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# Interactions of Tris Buffer with Nucleotides: The Crystal Structure of Tris(hydroxymethyl)methylammonium Adenosine 5′-Diphosphate Dihydrate†

Zippora Shakked,* M. A. Viswamitra, and Olga Kennard

ABSTRACT: The crystal and molecular structures of the Tris salt of adenosine 5′-diphosphate were determined from X-ray diffraction data. The crystals are monoclinic, space group $P2_1$, and $Z = 2$ with $a = 9.198$ (2) Å, $b = 6.894$ (1) Å, $c = 18.440$ (4) Å, and $\beta = 92.55$ (2)°. Intensity data were collected on an automated diffractometer. The structure was solved by the heavy-atom technique and refined by least squares to $R = 0.047$. The ADP molecule adopts a folded conformation. The conformation about the glycosidic bond is anti. The conformation of the ribose ring is close to a perfect C(2′)-endo–C(3′)-exo puckering. The conformation about C(4′)–C(5′) is gauche–gauche, similar to other nucleotide structures. The pyrophosphate chain displays a nearly eclipsed geometry when viewed down the P–P vector, unlike the staggered conformation observed in crystal structures of other pyrophosphates. The less favorable eclipsed conformation probably results from the observed association of Tris molecules with the polar diphosphate chain through electrostatic interactions and hydrogen bonds. Such interactions may play an important role in Tris-buffered aqueous solutions of nucleotides and metal ions.

The effect of the environment on the electronic and geometrical properties of nucleotides is important for understanding the reaction mechanisms of biological systems involving these molecules. The structure of the Tris [tris(hydroxymethyl)methylammonium] salt of adenosine 5′-diphosphate (ADP)[1] was investigated as a part of our crystallographic studies on the conformation of the ADP and ATP molecules as a function of external factors such as metal ions, buffering agents, and hydration. Other structures determined so far are those for the monorubidium salt of ADP (Viswamitra et al., 1976), ADP free acid (Viswamitra & Hosur, 1977), the potassium salt of ADP (Swaminathan & Sundaralingam, 1979), which is isostructural with the Rb–ADP structure, and the disodium salt of ATP (Kennard et al., 1971).

The Tris buffer is a widely used buffering agent in the pH range 7–9, important for studies of physiological media. The accurate dimensions of Tris and Tris-HCl in the crystalline state have been recently determined (Rudman et al., 1978, 1979). The possible formation of Tris–nucleotide complexes in solution was indicated in stability constant studies of Mg–ATP and Mg–ADP complexes using Tris buffer as the supporting electrolyte. Norby (1970) has shown that the stability constant of Mg–nucleotide complexes decreases with increasing ionic strength. O'Sullivan & Perrin (1964) have shown that the stability constant of the complex is considerably lower in Tris than in triethanolamine or *N*-ethylmorpholine buffers. The interactions between Tris and ADP molecules and their effect on the molecular conformation of the nucleotide might

Table I: Crystal Data

| | |
|---|---|
| stoichiometry | $C_{10}H_{14}N_5O_{10}P_2 \cdot C_4H_{12}NO_3 \cdot 2H_2O$ |
| space group | $P2_1$ |
| $Z$ | 2 |
| $a$ | 9.198 (2) Å |
| $b$ | 6.894 (1) Å |
| $c$ | 18.440 (4) Å |
| $\beta$ | 92.55 (2)° |
| $d_c$ | 1.65 g cm$^{-3}$ |
| $d_m$ | 1.66 g cm$^{-3}$ |
| $\mu$ (Cu K$\alpha$) | 22.6 cm$^{-1}$ |

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[1] Abbreviations used: AMP, ADP, and ATP, adenosine 5′-mono-, 5′-di-, and 5′-triphosphate; NMR, nuclear magnetic resonance.

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Table II: Nonhydrogen Atom Coordinates and Estimated Standard Deviations[a]

| atom | x | y | z | atom | x | y | z |
|---|---|---|---|---|---|---|---|
| N(1) | 5308 (6) | 1421 (11) | 6162 (3) | P(1) | 1880 (2) | −3 (0) | 1852 (1) |
| C(2) | 3866 (8) | 1412 (15) | 6251 (4) | O(11) | 634 (5) | 675 (8) | 1379 (3) |
| N(3) | 2816 (6) | 1493 (11) | 5747 (3) | O(12) | 3269 (5) | 1095 (8) | 1840 (3) |
| C(4) | 3374 (6) | 1600 (10) | 5077 (3) | O(6′) | 2143 (5) | −2196 (8) | 1636 (2) |
| C(5) | 4803 (7) | 1601 (11) | 4915 (3) | P(2) | 3350 (2) | −3748 (4) | 1945 (1) |
| C(6) | 5863 (7) | 1525 (11) | 5482 (3) | O(21) | 3007 (5) | −4218 (8) | 2714 (2) |
| N(6) | 7245 (7) | 1531 (13) | 5403 (4) | O(23) | 2954 (7) | −5444 (9) | 1430 (3) |
| N(7) | 4954 (5) | 1620 (11) | 4168 (3) | O(22) | 4819 (5) | −3046 (10) | 1825 (3) |
| C(8) | 3617 (7) | 1663 (12) | 3903 (3) | C(10) | 7852 (7) | 5061 (12) | 925 (4) |
| N(9) | 2588 (5) | 1674 (10) | 4418 (3) | N(10) | 6257 (6) | 4822 (11) | 749 (3) |
| C(1′) | 1006 (7) | 1604 (12) | 4330 (3) | C(101) | 8209 (9) | 4119 (13) | 1665 (4) |
| O(1′) | 598 (5) | −310 (8) | 4150 (2) | O(101) | 7988 (7) | 2090 (9) | 1636 (3) |
| C(2′) | 396 (7) | 2922 (11) | 3713 (3) | C(102) | 8148 (9) | 7230 (13) | 991 (5) |
| C(3′) | −923 (6) | 1753 (11) | 3436 (3) | O(102) | 7516 (6) | 8282 (9) | 392 (3) |
| C(4′) | −441 (7) | −334 (12) | 3525 (3) | C(103) | 8632 (8) | 4049 (14) | 310 (4) |
| O(3′) | −2069 (5) | 2212 (9) | 3894 (3) | O(103) | 10170 (6) | 4059 (13) | 464 (3) |
| O(2′) | 93 (6) | 4802 (9) | 3941 (3) | OW(1) | 4853 (6) | 5826 (9) | −634 (3) |
| C(5′) | 241 (8) | −1222 (13) | 2886 (4) | OW(2) | 6378 (10) | 88 (24) | 2651 (4) |
| O(5′) | 1450 (4) | −99 (8) | 2672 (2) | | | | |

[a] All values are multiplied by $10^4$.

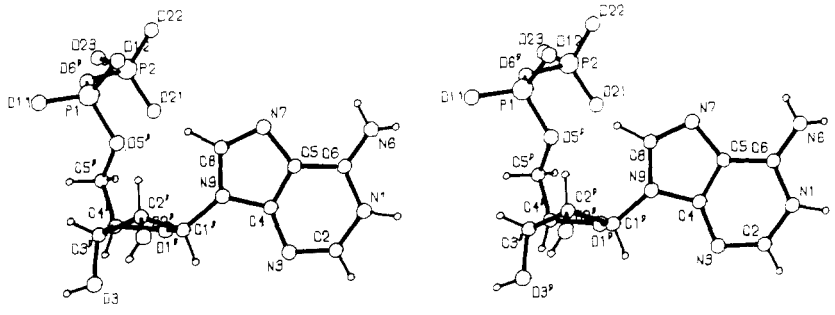


FIGURE 1: Stereoscopic view of ADP.

be relevant to biochemical studies which involve Tris-buffered solutions of nucleotides.

## Experimental Procedures

The Tris salt of ADP was crystallized as long plates by diffusing acetone into a Tris-buffered aqueous solution of ADP. A crystal of dimensions 0.02 × 0.10 × 1.08 $mm^3$ was mounted on a Syntex $P2_1$ diffractometer. Cell dimensions were calculated from 15 automatically centered reflections. Crystal data are in Table I. The measured density (1.66 g $cm^{-3}$) indicated the presence of two Tris–ADP molecules and four water molecules in the unit cell, which was later confirmed by analysis. Three-dimensional intensity data ($2\theta < 110°$, 1624 unique reflections) were collected at room temperature by using graphite monochromated Cu K$\alpha$ radiation. The intensities were measured by the $\omega/2\theta$ scan technique described previously (Shakked & Kennard, 1977). No significant reduction in the intensities was observed during data collection. The intensities were corrected for Lorentz and polarization effects. No absorption correction was applied since this effect was negligible (the transmission factors for most of the reflections were in the range of 0.90–0.95; for the other reflections they were in the range of 0.80–0.90) compared to the effects caused by the anisotropy of the data described later on.

An attempt to solve the structure by direct methods using MULTAN (Main et al., 1974) failed, and the structure was solved by the heavy-atom technique. The positions of the phosphorus atoms were located from a sharpened Patterson map. Two successive weighted difference Fourier maps yielded the positions of all nonhydrogen atoms. The structure was refined isotropically by full matrix least squares to $R = 0.15$. Further refinements with anisotropic temperature factors failed, as they yielded nonpositive thermal vibration tensors for most atoms. Inspection of the data revealed a systematic discrepancy between the observed and calculated structure factors; the $F_o/F_c$ ratio was nearly constant for any diffracting direction and was averaged to 0.9, 1.6, and 0.8 for the three axial directions $a^*$, $b^*$, and $c^*$, respectively. This anisotropy of the data manifested itself also by the large variation in the mosaic spread along different directions as measured by an $\omega$ scan of selected reflections. The profile widths varied from 1.0 to 3.5°. Consequently, the data were rescaled anisotropically in the way suggested by Shakked & Rabinovich (1977). The six components of the scaling tensor were refined together with the coordinates and isotropic temperature factors to $R = 0.08$. At this stage it was possible to locate all the hydrogens by means of difference Fourier synthesis. The structure was further refined with anisotropic temperature factors for nonhydrogen atoms and isotropic temperature factors for hydrogens to $R = 0.047$ and $R_w = 0.043$. The refined components ($K_{11}$, $K_{22}$, $K_{33}$, $K_{23}$, $K_{13}$, and $K_{12}$) and the estimated standard deviations of the anisotropic scaling tensor were 0.963 (6), 1.719 (11), 0.877 (7), −0.201 (7), 0.028 (5), and 0.009 (5), respectively. Atomic scattering factors were taken from *International Tables for X-ray Crystallography* (1974).

## Results and Discussion

The coordinates of the nonhydrogen atoms are given in Table II. Bond lengths, bond angles, and torsion angles are given in Tables III and IV. Tables of thermal parameters and hydrogen atom parameters are available (see paragraph at the end of paper regarding supplementary material). The average estimated standard deviations in bond lengths are

Table III: Bond Lengths and Bond Angles

| bond | length (Å) | bond | length (Å) |
|---|---|---|---|
| C(2)–N(1) | 1.343 (9) | C(6)–N(1) | 1.376 (8) |
| N(3)–C(2) | 1.311 (8) | C(4)–N(3) | 1.361 (7) |
| C(5)–C(4) | 1.361 (8) | N(9)–C(4) | 1.386 (7) |
| C(6)–C(5) | 1.398 (8) | N(7)–C(5) | 1.391 (8) |
| N(6)–C(6) | 1.286 (9) | C(8)–N(7) | 1.303 (8) |
| N(9)–C(8) | 1.371 (8) | C(1′)–N(9) | 1.458 (8) |
| O(1′)–C(1′) | 1.408 (9) | C(2′)–C(1′) | 1.543 (9) |
| C(4′)–O(1′) | 1.464 (7) | C(3′)–C(2′) | 1.526 (9) |
| O(2′)–C(2′) | 1.394 (9) | C(4′)–C(3′) | 1.512 (11) |
| O(3′)–C(3′) | 1.416 (8) | C(5′)–C(4′) | 1.491 (10) |
| O(5′)–C(5′) | 1.425 (9) | P(1)–O(5′) | 1.582 (5) |
| O(11)–P(1) | 1.483 (5) | O(12)–P(1) | 1.486 (5) |
| O(6′)–P(1) | 1.585 (6) | P(2)–O(6′) | 1.627 (5) |
| O(21)–P(2) | 1.501 (5) | O(23)–P(2) | 1.539 (6) |
| O(22)–P(2) | 1.461 (5) | N(10)–C(10) | 1.498 (8) |
| C(101)–C(10) | 1.534 (10) | C(102)–C(10) | 1.524 (12) |
| C(103)–C(10) | 1.536 (11) | O(101)–C(101) | 1.414 (11) |
| O(102)–C(102) | 1.423 (10) | O(103)–C(103) | 1.431 (9) |

| angle | deg | angle | deg |
|---|---|---|---|
| C(6)–N(1)–C(2) | 121.3 (5) | N(3)–C(2)–N(1) | 127.8 (6) |
| C(4)–N(3)–C(2) | 110.4 (5) | C(5)–C(4)–N(3) | 127.3 (5) |
| N(9)–C(4)–N(3) | 126.5 (5) | N(9)–C(4)–C(5) | 106.2 (5) |
| C(6)–C(5)–C(4) | 119.0 (6) | N(7)–C(5)–C(4) | 110.9 (5) |
| N(7)–C(5)–C(6) | 130.1 (6) | C(5)–C(6)–N(1) | 114.1 (5) |
| N(6)–C(6)–N(1) | 120.8 (6) | N(6)–C(6)–C(5) | 125.1 (6) |
| C(8)–N(7)–C(5) | 103.7 (5) | N(9)–C(8)–N(7) | 114.1 (5) |
| C(8)–N(9)–C(4) | 105.0 (5) | C(1′)–N(9)–C(4) | 125.1 (5) |
| C(1′)–N(9)–C(8) | 129.7 (5) | O(1′)–C(1′)–N(9) | 108.2 (6) |
| C(2′)–C(1′)–N(9) | 113.0 (5) | C(2′)–C(1′)–O(1′) | 107.1 (5) |
| C(4′)–O(1′)–C(1′) | 110.7 (5) | C(3′)–C(2′)–C(1′) | 101.2 (6) |
| O(2′)–C(2′)–C(1′) | 113.3 (5) | O(2′)–C(2′)–C(3′) | 115.2 (5) |
| C(4′)–C(3′)–C(2′) | 103.9 (5) | O(3′)–C(3′)–C(2′) | 106.5 (5) |
| O(3′)–C(3′)–C(4′) | 111.7 (6) | C(3′)–C(4′)–O(1′) | 104.7 (6) |
| C(5′)–C(4′)–O(1′) | 110.1 (5) | C(5′)–C(4′)–C(3′) | 115.8 (6) |
| O(5′)–C(5′)–C(4′) | 111.0 (6) | P(1)–O(5′)–C(5′) | 121.3 (4) |
| O(11)–P(1)–O(5′) | 110.8 (3) | O(12)–P(1)–O(5′) | 106.7 (3) |
| O(12)–P(1)–O(11) | 118.3 (3) | O(6′)–P(1)–O(5′) | 104.3 (3) |
| O(6′)–P(1)–O(11) | 106.0 (3) | O(6′)–P(1)–O(12) | 109.9 (3) |
| P(2)–O(6′)–P(1) | 130.5 (3) | O(21)–P(2)–O(6′) | 107.7 (3) |
| O(23)–P(2)–O(6′) | 98.3 (3) | O(23)–P(2)–O(21) | 111.4 (3) |
| O(22)–P(2)–O(6′) | 110.5 (3) | O(22)–P(2)–O(21) | 116.7 (3) |
| O(22)–P(2)–O(23) | 110.7 (4) | C(101)–C(10)–N(10) | 108.3 (6) |
| C(102)–C(10)–N(10) | 107.2 (6) | C(102)–C(10)–C(101) | 108.3 (6) |
| C(103)–C(10)–N(10) | 106.0 (6) | C(103)–C(10)–C(101) | 112.1 (6) |
| C(103)–C(10)–C(102) | 114.7 (6) | O(101)–C(101)–C(10) | 111.1 (6) |
| O(102)–C(102)–C(10) | 111.9 (6) | O(103)–C(103)–C(10) | 109.9 (6) |

Table IV: Torsion Angles

| angle | deg | angle | deg |
|---|---|---|---|
| C(8)–N(9)–C(1′)–O(1′) | 75.5 | C(4′)–C(5′)–O(5′)–P(1) | −149.1 |
| C(8)–N(9)–C(1′)–C(2′) | −42.9 | C(5′)–O(5′)–P(1)–O(6′) | −57.3 |
| C(4′)–O(1′)–C(1′)–C(2′) | −9.6 | C(5′)–O(5′)–P(1)–O(11) | 56.4 |
| O(1′)–C(1′)–C(2′)–C(3′) | 27.6 | C(5′)–O(5′)–P(1)–O(12) | −173.6 |
| C(1′)–C(2′)–C(3′)–C(4′) | −34.7 | O(5′)–P(1)–O(6′)–P(2) | −63.6 |
| C(2′)–C(3′)–C(4′)–O(1′) | 30.1 | O(11)–P(1)–O(6′)–P(2) | 180.0 |
| C(3′)–C(4′)–O(1′)–C(1′) | −12.9 | O(12)–P(1)–O(6′)–P(2) | 50.5 |
| O(1′)–C(4′)–C(5′)–O(5′) | −62.8 | P(1)–O(6′)–P(2)–O(21) | 68.2 |
| C(3′)–C(4′)–C(5′)–O(5′) | 55.7 | P(1)–O(6′)–P(2)–O(22) | −60.3 |
| | | P(1)–O(6′)–P(2)–O(23) | −176.1 |

0.005 Å for P–O bonds, 0.008 Å for other bonds involving nonhydrogen atoms, and 0.07 Å for bonds involving hydrogen atoms. The average estimated standard deviation in bond angles involving P–O bonds is 0.3° and is 0.6° for other nonhydrogen angles. Stereoscopic views of ADP and Tris molecules are given in Figures 1 and 2, respectively.

The analysis shows that the diphosphate chain is doubly ionized. The two protons are attached to the adenine nitrogen N(1) and the Tris nitrogen. The bond lengths and angles of the adenine base and ribose ring are in agreement with average values reported previously (Voet & Rich, 1970; Saenger, 1973). The values of the P–O distances and O–P–O angles depend on the state of protonation/substitution of the phosphate group. The P–O bonds may be classified into two main categories as follows: three internal or in-chain single bonds (~1.598 Å) and four external or out-of-chain bonds (~1.483 Å). The latter reflect a considerable degree of double-bond character. The P(2)–O(23) bond may be included in the first group since it carries a proton and is significantly longer (1.539 Å) than the other external bonds. However, it should be noted that the bridging bonds in the high-energy pyrophosphate linkage [P(1)–O(6′)–P(2)] are significantly different [1.585 (6) and 1.627 (5) Å], in agreement with other nucleoside 5′-diphosphate molecules (Viswamitra et al., 1975). This difference, which indicates different electronic properties of the two P–O bonds, is probably related to the reactivity of this system. A very similar pattern in P–O bonds was observed in the refined structure ($R$ = 0.08) of Rb–ADP (Z. Shakked, unpublished results).


FIGURE 2: Stereoscopic view of the Tris cation.

The angle at the bridging oxygen O(6′) in the pyrophosphate (130.5°) is considerably larger than the bond angle at O(5′) (121.3°), similar to values observed in ADP molecules and other pyrophosphate molecules (Viswamitra et al., 1975; Wood et al., 1975; Saenger et al., 1977). This angle widening which may be attributed to the repulsion between negatively charged phosphate oxygens probably contributes to the reactivity of this system.

The orientation of the base about the glycosidic linkage is anti; the corresponding dihedral angle [O(1′)–C(1′)–N(9)–C(8)] equals 75.5°, which is similar to the values (70 and 69°) observed in orthorhombic 5′-AMP (Neidle et al., 1976) and barium 5′-AMP (Sternglanz et al., 1976), respectively, but somewhat larger than those observed in other purine nucleotides (Sundaralingam, 1969).

The conformation of the ribose ring is close to a perfect C(2′)-endo–C(3′)-exo puckering; the deviations of C(2′) and C(3′) from the plane through C(1′)–O(1′)–C(4′) are +0.25 and −0.33 Å, respectively. The sugar conformation in Rb–ADP was found to be C(2′)-endo (Viswamitra et al., 1976), whereas in ADP free acid the ribose is disordered between C(2′)-endo and C(3′)-endo conformations (Viswamitra & Hosur, 1977). The sugar conformations observed in monoclinic 5′-AMP (Krout & Jensen, 1963) and in orthorhombic 5′-AMP (Neidle et al., 1976) are C(3′)-endo and C(2′)-endo, respectively. These conformations are most common in nucleosides and nucleotides. The possible correlation between the sugar pucker, the base type, and the glycosidic angle has been discussed by Sundaralingam (1969).

The conformation about the C(4′)–C(5′) bond is $g,g$, in agreement with that of the majority of nucleotides determined so far. The conformation about the C(5′)–O(5′) bond may be classified as trans with a torsion angle of −149° compared to 147 and 152° in Rb–ADP and ADP free acid, respectively. The staggering around the P–O bond is nearly perfect in the present structure (Table IV). The major conformational difference between this structure and the other two centers at the diphosphate chain. The conformation of the phosphodiester linkage O(5′)–P(1)–O(6′) is $g^-,g^-$ (the torsion angles equal −57.3 and −63.6°), whereas the conformation of this

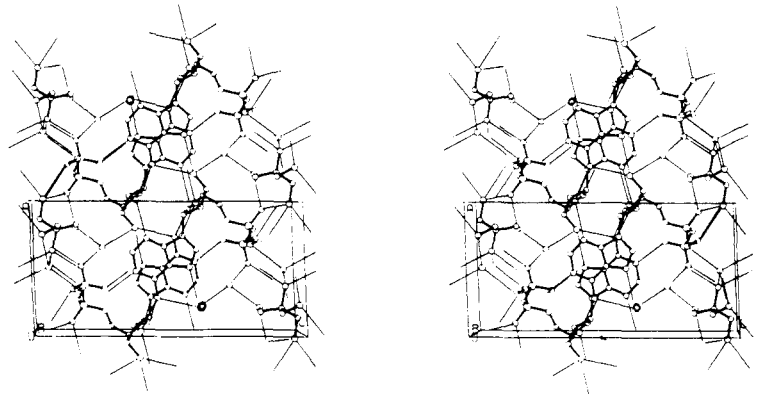

FIGURE 3: Stereoscopic view of the molecular packing viewed down the *b* axis. Hydrogen bond directions are shown by thin lines.

linkage in Rb–ADP and ADP free acid is $g^-,t$ (−67, 155° and −69, 164°, respectively). These conformational features lead to a nearly eclipsed orientation of the pyrophosphate moiety when viewed along the P–P vector in the present structure unlike the more favorable staggered conformation observed in the other ADP structures and also in other organic pyrophosphate molecules (Viswamitra et al., 1975; Wood et al., 1975; Carlisle & Cook, 1969). The less favorable eclipsed arrangement of the pyrophosphate observed here is caused by close interactions with Tris molecules as discussed below.

Aside from the terminal phosphate, the molecule adopts a folded conformation compatible with that of the other ADP structures, the disodium salt of adenosine 5′-triphosphate (Kennard et al., 1971), and the two adenosine 5′-monophosphate structures AMP (Neidle et al., 1976) and Ba–AMP (Sternglanz et al., 1976).

In this context it should be mentioned that two other extreme conformations of ADP were observed. An extended form of ADP when bound to either lactate dehydrogenase (Chandrasekhar et al., 1973) or phosphoglycerate kinase (Banks et al., 1979) was suggested from low-resolution crystallographic studies. An extremely folded conformation of ADP and ATP where an intramolecular hydrogen bond exists between the β-phosphate and the 6-amino nitrogen was suggested from $^{31}P$ nuclear magnetic resonance studies in nonaqueous media (Labotka et al., 1976).

The molecular conformation of the protonated Tris molecule is nearly identical with that of the neutral molecule studied by Rudman et al. (1978). These molecules possess a pseudomirror symmetry through N(10)–C(10)–C(103)–O(103) (Figure 2), thus leading to the maximum separation of the oxygens, which seems most favorable from energy considerations. The bond lengths and angles compare very well with those of crystalline Tris. It should be noted, however, that the Tris-$H^+$ ion observed in crystalline Tris-HCl (Rudman et al., 1979) possesses rather a perfect threefold symmetry probably due to interactions involving the chlorine ions.

The extended crystal structure consists of alternating layers of ADP molecules and Tris–water complexes parallel to the *ab* plane. The major types of interactions within the ADP layers are base stacking and hydrogen bonding involving the adenine nitrogens and the ribose and phosphate oxygens (Table V and Figure 3). The crystal structure of the ADP layers which is determined by the *a* and *b* translations as well as by the screw axis symmetry shows a striking resemblance to that of orthorhombic AMP (Neidle et al., 1976) and that of Ba–AMP (Sternglanz et al., 1976). In the three structures the adenine bases are nearly perpendicular to the short axis and stacked similarly in an antiparallel manner via the screw axis operation along this axis [*b* (6.89 Å) in ADP, *c* (6.60 Å) in AMP, and *b* (6.97 Å) in Ba–AMP]. Such a stacking pattern where the amino group is positioned over the imidazole ring of the adjacent base has been found in several other crystal structures of adenine derivatives (Bugg et al., 1971). The columns of stacked molecules are related within the layer by an *a* translation (9.20 Å) in Tris–ADP, a *b* translation (9.41 Å) in AMP, and a *c* translation (9.60 Å) in Ba–AMP. The feasibility of similar intralayer arrangements of these molecules results from the compatible molecular shapes of their AMP moieties and from the folded nature of the diphosphate chain.

Table V: Hydrogen Bond Distances[a]

| | sym-metry element[b] | translation along *a* | *b* | *c* | distance[c] (Å) |
|---|---|---|---|---|---|
| O(21)···N(1) | 2 | 1 | −1 | 1 | 2.57 |
| O(2′)···N(3) | 2 | 0 | 0 | 1 | 3.00 |
| O(2′)···N(6) | 2 | 1 | 0 | 1 | 2.94 |
| O(3′)···N(6) | 1 | −1 | 0 | 0 | 2.92 |
| O(3′)···N(7) | 1 | −1 | 0 | 0 | 2.84 |
| OW(2)···O(3′) | 1 | 1 | 0 | 0 | 3.02 |
| O(101)···O(11) | 1 | 1 | 0 | 0 | 2.68 |
| O(103)···O(11) | 1 | 1 | 0 | 0 | 2.90 |
| O(23)···O(12) | 1 | 0 | −1 | 0 | 2.52 |
| OW(1)···O(12) | 2 | 1 | 0 | 0 | 2.88 |
| N(10)···O(22) | 1 | 0 | 1 | 0 | 2.84 |
| OW(2)···O(22) | 1 | 0 | 0 | 0 | 2.98 |
| O(103)···O(23) | 1 | 1 | 1 | 0 | 3.07 |
| OW(1)···N(10) | 1 | 0 | 0 | 0 | 2.89 |
| OW(1)···N(10) | 2 | 1 | 0 | 0 | 2.94 |
| OW(2)···O(13) | 1 | 0 | 0 | 0 | 2.80 |
| O(103)···O(102) | 2 | 2 | −1 | 0 | 2.76 |
| OW(1)···O(102) | 2 | 1 | −1 | 0 | 2.85 |

[a] The second atom in each pair is related to the first by the corresponding symmetry and translation. [b] (1) *x*, *y*, *z*; (2) $-x$, $(1/2) + y$, $-z$. [c] The average esd is 0.02 Å.

The alternating layers of ADP molcules and Tris–water complexes are held together by hydrogen bonds between the phosphate oxygens and Tris and water molecules. The pyrophosphate chains are positioned between the adenosine moieties and Tris–water complexes in such a manner that two zigzag chains (related by the $2_1$ axis) of alternating Tris–water and diphosphate groups comprise a dense network of hydrogen bonds. The diphosphate chain is hydrogen bonded directly to three Tris molecules (Figure 3 and Table V) and indirectly to several more via water molecules. These strong interactions probably modify the present conformation with respect to that of the other ADP structures.

In any attempt to predict a reasonable hydrogen-bonding scheme in solution (pH ~8) which involves the pyrophosphate chain and Tris molecules, one should take into account the degree of protonation in ADP and Tris molecules. Since the

p$K$ values of the protonation of the terminal phosphate and the adenine ring ($N_1$+H) are 6.7 and 4.0, respectively, and the Tris p$K$ is 8.3, we would expect the terminal phosphate oxygen and the adenine base to be mainly deprotonated, whereas the Tris molecules are expected to adopt both the charged and uncharged forms at physiological pH. Such changes should not necessarily alter the hydrogen-bonding pattern between ADP and Tris molecules observed here since both the single phosphate proton and the adenine proton are involved in hydrogen bonds with phosphate oxygens of two adjacent molecules (Table V). These interactions may be substituted in solution by hydrogen bonds to additional Tris and water molecules. Hence, we may speculate that in solution a larger number of Tris molecules will bind to the polar diphosphate chain through electrostatic interactions and hydrogen bonds. Such interactions might interfere with the binding of metal ions which play an important role in biological processes involving the ADP–ATP system. The ability of the Tris ion in solution to compete with the metal ion for the nucleotide ligand was already indicated in studies of Mg–ATP complexes in Tris-buffered solutions (Norby, 1970; O'Sullivan & Perrin, 1964). The observation of O'Sullivan & Perrin (1964) that the stability constant of Mg–ATP was 4 times lower in Tris than in triethanolamine buffer may be rationalized on the basis of the enhanced stabilization of the ATP–Tris complex with respect to that of ATP–triethanolamine due to formation of additional hydrogen bonds involving the primary amine group in the former.

The exclusive binding of Tris molecules to the phosphate oxygens is probably influenced by the positively charged adenine base. The association of Tris molecules with other nucleotides which incorporate keto groups might be even larger. Therefore, we may conclude that the presence of polyhydric positively charged molecules such as Tris in biological systems which involve nucleotides and metal ions may affect significantly their biological activity.

## Supplementary Material Available

A listing of heavy-atom and hydrogen parameters and of observed and calculated structure factors (10 pages). Ordering information is given on any current masthead page.

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