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# The Structure, Chirality and Zwitterionic Character of Potassium Adenosine Diphosphate Dihydrate, KADP.$2H_2O$*

By P. Swaminathan and M. Sundaralingam†

*Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin–Madison, Madison, Wisconsin* 53706, *USA*

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## Abstract

Potassium adenosine diphosphate dihydrate, $K^+.C_{10}H_{14}N_5O_{10}P_2^-.2H_2O$, crystallizes in the orthorhombic space group $P2_12_12$ with four molecules in a unit cell of dimensions $a = 28{\cdot}491\ (6)$, $b = 10{\cdot}446\ (3)$ and $c = 6{\cdot}316\ (1)$ Å. The structure was solved by direct methods and refined to an $R$ index of 0·062 ($R_w$ = 0·076) on 1384 intensities. The mean e.s.d.'s in bond lengths and angles are 0·013 Å and 0·7° respectively. The crystal structure has revealed that the adenosine diphosphate exists as a zwitterion with N(1) of the base protonated and the pyrophosphate group dinegatively charged (one negative charge on each phosphate) and has provided details of the molecular conformation and chirality of the chelate ring. The two water molecules in the asymmetric unit are distributed over three positions, two on the same diad axis and one in a general position. The molecule is folded into one of the preferred compact conformations, *viz.* sugar pucker $^2E$ ($P$ = 163·3°, $\tau_m$ = 38·3°), the exocyclic C(4')–C(5') bond torsion angle $\psi$ *gauche*$^+$ (57·8°) and the glycosyl torsion angle $\chi$ *anti* (32·4°), with an anionic oxygen of the $\alpha$-phosphate and the hydroxyl oxygen of the $\beta$-phosphate ligated to the $K^+$ ion. A second anionic oxygen of the $\beta$-phosphate forms a slightly weaker coordination to the metal; thus, the metal ion is engaged in an $\alpha,\beta,\beta$ chelate complex. In addition, the metal ion is coordinated to the base N(3), the ribose O(2'), the anionic phosphate oxygen O(13) of neighboring nucleotides, and to one of the water molecules on the diad axis. Thus, the $K^+$ ion is surrounded by seven possible ligands. The chirality of the chelate ring corresponds to the $\Delta$ diastereomer. The N(1) and N(6) sites and N(6) and N(7) sites of the adenine base form pairs of hydrogen bonds with the oxygens of the $\beta$-phosphate groups of neighboring molecules. There are no base-stacking interactions observed in the structure.

## Introduction

X-ray crystallographic studies have shown that the free acids of three of the five common nucleotides of DNA and RNA involving the bases adenine, cytosine and guanine exist as zwitterions (pH range 2 to 4·5; Ts'o, 1974) with the bases protonated at N(1), N(3) and N(7) respectively neutralized by a net negative charge on the phosphate group (Sundaralingam & Prusiner, 1978). Here we report the structure of the potassium salt of ADP and show that the base is protonated at N(1) with a negative charge on each of the $\alpha$- and $\beta$-phosphate groups. The net negative charge on the ADP molecule is neutralized by the metal ion. KADP.$H_2O$ is isostructural with RbADP.$2H_2O$ (Muller & DeLuke, 1971) and RbADP.$H_2O$ (Viswamitra, Hosur, Shakked & Kennard, 1976). Although RbADP.$2H_2O$ was reported to be a trihydrate, it actually corresponds to a dihydrate since the two water molecules are distributed over three sites: one in a general position and the other two on a diad axis as in KADP.$2H_2O$. In RbADP.$H_2O$, the water molecules on the diad axis were not located. In both RbADP structures the protonation of the adenine base was not reported. Similarly, no protonation of adenine was reported in $Na_2ATP$ (Kennard, Isaacs, Motherwell, Coppola, Wampler, Larson & Watson, 1971; Larson, 1978).

## Experimental

A sample of the monopotassium salt of ADP (purchased from Boehringer Mannheim, Inc.) was crystallized by slow evaporation of a solution (pH of 6·3) in a water–ethanol mixture at 280 K. The crystals grew as thin needles elongated in the **c** direction and belong to the orthorhombic space group $P2_12_12$. The pertinent crystal data are given in Table 1. A crystal of approximate dimensions 0·08 × 0·2 × 0·5 mm was used for the data collection. Two sets of three-dimensional intensity data were collected, the first on a Picker FACS-I diffractometer and the second on a Nonius CAD-4 diffractometer using the $\theta$–$2\theta$ scan

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† To whom correspondence should be addressed.

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Table 1. *Crystal data for* KADP.$2H_2O$

KADP.$2H_2O$: $K^+.C_{10}H_{14}N_5O_{10}P_2^-.2H_2O$, $M_r = 501{\cdot}3$

| | KADP.$2H_2O$ | RbADP.$2H_2O$ | RbADP.$H_2O$ |
|---|---|---|---|
| $a$ | 28·491 (6) Å | 28·478 Å | 28·516 (9) Å |
| $b$ | 10·446 (3) | 10·598 | 10·594 (3) |
| $c$ | 6·316 (1) | 6·332 | 6·315 (2) |
| $V$ | 1879·7 $Å^3$ | 1911·1 $Å^3$ | 1907·8 $Å^3$ |
| Space group | $P2_12_12$ | $P2_12_12$ | $P2_12_12$ |
| $Z$ | 4 | | |
| $D_c$ | 1·771 Mg $m^{-3}$ | | |
| Crystal dimensions | 0·08 × 0·2 × 0·5 mm | | |
| $\mu$ | 4·75 $mm^{-1}$ | | |
| $\lambda$(Cu $K\alpha$) | 1·5418 Å | | |

technique out to a $2\theta$ limit of 127° (Picker) and 154° (CAD-4). The two data sets were reduced individually and averaged using the common reflections. The agreement index between the two data sets is 0·04. Of 1816 reflections, 1384 had $I > 1{\cdot}5\sigma(I)$, where $\sigma(I)$ is given by $\sigma(I) = [I_{\rm scan} + I_{\rm bkg} + 0{\cdot}03\, I^2_{\rm scan}]^{1/2}$ and these were used in the structure analysis.

## Structure determination and refinement

The structure was determined by the multisolution method (Main, Woolfson & Germain, 1971) using 286 normalized structure amplitudes $|E|$ greater than 1·2. The starting set of phases generated 32 phase sets. The $E$ map computed with the set having the highest combined figure of merit revealed the positions of the pyrophosphate group. The rest of the structure was obtained by cyclic tangent refinement (Karle & Hauptman, 1956) of the phases obtained from the partial structure and difference Fourier maps. The structure contains three water molecules; one is in a general position and the remaining two are on a twofold axis. The water molecules on the twofold axis were assigned a multiplicity of 0·5. The structure was refined by the block-diagonal least-squares method, initially using isotropic temperature factors for the non-hydrogen atoms. For further refinement, anisotropic thermal parameters were applied. The positions of all the H atoms except those bonded to the water molecules were revealed ($0{\cdot}4$ e $Å^{-3} < \rho < 0{\cdot}7$ e $Å^{-3}$) in subsequent difference Fourier maps and these were included with isotropic temperature factors in the final refinement cycles. The final $R$ index was 0·062 ($R_w$ = 0·076). The weights used for the individual reflections were calculated using the expression $w = [\sigma_F^2 + (0{\cdot}1|F_o|)^2]^{-1}$. The final difference Fourier map did not contain any residual peak greater than 0·4 e $Å^{-3}$.

All the calculations described above were performed on an in-house PDP 11/35 minicomputer using programs developed in these laboratories (Rao, McAlister & Merritt, 1979, unpublished). The scattering factors used for $K^+$, P, C, N and O are those given by Cromer & Waber (1965) and, for H atoms, those of Stewart, Davidson & Simpson (1965).

## Results and discussion

The positional and thermal ($B$) parameters of all the atoms are given in Table 2.* The bond lengths and

Table 2. *Fractional positional parameters of all the atoms* ($\times 10^4$, *for* H $\times 10^3$), *equivalent isotropic temperature factors* ($B_{\rm eq}$) *of the nonhydrogen atoms and the isotropic temperature factors of the hydrogen atoms* ($B_{\rm iso}$)

| | $x$ | $y$ | $z$ | $B^*_{\rm eq}/B_{\rm iso}$ ($Å^2$) |
|---|---|---|---|---|
| K | 1051 (2) | 5132 (4) | 305 (11) | 11·80 |
| P(2) | 3168 (1) | 1976 (2) | 2975 (4) | 1·68 |
| O(21) | 2721 (2) | 2585 (6) | 3670 (10) | 2·39 |
| O(22) | 3253 (2) | 641 (7) | 3659 (12) | 2·88 |
| O(23) | 3215 (3) | 2075 (8) | 452 (10) | 3·17 |
| O(6′) | 3570 (2) | 2916 (7) | 3861 (11) | 2·68 |
| P(1) | 4132 (1) | 2711 (3) | 4150 (4) | 2·25 |
| O(11) | 4321 (2) | 2012 (8) | 2285 (12) | 3·18 |
| O(13) | 4230 (3) | 2191 (10) | 6286 (12) | 4·55 |
| O(5′) | 4287 (3) | 4161 (7) | 4142 (13) | 3·09 |
| O(1′) | 3654 (2) | 6307 (7) | 3959 (10) | 2·26 |
| O(2′) | 4320 (2) | 7823 (8) | 7946 (11) | 2·72 |
| O(3′) | 4436 (3) | 8349 (7) | 3710 (14) | 3·74 |
| C(1′) | 3692 (3) | 6930 (8) | 5920 (13) | 1·57 |
| C(2′) | 4214 (3) | 6871 (9) | 6483 (12) | 2·07 |
| C(3′) | 4445 (3) | 7037 (10) | 4276 (17) | 2·70 |
| C(4′) | 4096 (3) | 6319 (9) | 2902 (17) | 2·33 |
| C(5′) | 4218 (4) | 4942 (11) | 2307 (17) | 2·99 |
| N(1) | 2688 (3) | 7208 (7) | 12571 (12) | 1·91 |
| C(2) | 2926 (3) | 8132 (8) | 11578 (18) | 2·14 |
| N(3) | 3174 (3) | 7966 (8) | 9819 (12) | 2·18 |
| C(4) | 3172 (3) | 6752 (8) | 9263 (13) | 1·62 |
| C(5) | 2962 (3) | 5713 (9) | 10213 (15) | 2·04 |
| C(6) | 2691 (3) | 5965 (9) | 12002 (15) | 2·01 |
| N(6) | 2451 (3) | 5124 (8) | 13042 (16) | 2·77 |
| N(7) | 3056 (3) | 4571 (9) | 9101 (15) | 3·14 |
| C(8) | 3317 (3) | 4932 (12) | 7476 (19) | 2·76 |
| N(9) | 3400 (3) | 6228 (7) | 7469 (11) | 1·82 |
| O(*W*1) | 0 (0) | 5000 (0) | 9455 (31) | 7·88 |
| O(*W*12) | 5000 (0) | 10000 (0) | 6026 (24) | 7·91 |
| O(*W*2) | 5146 (3) | 6981 (9) | 9188 (14) | 4·46 |
| H(O23) | 305 (5) | 312 (15) | 21 (24) | 9·77 |
| H(5′)1 | 448 (5) | 528 (15) | 230 (27) | 3·71 |
| H(5′)2 | 397 (5) | 458 (14) | 169 (25) | 6·20 |
| H(4′) | 395 (5) | 690 (16) | 190 (24) | 4·00 |
| H(3′) | 471 (5) | 655 (15) | 340 (26) | 10·29 |
| H(2′) | 411 (5) | 594 (13) | 602 (26) | 7·82 |
| H(1′) | 360 (4) | 790 (12) | 602 (26) | 4·06 |
| H(O3′) | 462 (5) | 918 (13) | 451 (27) | 7·50 |
| H(O2′) | 463 (4) | 799 (13) | 785 (27) | 6·59 |
| H(2) | 283 (5) | 885 (13) | 1142 (25) | 7·05 |
| H(8) | 355 (5) | 416 (15) | 705 (25) | 6·41 |
| H(N6)1 | 249 (4) | 409 (13) | 1287 (25) | 6·01 |
| H(N6)2 | 221 (5) | 561 (14) | 1288 (24) | 4·76 |
| H(N1) | 247 (3) | 798 (11) | 1337 (19) | 3·06 |

* $B_{\rm eq} = \frac{4}{3}\sum_i \sum_j \mathbf{a}_i.\mathbf{a}_j\beta_{ij}$ where the $a_i$'s are the cell constants in direct space.

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* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 35450 (8 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

bond angles involving nonhydrogen atoms are given in Table 3. The mean standard deviation in the bond lengths of the pyrophosphate group is 0·008 Å and in the bond angles 0·4°. The mean standard deviations in the C—C, C—O and C—N bond lengths are 0·014, 0·013 and 0·013 Å, respectively, and the standard deviation in the bond angles is 0·7°. An *ORTEP* (Johnson, 1965) drawing of the molecule showing the anisotropic thermal vibrations and the overall conformation is given in Fig. 1.

Table 3. *Bond lengths* (Å) *and angles* (°) *of* KADP.-$2H_2O$

The mean standard deviations in the bond lengths and angles of the pyrophosphate group are 0·008 Å and 0·4° and those of the C—C, C—N and C—O bond lengths are 0·014, 0·013 and 0·013 Å. The mean standard deviation in the bond angles is 0·7°.

Base

| | | | |
|---|---|---|---|
| N(1)–C(2) | 1·336 | C(5)–C(6) | 1·394 |
| N(1)–C(6) | 1·347 | C(5)–N(7) | 1·410 |
| C(2)–N(3) | 1·328 | C(6)–N(6) | 1·293 |
| N(3)–C(4) | 1·316 | N(7)–C(8) | 1·322 |
| C(4)–C(5) | 1·377 | C(8)–N(9) | 1·374 |
| C(4)–N(9) | 1·416 | N(9)–C(1′) | 1·479 |
| C(2)–N(1)–C(6) | 124·6 | C(5)–C(6)–N(6) | 125·2 |
| N(1)–C(2)–N(3) | 124·6 | N(1)–C(6)–C(5) | 113·7 |
| C(2)–N(3)–C(4) | 110·3 | N(1)–C(6)–N(6) | 121·1 |
| N(3)–C(4)–C(5) | 130·2 | C(5)–N(7)–C(8) | 104·6 |
| N(3)–C(4)–N(9) | 125·7 | N(7)–C(8)–N(9) | 112·3 |
| C(5)–C(4)–N(9) | 104·1 | C(4)–N(9)–C(8) | 107·4 |
| C(4)–C(5)–C(6) | 116·4 | C(1′)–N(9)–C(4) | 126·6 |
| C(4)–C(5)–N(7) | 111·6 | C(1′)–N(9)–C(8) | 126·0 |
| C(6)–C(5)–N(7) | 132·0 | | |

Ribose

| | | | |
|---|---|---|---|
| C(1′)–O(1′) | 1·403 | C(2′)–O(2′) | 1·391 |
| C(1′)–C(2′) | 1·530 | C(3′)–O(3′) | 1·417 |
| C(2′)–C(3′) | 1·551 | C(4′)–C(5′) | 1·527 |
| C(3′)–C(4′) | 1·518 | C(5′)–O(5′) | 1·431 |
| C(4′)–O(1′) | 1·425 | | |
| C(2′)–C(1′)–O(1′) | 105·2 | C(1′)–C(2′)–O(2′) | 109·7 |
| C(2′)–C(1′)–N(9) | 111·9 | C(2′)–C(3′)–O(3′) | 109·1 |
| O(1′)–C(1′)–N(9) | 108·1 | C(4′)–C(3′)–O(3′) | 108·8 |
| C(1′)–C(2′)–C(3′) | 101·5 | C(5′)–C(4′)–O(1′) | 108·0 |
| C(2′)–C(3′)–C(4′) | 100·4 | C(5′)–C(4′)–C(3′) | 117·2 |
| C(3′)–C(4′)–O(1′) | 108·4 | C(4′)–C(5′)–O(5′) | 111·7 |
| C(4′)–O(1′)–C(1′) | 110·0 | P(1)–O(5′)–C(5′) | 120·8 |
| C(3′)–C(2′)–O(2′) | 115·2 | | |

Pyrophosphate

| | | | |
|---|---|---|---|
| O(5′)–P(1) | 1·578 | O(6′)–P(2) | 1·609 |
| P(1)–O(13) | 1·481 | P(2)–O(22) | 1·480 |
| P(1)–O(11) | 1·487 | P(2)–O(21) | 1·490 |
| P(1)–O(6′) | 1·626 | P(2)–O(23) | 1·602 |
| O(13)–P(1)–O(5′) | 107·6 | O(22)–P(2)–O(6′) | 110·9 |
| O(13)–P(1)–O(11) | 118·3 | O(22)–P(2)–O(21) | 117·2 |
| O(13)–P(1)–O(6′) | 109·7 | O(22)–P(2)–O(23) | 109·7 |
| O(11)–P(1)–O(5′) | 111·6 | O(21)–P(2)–O(23) | 109·7 |
| O(11)–P(1)–O(6′) | 109·4 | O(21)–P(2)–O(6′) | 104·2 |
| O(6′)–P(1)–O(5′) | 98·6 | O(23)–P(2)–O(6′) | 104·3 |
| P(1)–O(6′)–P(2) | 131·3 | | |

### KADP *is a zwitterion*

The adenine base is found to be protonated at the ring nitrogen atom N(1) and the pyrophosphate group is dinegatively charged with the α- and β-phosphate groups each carrying a negative charge. The net negative charge of the molecule is neutralized by the $K^+$ ion. This result suggests that the adenine base should be similarly protonated in the Rb salts of ADP as well as the disodium salt of ATP. By a similar reasoning the guanosine and cytidine polyphosphate structures would also be expected to be protonated at N(7) of guanine and N(3) of the cytosine bases (see Sundaralingam & Prusiner, 1978).

### *Molecular dimensions*

The bond lengths and bond angles of the base agree well with the values reported for the N(1) protonated adenine (Sundaralingam, 1966). For the protonated adenine, the preferred site for the metal binding is N(7); however, in the present structure the metal ion is coordinated to N(3) (see also Swaminathan & Sundaralingam, 1979). This may be partly due to the competition of the phosphate oxygens for hydrogen bonding with the Watson–Crick [N(1) and N(6)] and the Hoogsteen [N(7) and N(6)] sites of the base. Similar phosphate–base hydrogen bonding has been observed in the structure of $RbADP.H_2O$ (Viswamitra *et al.*, 1976).

The bridge P—O bond lengths of the pyrophosphate group are unequal, P(2)—O(6′) = 1·609 (8) and O(6′)—P(1) = 1·626 (8) Å. A similar feature is observed in dipotassium uridine diphosphate ($K_2UDP$),

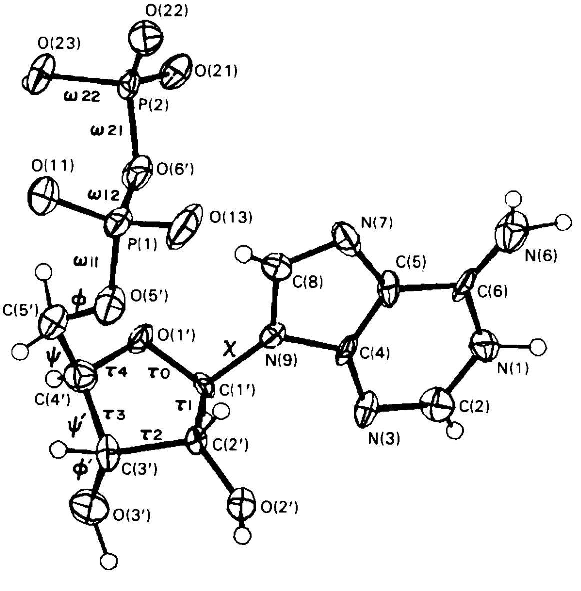

Fig. 1. An *ORTEP* drawing (Johnson, 1965) of the KADP molecule showing the molecular conformation, atom numbering and the torsion-angle notations (Sundaralingam, 1969).

1·617 (8) and 1·566 (7) respectively (Viswamitra, Post & Kennard, 1979). It may be noted that in the less accurate RbADP structures (Muller & DeLuke, 1971; Viswamitra *et al.*, 1976) the asymmetry of the P–O bonds is reversed (see also Merritt & Sundaralingam, 1980).

### *The molecular conformation*

The nucleotide exhibits one of the basic conformations, with the sugar pucker, the sugar–base and sugar–phosphate bond torsion angles within the preferred ranges of values reported for the mononucleotides themselves (Sundaralingam, 1969, 1973). The orientation of the base with respect to the sugar is *anti*, $\chi$ = 32·4° (Sundaralingam, 1969). The sugar-ring pucker is C(2′)-*endo*. The endocyclic torsion angles, the pseudorotation phase angle $P$ and the amplitude of puckering $\tau_m$ (Altona & Sundaralingam, 1972) of the furanoside ring are given in Table 4. The conformation about the exocyclic C(4′)–C(5′) bond $\psi$ is *gauche*$^+$ (58°). The torsion angle $\varphi$ [C(4′)–C(5′)–O(5′)–P(1)] is 144·6°, while in $Na_2ATP$ this torsion angle is −138·4° (molecule *A*) and −142·4° (molecule *B*) (Kennard *et al.*, 1971; Larson, 1978) which permits the metal bridge between the phosphate chain and the base. The torsion angle $\varphi$ shows a greater tendency to deviate from 180° in nucleoside di- and polyphosphates than in nucleoside monophosphates. This is because the base–phosphate attractive interactions while stabilizing the *gauche*$^+$ conformation about the C(4′)–C(5′) bond ($\psi$) (Yathindra & Sundaralingam, 1973) fold the phosphate chain towards the base, leading to $\varphi$ values that deviate from the ideal *trans* value (see also Sundaralingam, 1969). The torsion angle, $\omega$ [C(5′)–O(5′)–P(1)–O(6′)] is *gauche*$^-$ (−63·6°) which is characteristic of the nucleic acid right-handed helical conformations (Sundaralingam, 1969). The pyrophosphate O atoms exhibit the usual staggered conformations about the P(1)···P(2) virtual bond (Fig. 3) (Pletcher & Sax, 1972; Pletcher, Wood, Blank, Shin & Sax, 1977). The P atoms of the pyrophosphate lie on opposite sides of the base plane: P(1) is 1·06 Å above and P(2) 1·47 Å below the base plane as shown in Fig. 2(*a*).

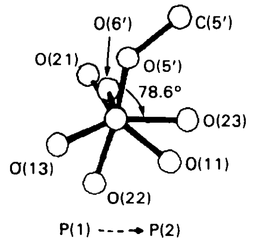

Fig. 3. View down the P(1)···P(2) virtual bond showing the staggered conformation of the P–O bonds of the pyrophosphate group.

### *Potassium coordination, chirality and pucker of chelate ring*

The metal ion has seven neighbors within a sphere of radius 3·25 Å. The ligands to the K$^+$ ion and the metal pyrophosphate chelate ring are shown in Fig. 4. The metal ion is coordinated to the pyrophosphate oxygens O(23), O(11) and O(22) of the same molecule, O(13), N(3) and O(2′) of the neighboring molecules and the water molecule O(*W*1) on the twofold axis. The

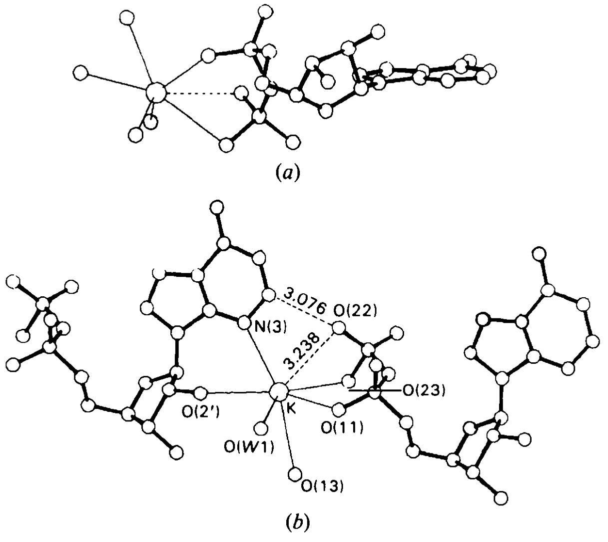

Fig. 2. The potassium coordination to the ADP molecule (*a*) viewed parallel to the plane of the base and (*b*) showing the coordination sphere around the metal ion involving atoms of the translation-related molecules (distances in Å).

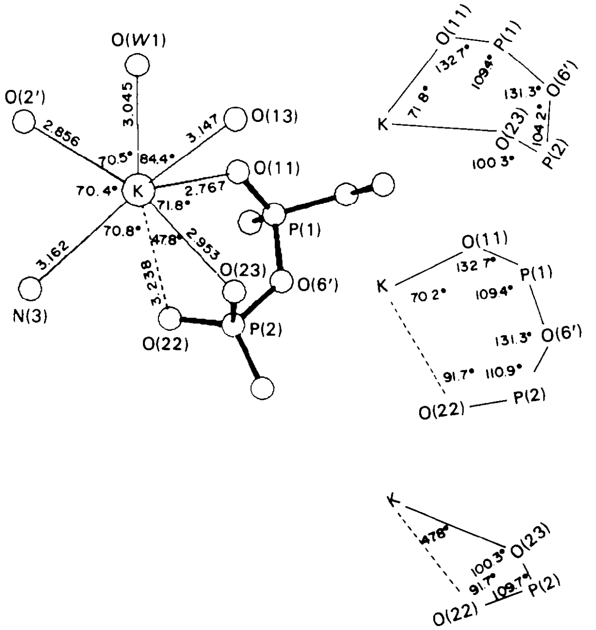

Fig. 4. The coordination distances (Å) and angles involving the potassium ion.

shortest phosphate–metal bond is to O(11) (2·767 Å) while the longest is to O(22) (3·238 Å). The interaction with O(23) (2·953 Å) is of intermediate length. Thus, there is no metal bridge between the base and the phosphate group of the same molecule as observed in the case of the $Na_2ATP$ complex (Kennard *et al.*, 1971; Larson, 1978). The weak interaction of O(22) with the $K^+$ ion probably results from the steric hindrance produced by the base atom C(2) of a neighboring molecule. The distance between C(2) and O(22) is 3·076 Å, and between H(2) [proton bonded to C(2)] and O(22) is 2·64 Å (Fig. 2*b*).

The ligated oxygens O(2′), O(23), O(11) and the $K^+$ ion are nearly coplanar. The deviations of the water molecule O(*W*1), O(13), O(22) and N(3) atoms from this plane are 0·42, 3·07, −2·36 and −1·90 Å respectively. Excluding the weak O(22) ligand, the coordination geometry around the metal ion is a distorted octahedron. The coordination spheres of the diad-related metal ions are bridged by the water molecule on the twofold axis (Fig. 5). This type of sharing of the edges of the coordination spheres through the water molecule has been observed in several other structures, *e.g.* monosodium inosine 5′-monophosphate (Rao & Sundaralingam, 1969), anhydrous sodium triphosphate (Davies & Corbridge, 1958) and disodium dihydrogen pyrophosphate (Collin & Willis, 1971).

The torsion angles of the six-membered metal–chelate ring shown in Table 4 indicate that the ring is a distorted chair flattened at the metal end and sharpened at the bridge oxygen. The chirality of the chelate ring, with reference to the D-ribose, corresponds to the Δ diastereomer (Merritt, Sundaralingam, Cornelius & Cleland, 1978); thus, the RbADP structures are also Δ diastereomers.

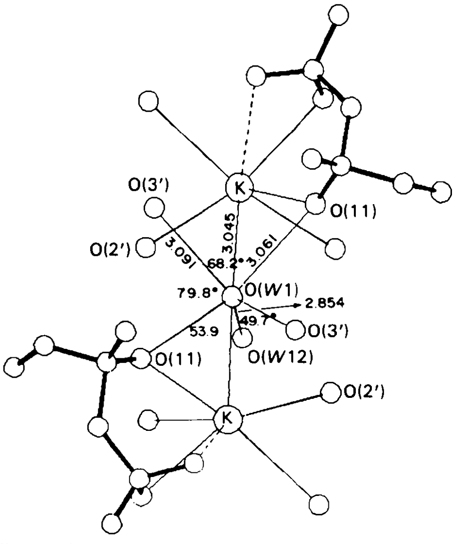

Fig. 5. The environment of the water molecule on the twofold axis which bridges the potassium coordination spheres (distances in Å).

$KADP.2H_2O$, $RbADP.2H_2O$ and $RbADP.H_2O$ structures are isostructural. The r.m.s. distance between the three structures referred to a common origin is about 0·5 Å. The difference between the $KADP.2H_2O$ and $RbADP.H_2O$ (Viswamitra *et al.*, 1976) structures is the missing water which is distributed over two sites on the same twofold axis. The absence of the water molecules on the diad axis leaves a rather large opening of about 5–6 Å diameter and also leaves the Rb coordination centers unbridged. Therefore, it appears unlikely that these half-water molecules have actually completely evaporated from the crystal lattice in the $RbADP.H_2O$ structure.

### *Molecular packing and hydrogen bonding*

The packing of the molecules viewed down the *c* axis is shown in Fig. 6. The molecules are arranged in layers along the *a* axis and molecules within a layer are bridged through the metal ion and the adjacent layers are bridged by the water molecules. The hydrogen-bond parameters are listed in Table 5.

As noted by the previous authors in their work on $RbADP.H_2O$ (Viswamitra *et al.*, 1976), there is an absence of base stacking or base pairing in this structure. Instead, the potential hydrogen-bonding sites of the base, N(1), N(6) and N(7), interact preferentially with the phosphate oxygens. Both the Watson–Crick hydrogen-bonding sites $N^+(1)$ and N(6) and the Hoogsteen sites N(6) and N(7) form pairs of hydrogen bonds to the β-phosphate oxygens of the neighboring molecules (Fig. 7*a*). The pairing to the Watson–Crick sites is accomplished through the anionic oxygens of P(2) such that the six atoms O(22), P(2), O(21), N(1), C(6) and N(6) forming the six-membered hydrogen-bonding ring lie roughly in a plane. The deviation of P(2) from this plane is 0·23 Å. The pairing to the Hoogsteen sites is accomplished through one of the

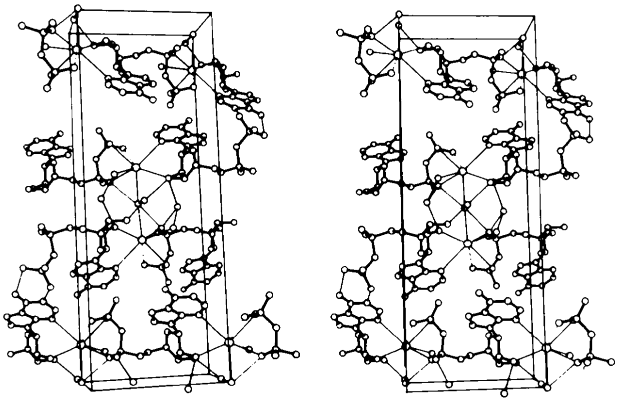

Fig. 6. Stereoview of the packing of the molecule along the *c* axis.

Table 4. *Comparison of the molecular conformations of ADP and ATP in their metal complexes*

Torsion-angle notations for the nucleotide are after Sundaralingam (1969) and for pseudorotation parameters after Altona & Sundaralingam (1972). The pyrophosphate backbone torsion-angle notations are: $\omega_{11}$ = C(5')–O(5')–P(1)–O(6'), $\omega_{12}$ = O(5')–P(1)–O(6')–P(2), $\omega_{21}$ = P(1)–O(6')–P(2)–O(23), $\omega_{22}$ = O(6')–P(2)–O(23)–H(O23).

| | | | | $Na_2ATP$ | |
|---|---|---|---|---|---|
| | $KADP.2H_2O$ | $RbADP.2H_2O$ | $RbADP.H_2O$ | Mol. *A* | Mol. *B* |
| Glycosyl $\chi$ (°) | 32·4 | 37·9 | 40 | 57·8 | 27·9 |
| Ribose pucker | $^2E$ | $^2E$ | $^2E$ | $^3T_4$ | $^2T_3$ |
| Pseudorotation parameters (°) | | | | | |
| Phase angle *P* | 163·3 | 163·0 | 163 | 29·8 | 174·8 |
| Max. amplitude of pucker $\tau_m$ | 38·3 | 36·0 | 36 | 44·0 | 41·4 |
| Backbone torsion angles (°) | | | | | |
| $\psi$ | 57·8 | 57·4 | 57 | −64·7 | 61·0 |
| $\varphi$ | 144·6 | 148·5 | 147 | −138·4 | −142·4 |
| $\omega_{11}$ | −63·6 | −72·1 | −67 | −66·2 | −54·9 |
| $\omega_{12}$ | 157·3 | 153·1 | 155 | 158·4 | −69·2 |
| $\omega_{21}$ | −80·1 | −76·8 | −77 | −115·8 | 51·3 |
| $\omega_{22}$ | −74·1 | | | −82·4 | 113·5 |
| Chelate-ring torsion angles (°) | | | | | |
| P(1)–O(11)–K–O(23) | 43·0 | 40·8 | 38·1 | | |
| O(6')–P(1)–O(11)–K | −33·8 | −30·1 | −28·5 | | |
| P(2)–O(12)–P(1)–O(11) | 40·8 | 37·0 | 36·5 | | |
| O(23)–P(2)–O(6')–P(1) | −80·1 | −76·2 | −76·5 | | |
| K–O(23)–P(2)–O(6') | 85·7 | 87·0 | 83·7 | | |
| O(11)–K–O(23)–P(2) | −60·9 | −62·7 | −60·4 | | |

Table 5. *Hydrogen-bond distances* (Å) *and angles* (°) *in* $KADP.2H_2O$

| Bond *D*–H···*A* | Symmetry code | Translation for *A* (*x*,*y*,*z*) | *D*–H | *D*···*A* | H···*A* | *D*–H···*A* |
|---|---|---|---|---|---|---|
| O(23)–H(O23)···N(7) | 1 | (0,0,−1) | 1·20 | 2·781 | 1·67 | 150 |
| O(3')–H(O3')···O(*W*12) | 3 | (0,1,1) | 1·14 | 2·774 | 1·68 | 161 |
| O(2')–H(O2')···O(*W*2) | 4 | (1,0,1) | 0·89 | 2·632 | 2·01 | 126 |
| N(1)–H(N1)···O(21) | 4 | (1,0,2) | 1·13 | 2·674 | 1·99 | 115 |
| N(6)–H(N6)1···O(21) | 1 | (0,0,1) | 1·10 | 2·790 | 1·78 | 152 |
| N(6)–H(N6)2···O(22) | 4 | (1,0,1) | 0·87 | 2·942 | 2·55 | 109 |
| *O(*W*2)···O(11) | 3 | (−1,0,0) | | 2·691 | | |
| O(*W*2)···O(13) | 3 | (−1,0,1) | | 2·696 | | |
| O(*W*12)···O(*W*1) | 3 | (−1,0,0) | | 2·854 | | |
| O(*W*12)···O(11) | 1 | (0,0,0) | | 3·061 | | |
| O(*W*1)···O(3') | 2 | (2,1,0) | | 3·091 | | |
| Mean $\sigma$ | | | (0·11) | (0·012) | (0·14) | (9) |

Symmetry code

(1) $x,y,z$
(2) $\bar{x},\bar{y},z$
(3) $\frac{1}{2}-x, \frac{1}{2}+y, \bar{z}$
(4) $\frac{1}{2}+x, \frac{1}{2}-y, \bar{z}$

* Hydrogens attached to the water molecules could not be located from the difference Fourier map.

anionic oxygens and the hydroxyl oxygen of P(2). Here, P(2) is significantly out of the least-squares plane (0·71 Å) passing through the six atoms [N(7), C(5), C(6), N(6), O(21) and O(23)] of the seven-membered hydrogen-bonding ring. Similar phosphate–base hydrogen-bond interactions have been frequently found in crystal structures of nucleotides. This type of phosphate–base hydrogen bonding may be an important mode of interaction or recognition between nucleic acids or nucleic acid–protein complexes where the phosphate group may be hydrogen bonded to the bases or to a base and an amino acid side chain, *e.g.* arginine or aspartic acid. Note that the base N(1) and N(6) sites [and to some extent the N(7) and N(6) sites] are reminiscent of the guanidinium group of arginine (Cotton, Day, Hazen, Larsen & Wong, 1974).

The sugar-ring O(4') atom lies under a neighboring base and exhibits some short non-bonded contacts to the base (Fig. 7*b*). Such sugar–base interactions are frequently observed in the crystal structures of nucleosides and nucleotides having a protonated base (Sundaralingam, 1968; Bugg, Thomas, Sundaralingam & Rao, 1971).

An interesting feature of the crystal packing is the presence of seven neighbors within a sphere of radius 3·1 Å around the water molecule *W*(1) on the diad axis. This suggests that the water molecule is in dynamic disorder or is tumbling.

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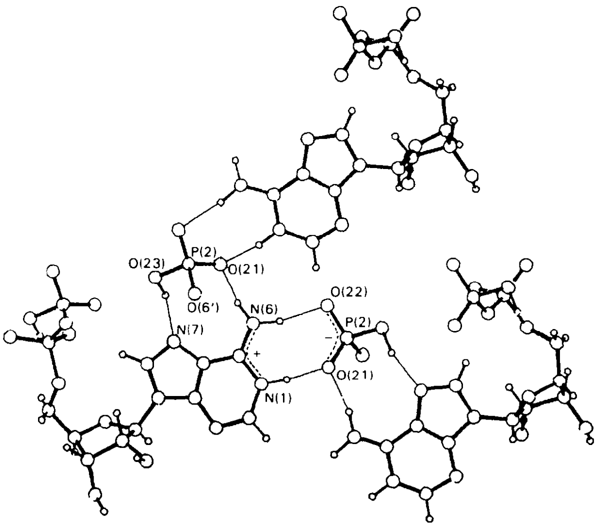
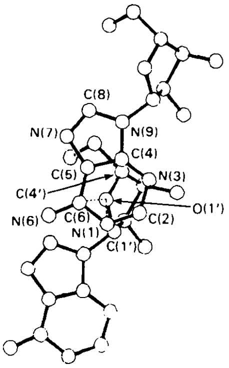


(*a*)



(*b*)

Fig. 7. (*a*) The base–phosphate pairing interactions in KADP.-$2H_2O$. (*b*) The overlapping of the base on the neighboring ribose; the shorter O(1')···N(1), O(1')···C(6), O(1')···C(2), O(1')···C(4), and O(1')···C(5) distances are 3·04 (1), 3·03 (1), 3·19 (1), 3·30 (1) and 3·14 (1) Å respectively.

*Note:* We thank one of the referees for drawing our attention to a recent preliminary communication on the same structure (Viswamitra, 1979).

We have also learned from Dr W. Saenger, after this paper was submitted for publication, that he too had submitted a paper on the same crystal structure (Adamiak & Saenger, 1980) (previous paper). The overall features of the structure are the same in the two determinations with the exception of some significant differences in the geometrical parameters. The precision and the *R* index are slightly better in our work: $R = 0{\cdot}062$ *vs* 0·124, $\langle\sigma_l\rangle = 0{\cdot}013$ Å *vs* 0·02 Å and $\langle\sigma_\theta\rangle = 0{\cdot}7°$ *vs* 1·2°. All the H atoms of the molecule were determined and refined in our work, while the protonation of the base and the ionized state of the pyrophosphate group were inferred from the molecular geometries by Adamiak & Saenger. In the latter work the water molecules O(*W*1) and O(*W*12) are positioned on either side of the diad axis, while we have located them on the diad axis with multiplicity factors of 0·5 each. The half-normal probability plot (Abrahams & Keve, 1971) of the two sets of positional parameters has a slope of 1·68. The largest $|\Delta P|$ of 6·507, where $\Delta P = |P_1 - P_2|/(\sigma^2_{p_1} + \sigma^2_{p_2})^{1/2}$ is for the atom O(22) which has a probability of occurrence less than $7 \times 10^{-6}$. Even after the application of the factor of 1·68, the value of $|\Delta P|$ (3·87) is statistically significant with a probability of occurrence of 0·001. Similarly, the positional parameter of O(11) is significantly different with a $|\Delta P| = 3{\cdot}11$. A comparison of the bond lengths indicates differences ranging from 0·005 to 0·07 Å between the two determinations, but the half-normal probability analysis indicates that these differences are not significant.

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# Calcium-Binding to Nucleotides: Structure of a Hydrated Calcium Salt of Inosine 5′-Monophosphate

BY EDWIN A. BROWN AND CHARLES E. BUGG

*Department of Biochemistry, Institute of Dental Research and Comprehensive Cancer Center, University of Alabama in Birmingham, University Station, Birmingham, Alabama* 35294, *USA*

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## Abstract

The crystal and molecular structure of a hydrated calcium salt of inosine 5′-phosphate was determined from X-ray diffraction data. Crystals of $Ca^{2+}.C_{10}H_{11}N_4O_8P^{2-}.6{\cdot}5H_2O$ are monoclinic, space group $P2_1$, with $a = 10{\cdot}929$ (4), $b = 21{\cdot}315$ (2), $c = 8{\cdot}622$ (4) Å, $\beta = 98{\cdot}26$ (5)° and $Z = 4$. Intensity data were collected with an automated diffractometer. The structure was solved by Patterson methods and refined by least squares to $R = 0{\cdot}023$. One of the crystallographically independent nucleotides binds $Ca^{2+}$ through atom N(7), the site that is generally used in interactions of transition metals with purine nucleotides; additional $Ca^{2+}$ ions are directly coordinated to this nucleotide through the O(2′)—O(3′) pair of hydroxyl groups, and through an O atom of the phosphate group. The second nucleotide forms only outer-sphere, water-mediated contacts with $Ca^{2+}$ ions.

## Introduction

Despite the general importance of interactions between alkaline-earth metals and nucleotides in biological systems, little is known about the specific factors that are involved in these interactions. Most of the current models of metal–nucleotide complexes have been based on solution and crystallographic studies of transition-metal-ion interactions with purines, pyrimidines, nucleosides and nucleotides (Phillips, 1966; Izatt, Christensen & Rytting, 1971; Frey & Stuehr, 1974; Tu & Heller, 1974; Swaminathan & Sundaralingam, 1979). Unlike the transition metals, alkaline-earth-metal ions have no vacant atomic orbitals that can be used in covalent bonding to nucleotides. Consequently, there has been a tendency to assume that ions of this type interact with nucleotides through sites that are different from those occupied by transition metals. It is generally assumed that alkaline-earth-metal ions form relatively

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