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Metal Binding to Nucleic Acid Constituents. The Crystal Structure of Catenadichloro-μ-(9-methyladenine)zinc(II)[*]

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The crystal structure of catenadichloro-μ-(9-methyladenine)zinc(II) has been determined from the intensities of 2156 reflections measured with $\theta/2\theta$ scans on an automatic diffractometer using Cu $K\alpha$ radiation. The compound crystallized from an aqueous solution of zinc chloride and 9-methyladenine at $p\text{H} \sim 6$ in the monoclinic space group $P2_1/n$ with $a = 9{\cdot}938$ (1), $b = 13{\cdot}863$ (1), $c = 7{\cdot}453$ (1) Å, $\beta = 94{\cdot}93$ (1)°. Full-matrix least-squares refinement of all atomic positional coordinates and anisotropic temperature parameters for all atoms except H has resulted in an R index of 0·03. Each zinc ion is tetrahedrally coordinated to two Cl atoms and N(1) and N(7) of neighboring adenine moieties, with the –[Zn–N(1).9-methyladenine.N(7)]– unit being repeated by the n glide to form infinite chains of metal-linked bases throughout the crystals. The similarity in the lengths of the Zn–N(1), 2·068, and Zn–N(7), 2·040 Å, bonds is an indication that the metal ion binds with comparable strength at each of these sites. A model is presented for the bonding of zinc ions to adenine bases in DNA.

Introduction

Metal ions have a significant effect on the conformation of DNA and related molecules. Different effects are produced by different metal ions when parameters such as pH, ionic strength, and temperature of the solution are varied (Eichhorn, Berger, Butzow, Clark, Rifkind, Shin & Tarien, 1971; Shin, Heim & Eichhorn, 1972). Thus it is important to establish the binding sites of the metal ions under a variety of conditions.

DNA can be reversibly denatured by temperature manipulation in the presence of zinc ions. Shin & Eichhorn (1968) have postulated that the zinc ions are bound to the bases during this process. Crystal structure analyses of several zinc–purine complexes have established N(7) as a primary coordination site in N(9)-substituted purine derivatives in the solid state [Taylor, 1973; De Meester, Goodgame, Jones & Skapski, 1974; dichloroaquo-(9-ethylguanine)zinc(II), Taylor, unpublished]. In one other structure the sole binding site is N(1) (McCall & Taylor, 1975). The crystal structure of a zinc-9-methyladenine complex is described here. This compound was crystallized from a weakly acidic aqueous solution. Both the N(1) and N(7) binding sites of adjacent adenine bases are utilized by the zinc ion in the complex, thus confirming N(7) as a favored binding site, and endorsing the possibility of N(1) being a likely site for metal coordination in solution of weakly acidic to neutral pH. De Meester, Goodgame, Skapski & Warnke (1973) have determined the structure of an isomorphous Co complex crystallized from ethanol.

Experimental

Colorless chunky crystals of catenadichloro-μ-(9-methyladenine)zinc(II) were prepared by mixing warm, near-saturated, aqueous solutions of zinc chloride and 9-methyladenine (mole ratio ~4:1). Sufficient nitric acid was added to keep the pH at approximately 6. The space group was determined from examination of systematic absences on precession photographs obtained with Mo $K\alpha$ radiation. A few very weak reflections of the types $h0l$ ($h+l=2n+1$) and $0k0$ ($k=2n+1$) were observed to be significantly higher than background during intensity data collection using Cu $K\alpha$ radiation: of the 89 monitored 10 had intensities $I > 3\sigma(I)$. The three strongest reflections of this type were 050, $|F| = 7{\cdot}5$ (2); 300, $|F| = 5{\cdot}7$ (2); and 601, $|F| = 6{\cdot}6$ (2), where the $|F|$'s are on the absolute scale. From the refinement of the structure it was concluded that the deviations from the symmetry of space group $P2_1/n$ were not significant. Unit-cell parameters were obtained from a least-squares fit of

Table 1. *Crystallographic data*

Catenadichloro-μ-(9-methyladenine)zinc(II) $[C_6H_7N_5Cl_2Zn]_n$

Monoclinic	Space group: $P2_1/n$
$a = 9{\cdot}938$ (1) Å	F.W. 285·43
$b = 13{\cdot}863$ (1)	$F(000) = 564$
$c = 7{\cdot}453$ (1)	$V = 1022{\cdot}9$ (3) Å^3
$\beta = 94{\cdot}93$ (1)°	$Z = 4$
$D_x = 1{\cdot}853$ g cm^{-3}	$\mu(\text{Cu } K\alpha) = 79{\cdot}77$ cm^{-1}
$D_m = 1{\cdot}86$ (1)	$\lambda(\text{Cu } K\alpha) = 1{\cdot}5418$ Å

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the $\sin^2\theta$ values of 15 high-angle Cu $K\alpha_1$ reflections measured on the diffractometer from the crystal employed for data collection. The density was measured by flotation in mixtures of carbon tetrachloride and dibromomethane. Crystallographic data are given in Table 1.

The intensities of 4264 reflections in one-half of the sphere were measured on a G.E. quarter-circle diffractometer using Ni-filtered Cu $K\alpha$ radiation with the crystal mounted in a general direction, near the *a* axis. The $\theta/2\theta$ scan method was used with scan ranges of 1·6 to 3·0°, a scan speed of 1° per min, and a background counting time of 40 to 60 s. The crystal was of irregular shape with many faces developed – the maximum dimension being 0·17, and the minimum 0·13 mm. Two check reflections measured after every 50 reflections showed no significant change in intensity.

The data were averaged to give intensities for a set of 2156 independent reflections of which 44 had negative intensities. Each reflection was assigned a variance $\sigma^2(I)$, based on counting statistics plus an additional term $(0{\cdot}02S)^2$, where S is the scan count. The data were not corrected for absorption because of the difficulty in accurately defining the crystal. The agreement index $(\sum|\Delta F|/\sum F)$ between symmetry-equivalent reflections was 0·04.

Structure determination and refinement

The atomic scattering factors for Zn^{2+}, Cl^-, N, and C were taken from Cromer & Waber (1965), and for H from Stewart, Davidson & Simpson (1965). The real and imaginary parts of the anomalous dispersion correction were applied for Zn^{2+} ($\Delta f' = -1{\cdot}7$, $\Delta f'' = 0{\cdot}8$) and Cl^- ($\Delta f' = 0{\cdot}3$, $\Delta f'' = 0{\cdot}7$) as listed in *International Tables for X-ray Crystallography* (1962). Computer programs used in this analysis were the initial data processing programs of the CRYM system (Duchamp, 1964) and locally modified programs from a variety of sources referred to in an earlier paper (Taylor, 1973).

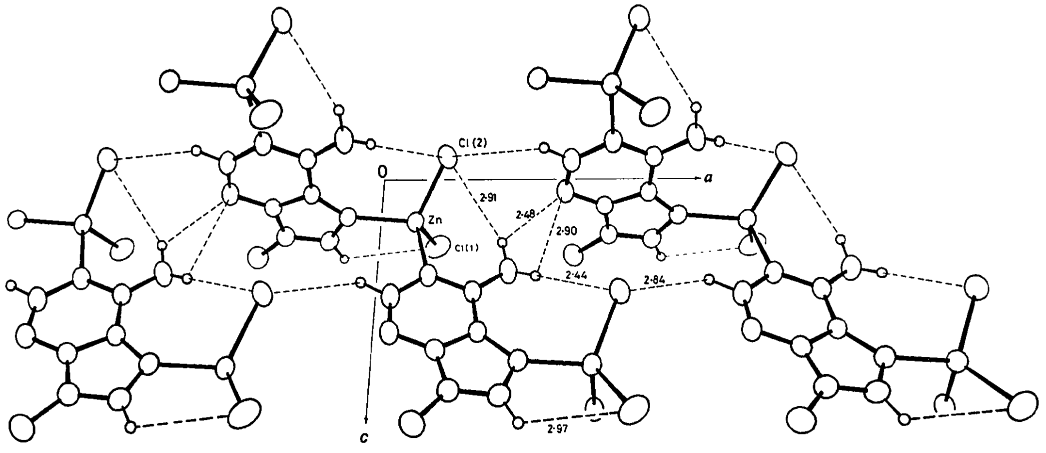

Fig. 1. A perspective view of the structure showing portions of three chains with dashed lines representing contacts shorter than 3 Å involving H atoms. The view is along the **b** direction.

Table 2. *The final atomic parameters*

(*a*) Non-hydrogen atoms

All values (× 10^4). Standard deviations are in parentheses.
Temperature factor $= \exp[-(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+2\beta_{12}hk+2\beta_{13}hl+2\beta_{23}kl)]$.

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
Zn	5992 (1)	3677 (1)	6640 (1)	49 (1)	43 (1)	119 (1)	−1 (1)	−1 (1)	3 (1)
Cl(1)	6808 (1)	5059 (1)	7772 (1)	121 (1)	48 (1)	174 (1)	−24 (1)	−36 (1)	8 (1)
Cl(2)	6778 (1)	3126 (1)	4114 (1)	60 (1)	75 (1)	159 (1)	−3 (1)	25 (1)	−22 (1)
N(1)	1427 (2)	2360 (1)	3600 (2)	47 (2)	42 (1)	139 (3)	−3 (1)	6 (2)	−10 (1)
C(2)	370 (2)	2795 (2)	4291 (3)	48 (2)	50 (1)	152 (4)	−1 (1)	6 (2)	−14 (2)
N(3)	396 (2)	3495 (1)	5484 (2)	50 (2)	50 (1)	138 (3)	2 (1)	10 (2)	−6 (1)
C(4)	1671 (2)	3750 (1)	6040 (2)	54 (2)	39 (1)	101 (3)	4 (1)	9 (2)	4 (1)
C(5)	2842 (2)	3344 (1)	5515 (2)	46 (2)	37 (1)	102 (3)	0 (1)	4 (2)	3 (1)
C(6)	2707 (2)	2628 (1)	4177 (2)	49 (2)	36 (1)	113 (3)	−2 (1)	7 (2)	2 (1)
N(6)	3743 (2)	2205 (1)	3486 (3)	51 (2)	53 (1)	191 (4)	1 (1)	19 (2)	−33 (2)
N(7)	3940 (2)	3806 (1)	6430 (2)	52 (2)	41 (1)	111 (3)	1 (1)	0 (2)	−1 (1)
C(8)	3398 (2)	4458 (2)	7439 (3)	64 (2)	46 (1)	111 (3)	4 (1)	−5 (2)	−11 (2)
N(9)	2044 (2)	4458 (1)	7252 (2)	60 (2)	45 (1)	117 (3)	7 (1)	3 (2)	−12 (1)
C(9)	1126 (2)	5077 (2)	8163 (3)	82 (2)	66 (2)	164 (4)	16 (1)	17 (3)	−31 (2)

Table 2 (*cont.*)

(*b*) Hydrogen atoms

Positional parameters ($\times 10^3$). Standard deviations are in parentheses. Temperature factor = exp ($-B \sin^2 \theta/\lambda^2$).

	x	*y*	*z*	*B*
H(2)	−51 (3)	256 (2)	382 (3)	2·9 (4)
H(8)	388 (2)	491 (2)	824 (3)	2·2 (4)
H(6-1)	356 (3)	187 (2)	243 (4)	5·2 (7)
H(6-2)	451 (3)	244 (2)	365 (4)	4·8 (7)

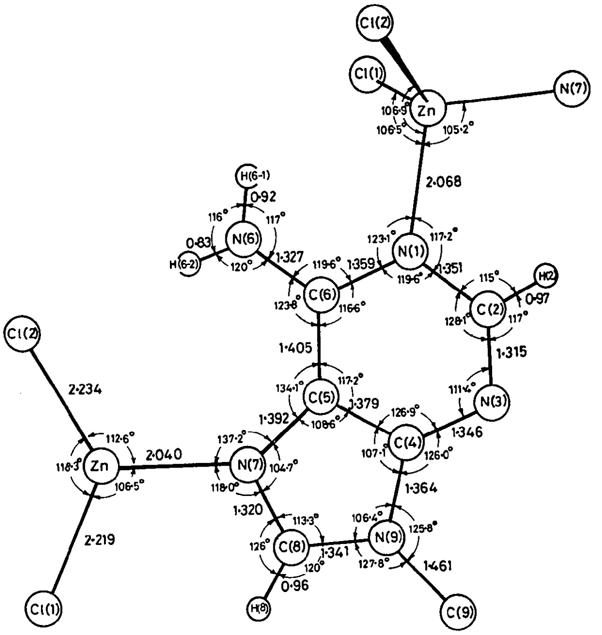

Fig. 2. Interatomic distances and angles in the complex. Standard deviations in interatomic distances are: Zn–Cl, 0·001; Zn–N, 0·002; C–C(N), 0·003; H–C(N), 0·03 Å; and 0·2° in angles not involving H atoms; 2° in angles involving H atoms.

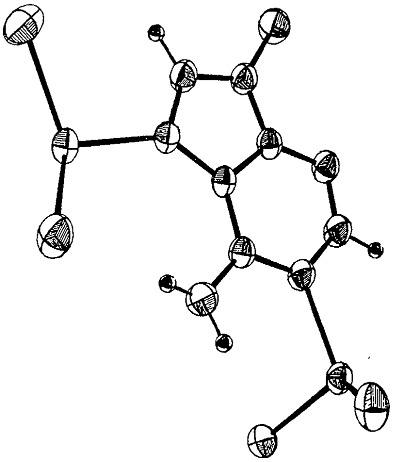

Fig. 3. A perspective view of the complex showing thermal ellipsoids drawn at the 50% probability level for all atoms, except H, which are arbitrary spheres (Johnson, 1965).

The function minimized in full-matrix least-squares refinement on a CDC6400 computer was $\sum w(F_o^2 - F_c^2)^2$, where $w = 1/\sigma^2(F_o^2)$. All reflections, including those with negative F^2, were used in the refinement. All *R* values quoted are based on $|F|$.

The coordinates of all non-hydrogen atoms were found from a Patterson map and were used to phase a Fourier synthesis in which all large peaks could be accounted for in terms of the proposed model. A structure factor calculation using the new atomic coordinates from the Fourier map resulted in an *R* index of 0·18. This model was refined with individual isotropic temperature factors by full-matrix least squares to an *R* of 0·11. Two further cycles with individual anisotropic temperature factors for the atoms reduced *R* to 0·05.

A difference map revealed the positions of four of the H atoms but not those of the methyl group. Subsequent difference maps calculated in sections parallel to the plane expected to contain the methyl H atoms showed a horseshoe area of positive electron density – the maximum density at a point in this area being 0·34 e Å^{-3}. Corrections for anomalous scattering by the Zn and two Cl atoms, the inclusion of the parameters for the four located H atoms in least-squares calculations, and adjustments to the C(9) parameters to reduce the effect of compensating for the missing atoms, did not appreciably alter the form of the difference maps.

Although the results obtained could be explained on the basis of a rotating methyl group, there remained the possibility that the apparent disorder in the methyl H positions was related to the deviations from space group $P2_1/n$ noted earlier. Since the intensities of the observed 'systematic absences' as well as data from one-half of the sphere had been measured, a set of structure factors [corrected for secondary extinction effects (Larson, 1967)] was calculated with the model extended to space group $P1$. Difference maps calculated in each of the four planes expected to contain the methyl H atoms showed little variation from the map calculated in $P2_1/n$.

It was concluded that within the limits of uncertainty of the experimental data the structure could be defined in $P2_1/n$ with six half-hydrogen atoms approximating the methyl group.

Further full-matrix least-squares calculations in $P2_1/n$ using the reduced data set and refining the scale factor, the secondary extinction parameter, and all atomic parameters (anisotropic refinement for the heavy atoms, isotropic for the H atoms, except for the methyl H atoms whose parameters were not refined) resulted in the final shifts all being less than one-fifth of their standard deviations, except for the extinction parameter. The final value of *g* was 1×10^{-7} ($\sigma = 2 \times 10^{-10}$, final shift $= 2 \times 10^{-9}$).

The *R* index for the complete data set used in the final refinement was 0·030, R_w was 0·058, and the goodness of fit was 2·00 for 144 variables and 2156 observations.* At the conclusion of the refinement a difference map in the plane of the adenine moiety was calculated. A prominent feature was a peak of 0·36 e Å^{-3} near the midpoint of the Zn–N(1) bond. All other irregularities were within $\pm$0·2 e Å^{-3} [$\sigma(\varrho)\simeq$ 0·1 e Å^{-3}]. The final atomic parameters and their standard deviations estimated from the inverted full-matrix, are listed in Table 2.

Description and discussion

The metal-ligand structure

Each zinc ion is coordinated to N(1) and N(7) of two neighboring adenine moieties, with the –[Zn–N(1).9-methyladenine.N(7)]– unit being repeated by the *n* glide to form infinite chains throughout the crystals (Fig. 1). Coordination about the Zn atom is approximately tetrahedral with two Cl atoms in the other positions. Although the Zn–N(1) and Zn–N(7) bond lengths differ by some 10 standard deviations (Fig. 2) the near equality in their lengths is a measure of the similarity in the strength of the metal interaction at the two binding sites. The two Zn–Cl bond lengths are also significantly different. Participation of Cl(2) in strong hydrogen bonds with the protons of the amino group contributes to the distortion from tetrahedral symmetry about the Zn atom and to the differences in the Zn–Cl bond lengths.

The bond lengths and angles within the adenine ring are in agreement with those observed in other neutral adenine systems, such as in adenosine (Lai & Marsh, 1972) where the maximum differences in bond lengths and angles between the two structures are 0·2 Å and 2·2° respectively. Taylor (1973) has observed that Zn coordination at N(7) does not appreciably perturb the interatomic distances and angles of the adenine ligand in trichloroadeniniumzinc(II). The same is true for Zn coordination at both N(7) and N(1) in this structure. This contrasts with the usual observation that protonation at either of these sites increases the internal ring bond angle at N by several degrees. The information available on the isostructural Co complex (De Meester *et al.*, 1973) is insufficient to allow a detailed comparison with the structure presented here.

Least-squares planes calculations have shown that the atoms of the imidazole ring, together with H(8) and C(9), are planar within experimental error. The Zn atom is 0·08 Å from this plane. The pyrimidine ring is folded about the C(2)–C(5) line so that the amino group is closer to Cl(2), thus strengthening the hydrogen-bonded interactions between these atoms. The Zn atom and the amino N atom, N(6), are 0·01 Å from the plane defined by C(2), N(1), C(6), and C(5), while H(6–1) and H(6–2) are displaced by 0·24 Å and 0·19 Å respectively, all in the same direction from this plane. The significant out-of-plane displacements of the amino protons is presumably caused by hydrogen bonding with Cl(2). The dihedral angle between the planes in the folded pyrimidine ring is 2·7°. The imidazole ring is almost coplanar with the plane defined by the atoms C(2), N(3), C(4), and C(5).

A thermal ellipsoid plot (Fig. 3) shows that, in general, out-of-plane vibrations of the base are slightly larger than in-plane vibrations, as is to be expected. The ellipsoids all appear reasonable and give no indication of abnormal displacements of atoms from their refined positions in space group $P2_1/n$.

Hydrogen bonding

Data for interactions involving H atoms are given in Table 3.

Table 3. *Interactions involving hydrogen atoms*

Donor atom	Acceptor atom*	Distances (Å) $D\cdots A$	$\text{H}\cdots A$	Angle (°)	
N(6)	Cl(2)$^{\text{i}}$	3·271	2·44	N(6)–H(6–2)–Cl(2)	179
N(6)	Cl(2)$^{\text{ii}}$	3·681	2·91	N(6)–H(6–1)–Cl(2)	142
N(6)	N(3)$^{\text{iii}}$	3·045	2·48	N(6)–H(6–1)–N(3)	120
N(6)	N(3)$^{\text{iii}}$	3·045	2·90	N(6)–H(6–2)–N(3)	92
C(8)	Cl(1)$^{\text{i}}$	3·477	2·97	C(8)–H(8)—Cl(1)	114
C(2)	Cl(2)$^{\text{iv}}$	3·591	2·84	C(2)–H(2)—Cl(2)	136

* Superscripts indicate position according to: i x,y,z; ii $x-\frac{1}{2},\frac{1}{2}-y,z-\frac{1}{2}$; iii $x+\frac{1}{2},\frac{1}{2}-y,z-\frac{1}{2}$; iv $x-1,y,z$.

Strands of metal–base complex are held together in the structure by electrostatic forces between Cl(2) and H(2), and between the amino protons and N(3). Along each strand there are hydrogen bonds between Cl(2) and different amino protons of adjacent adenine molecules (Fig. 1). These interactions may also be represented by the chain of bonds N(1)–Zn–Cl(2)$\cdots$ H(6–1)–N(6)–H(6–2)$\cdots$Cl(2)–Zn–N(7), where N(1) and N(7) are atoms of the same 9-methyladenine molecule. A segment of this chain, Cl(2)$\cdots$amino group$\cdots$Cl(2), is the basic unit in a sequence of hydrogen bonds extending continuously throughout the structure. This sequence is a major stabilizing influence in the crystal structure and is one of the factors holding the planes of adjacent bases within a strand approximately at right angles to each other.

Comments

A strong hydrogen bond between one proton of the amino group and a metal ligand is a common feature in the structures of metal–nucleic acid base complexes which have so far been determined (McCall & Taylor, 1975, and references therein). It is interesting to note that in this structure where metal ions are bonded at two well-separated sites on the same adenine molecule, the metal-ligand to amino-proton hydrogen bonds which form involve both protons of the amino

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* A list of structure factors (including data for the 'systematically absent' reflections) has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 31477 (14 pp., 1 microfiche). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

group. This is further evidence for the formation of this type of hydrogen bond being highly favored in the crystalline state. When these results are combined with those from NMR work on similar complexes in the liquid state (Hadjiliadis, Kourounakis & Theophanides, 1973) there seems to be little doubt that such hydrogen bonds also exist in solution.

It is interesting to consider the possibility that the linking of bases by metal ions through N(1) and N(7) as demonstrated here in the solid state, occurs in aqueous solution. We have no direct evidence on this matter, but some observations from crystal structure, and NMR, analyses may be relevant. Kong & Theophanides (1974) have deduced from PMR spectra that N(1) and N(7) of adenosine are coordinated simultaneously to two different Pt atoms in aqueous solution. In crystals, Zn and other metals can bind to the adenine moiety at N(7) alone (*e.g.* Taylor, 1973) and Zn at N(1) alone (McCall & Taylor, 1975) and further, the latter compound crystallizes from a solution containing the same components as the one described herein. It would seem reasonable to assume that there must be significant concentrations of 9-methyladenine.N(1)–Zn–N(7).9-methyladenine and Zn–N(1).9-methyladenine.N(7)–Zn complexes in solution in equilibrium with other metal–base species.

It is possible that the base–metal–base stereochemistry observed in this structure could be adopted when zinc ions interact with the bases of DNA. Examination of a CPK model shows that whenever two adenine–thymine base pairs are adjacent on the double-helix strands, small movements of a part of one strand (relative to the other strand assumed fixed) will position the bases to allow crosslinking by a zinc ion. The required configuration arises if the phosphate–ribose backbone is twisted to move a thymine base away from its hydrogen-bonded partner, and the adjacent adenine base is rotated about the glycosidic bond and moved towards the other adenine base into the space created by the displacement of thymine. All of these movements are possible in the denatured state of DNA. The zinc ion is then able to crosslink the strands by binding to the adenine bases at N(1) and N(7). The amino groups are also in position for hydrogen-bonded interactions with another ligand of the metal ion. Hence this structure may model the way in which zinc ions are able to hold together the strands of a DNA molecule in its denatured state.

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