-C(4′)	1.519(4)	C(9)-O(2′)	1.353(4)
C(3′)-O(3′)	1.433(4)	C(9)-O(8)	1.198(4)
C(4′)-C(5′)	1.494(6)	C(9)-C(10)	1.501(5)
C(4′)-O(4′)	1.428(4)	C(10)-C(11)	1.511(6)
C(5′)-O(5′)	1.451(4)	C(11)-C(12)	1.510(5)
N(1)-C(2)	1.352(5)	C(12)-O(9)	1.204(4)
N(1)-C(6)	1.351(4)	C(12)-O(10)	1.322(4)

Angle	Φ/°	Angle	Φ/°
O(6)-P(1)-O(7)	118.6(1)	N(1)-C(2)-N(3)	125.8(3)
O(6)-P(1)-O(5′)	109.0(1)	C(2)-N(3)-C(4)	111.5(3)
O(6)-P(1)-O(3′)	108.8(1)	N(3)-C(4)-C(5)	128.2(3)
O(7)-P(1)-O(5′)	108.1(1)	N(3)-C(4)-N(9)	126.7(3)
O(7)-P(1)-O(3′)	107.6(1)	C(5)-C(4)-N(9)	105.1(2)
O(3′)-P(1)-O(5′)	103.6(1)	C(4)-C(5)-C(6)	116.6(3)
P(1)-O(3′)-C(3′)	111.9(2)	C(4)-C(5)-N(7)	111.3(3)
C(2′)-C(3′)-C(4′)	102.2(2)	C(6)-C(5)-N(7)	132.0(3)
O(3′)-C(3′)-C(2′)	116.2(2)	C(5)-C(6)-N(1)	114.5(3)
O(3′)-C(3′)-C(4′)	110.5(2)	C(5)-C(6)-N(6)	125.4(3)
C(3′)-C(4′)-O(4′)	101.1(3)	N(1)-C(6)-N(6)	120.0(3)
C(3′)-C(4′)-C(5′)	110.5(3)	C(5)-N(7)-C(8)	103.8(3)
O(4′)-C(4′)-C(5′)	114.3(3)	N(7)-C(8)-N(9)	113.6(3)
C(4′)-C(5′)-O(5′)	104.1(3)	C(8)-N(9)-C(4)	106.1(2)
P(1)-O(5′)-C(5′)	118.5(2)	C(8)-N(9)-C(1′)	125.4(2)
C(4′)-O(4′)-C(1′)	105.4(2)	C(4)-N(9)-C(1′)	128.5(2)
O(4′)-C(1′)-C(2′)	107.6(2)	O(2′)-C(9)-O(8)	123.4(3)
O(4′)-C(1′)-N(9)	106.7(2)	O(8)-C(9)-C(10)	124.4(3)
N(9)-C(1′)-C(2′)	114.8(2)	O(2′)-C(9)-C(10)	112.1(3)
C(1′)-C(2′)-C(3′)	101.0(2)	C(9)-C(10)-C(11)	116.6(3)
C(1′)-C(2′)-O(2′)	106.1(2)	C(10)-C(11)-C(12)	114.6(3)
O(2′)-C(2′)-C(3′)	111.4(2)	C(11)-C(12)-O(9)	123.4(3)
C(2′)-O(2′)-C(9)	116.6(2)	C(11)-C(12)-O(10)	116.9(3)
C(6)-N(1)-C(2)	123.3(3)	O(9)-C(12)-O(10)	119.6(3)

final positional parameters with equivalent isotropic temperature factors for the non-H atoms are given in Table 2.[15] Bond lengths and bond angles are listed in Table 3.

Base: The molecule is found to be in zwitter ionic form with the adenine base protonated at N(1) and a single negative charge on the phosphate group. The bond lengths and bond angles in the base are in good agreement with the average values reported[16] for structures with protonated adenine base. The base shows syn conformation[17] with χ (C(8)-N(9)-C(1′)-O(4′))=−95.8°.

Ribose: The ribose in the present structure is covalently linked to succinic acid moiety at 2′-position and is cyclized between 3′ and 5′ ends by the phosphate group. The ribose ring shows C(3′)-

endo-C(4′)-*exo* pucker, a conformation generally found in cyclic 3′,5′-phosphate nucleotide structures.[5,18,19] The pseudorotation parameters[20] of the furanose ring are P=49.9° and τ_m=47.8°.

Phosphate: The six-membered ring formed by phosphate group and ribose is in the chair form with the best mirror bisecting C(4′) and P(1) atoms and with asymmetry parameter[21] ΔC_s[C(4′)-P(1)]=3.3°. The torsion angles ω' (O(5′)-P-O(3′)-C(3′)) and ω (C(5′)-O(5′)-P-O(3′)) fall in the region for 3′,5′-cyclic monophosphate nucleotides on ω' vs. ω map.[22]

Succinic Acid: The succinic acid moiety in the present structure is neutral. It shows a bent skeleton conformation (C(9)-C(10)-C(11)-C(12)=59.5°), which is not uncommon to this moiety.[23,24] In an analysis carried out on the energetically favored side-chain conformations of amino acids,[25] the glutamine and glutamic acid side chains which have a similar carbon backbone structure as that of succinic acid, show this bent conformation in about 50% of these residues in the total sample of oligopeptides considered. Also, this folded or gauche conformation is observed in many of the crystal structures of the compounds containing glutamic acid.[26]

A Comparison with the Biologically Important cAMP: The conformation of the nucleotide part in the present structure (χ=−95.7°, P=49.9°, and τ_m=47.8°) is close to that of one of the molecules in the asymmetric unit (χ=−90°, P=50°, and τ_m=48°) in the crystal structure of cAMP free acid.[5]

The ribose in the cyclic 3′,5′-phosphate nucleotides generally shows a small range of conformations ${}_4T^3 \leftrightarrow {}^3T_4$ and the six-membered ring containing the phosphate group is observed to have chair conformation with a slight flattening at the phosphate end. The flexibility of the cyclic nucleotide seems to lie mostly about the glycosidic bond, C(1′)-N(9), as can be seen from the fact that the cAMP and its analogues have been observed to be in anti or syn conformation and not generally restricted to the anti conformer as in the crystal structures of cyclic monophosphates of pyrimidines.

Hydrogen Bonding Pattern: The crystal structure is stabilized by a number of hydrogen bonds, the details of which are shown in Fig. 2 and Table 4. No base stacking has been observed.

In the present structure, a water molecule forms hydrogen-bonded bridge between the N(6) and N(7) of the same adenine base. This pattern has also been observed in crystal structure of a modified cAMP.[18] In a recent review,[27] on hydration of oligonucleotides in crystals, it is cited that such intra-residue bridges

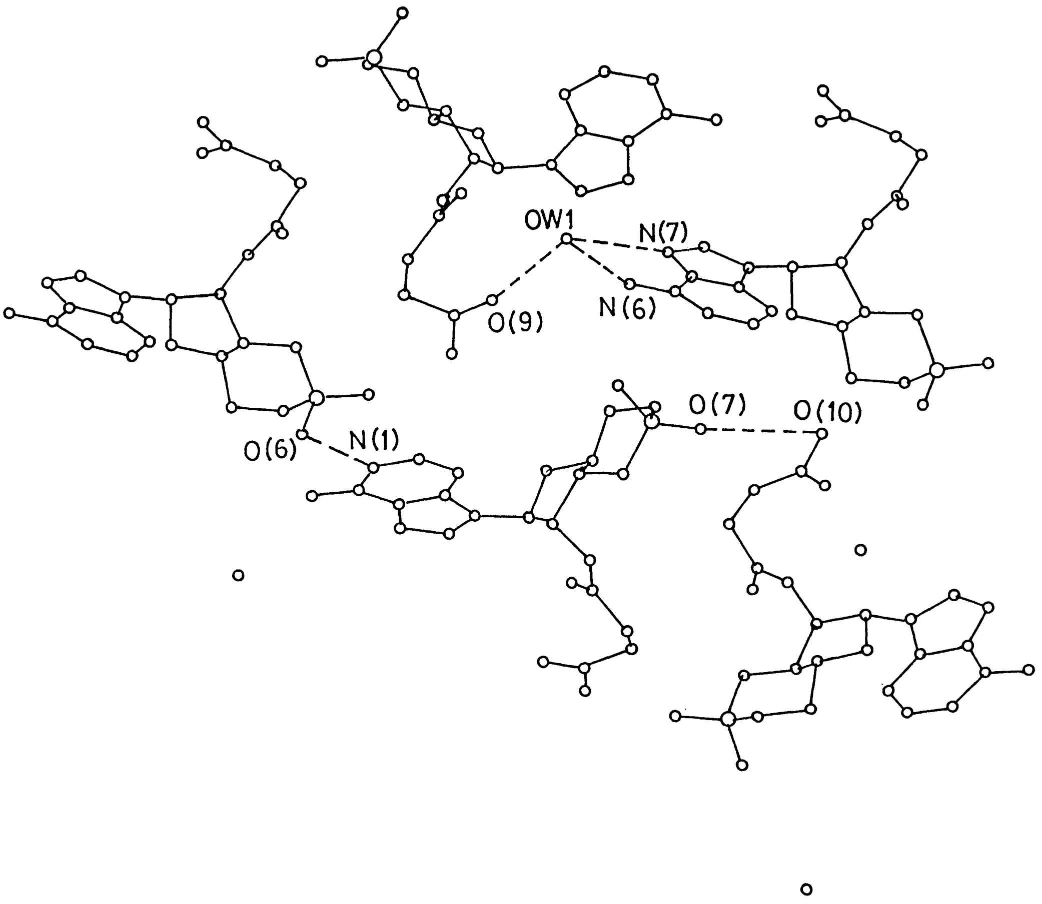

Fig. 2. Hydrogen bonding pattern in the crystal structure of the title compound.

Table 4. Details of Hydrogen Bonding

	D···A(l/Å)	H···A(l/Å)	D-H···A(Φ/°)
Base-phosphate			
N(1)-H···O(6^{i})	2.60	1.58	176.4
Succinic acid-phosphate			
O(10)-H···O(7^{ii})	2.57	1.50	166.7
Water-Succinic acid			
OW1-H···O(9^{iii})	2.84	2.06	171.4
Water-base			
OW1···H-N(6^{iv})	2.97	2.12	166.8
OW1-H···N(7^{iv})	2.86	1.99	154.2

(i) $-x$, $0.5+y$, $1.5-z$: (ii) $0.5+x$, $0.5-y$, $1.0-z$; (iii) $0.5-x$, $-y$, $0.5+z$; (iv) $1.0-x$, $0.5+y$, $1.5-z$.

have been observed for purines in crystals of all the three (A, B, and Z) forms of DNA. In general, bridging water molecules in several nucleic acid structures[28,29] are believed to play a significant role.

The hydroxyl oxygen atom, O(10), of the succinic acid moiety forms a direct hydrogen bond with the phosphate oxygen, O(7), and the carbonyl oxygen atom, O(9), hydrogen bonds to the water molecule (Fig. 2, Table 4) which bridges the N(6) and N(7) atoms of the adenine base. It is interesting to note that a similar mode of interaction, although not involving a water molecule, is observed in the crystal structures of cAMP-Catabolite activator protein (CAP) complex[30] and cAMP and adenosine-CAP mutant complex,[4] where the hydroxyl group of Ser side chain is at hydrogen-bonding distance from the same two nitrogens, N(6) and N(7) of adenine base. This mode of interaction could be a recurring feature, considering the fact that these nitrogens are free to take part in additional hydrogen-bonding interactions even for an adenine base involved in Watson-Crick base pairing. Water mediated interaction between the nucleotide part and the amino acid-like succinic acid moiety observed in the present structure seems to be a biologically important mode of interaction. A similar interaction has been observed in the crystal structure of 1 : 1 cytosine-*N*-(benzyloxycarbonyl)-glutamic acid dihydrate,[31] where in a water molecule oxygen is at hydrogen-bonding distance from both carboxyl oxygen of glutamic acid and O(2) atom of the cytosine base. In fact, such indirect interactions have also been observed between amino acid side chains and the nucleotides in the crystal stuctures of certain repressor-operator complexes.[32] In the crystal structure of 434 phage repressor-DNA operator complex,[33] four of the six water molecules present are involved in bridging the residues Arg 43, Gln 33, and Ser 30 to base pairs and phosphates of the DNA operator. Also, in the crystal structure of Trp repressor-DNA operator complex,[34] water mediated polar contacts to the bases appear to contribute part of the specificity. The structure shows three water mediated hydrogen bonds, two of which bridging peptide nitrogens of Ile 79 and Ala 80 to adenine and guanine bases, and the third water molecule interacts with hydroxyl of Thr 83 and both the nitrogens N(6) and N(7) of adenine 7 of the DNA operator.

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