Synthesis and X-ray Structure of a Hg(II) Complex with Adenine N(1)-Oxide. A Model for Mercury Binding to DNA

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Abstract

The synthesis and crystal structure of the adenine N(1)-oxide complex with mercury(II) chloride, $(C_5H_5N_5O)HgCl_2$ are reported. Crystals of the coordination compound belong to the monoclinic system, space group $P2_1/n$ with the following primary crystallographic data: a = 6.685(1) Å, b = 11.798(2) Å, c = 10.155(1) Å, β = 100.22(1)°, V = 906.04 Å^3, Z = 4. The structure was elucidated by conventional Patterson and Fourier methods and refined by the full matrix least-squares technique on the basis of 1977 observed reflections to an R value of 0.074. The basic unit of the structure is a dimer, with a centre of symmetry, consisting of two $HgCl_2$ moieties and two adenine N(1)-oxide ligands. A polymeric structure results from the bridging interactions of chloride ions. Adenine N(1)-oxide acts as a bidentate bridging ligand, coordinating through N(7) and O(1). The coordination geometry around the mercury ion is a distorted square pyramid with N(7) and three chlorines (two of which are centrosymmetrically related) forming the square plane and O(1) occupying the axial position. Hg also interacts indirectly with N(6) through a Cl····H–N hydrogen bond. Principal intracomplex geometrical parameters are as follows: Hg–N(7) = 2.61(1) Å, Hg–O(1) = 2.55(1) Å, Hg–Cl(1) = 2.330(3) Å, Hg–Cl(2) = 2.318(3) Å, Hg–Cl(2′) = 3.347(3) Å. The *cis* angles range from 77.5° to 107.9° and the two *trans* angles are 155.5° and 163.1°. The centrosymmetrically related bases overlap partially and pack at a distance of 3.2 Å. The glide-related bases are linked by a hydrogen bond, N(9)–H····O(1) and are inclined to one another by 109.7°. The results are compared with those derived from spectroscopic and other physicochemical studies on metal interaction with adenine N(1)-oxide. Based on the present structural observations and earlier experimental results a possible mechanism is proposed for mercury interaction with DNA.

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Introduction

Nucleic acids are the basis on which cells exist, function and reproduce. Consequently a considerable amount of effort is being devoted to the study of these polymers. Because of limitations in the study of macromolecular and fibrous nucleic acids, *viz.*, DNA and RNA, efforts have been directed towards the study of nucleic acid constituents [1] – nucleotides, nucleosides and bases. These studies have in turn yielded data regarding hydrogen bonding and stacking interactions [2], as well as the conformational effects that exist within the nucleic acids. The study of metal ion interactions with these nucleic acid constituents [3] is also of great importance due to the variety of metal ion–nucleic acid interactions observed in biological systems [4]. A great wealth of information has been accumulated regarding the binding sites on these ligands for a variety of metal ions.

The interaction of Hg(II) with nucleic acid constituents is of special interest as the former is unique in its ability to form complexes with native DNA at room temperature [5]; other metals such as Cu(II) require thermal assistance for binding. Extensive studies have been carried out on mercury–DNA interactions [5–10] and a general conclusion drawn was that Hg(II) binds to the nitrogen atom of the heterocyclic bases. Moreover it was reported [11] that the binding is selective and AT rich DNA's bind more strongly than GC rich DNA's. Further , it was observed that the original DNA is recovered by the addition of Cl^- or CN^- to the Hg(II)–DNA complex and the biological activity is not lost by this reversible process [11, 12]. A knowledge of mercury binding sites for these DNA constituents is, therefore, of great value in understanding the mechanism of such a complex formation.

Modified nucleotides, nucleosides and their heterocyclic bases, in general, are not only biologically active compounds, but also of potential chemotherapeutic value. For example, it has been suggested that adenine N(1)-oxide and certain of its derivatives may either act as antimetabolites or

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be metabolized to normal cellular purines [13]. They may also be significant in the metabolic roles of some coenzymes in oxidation-reduction systems, as well as in the enzymatic hydroxylation of purines [14]. The study on purine N(1)-oxides, in general, is of special interest in view of the established oncogenic activity of these ligands [15]. Another aspect of fundamental interest is the change in coordination behaviour of these modified entities. For these reasons we have taken up the structural study on a mercury complex with adenine N(1)-oxide. As far as we are aware this is the first crystallographic study on a metal–adenine N(1)-oxide complex.

Experimental

Synthesis

The complex $Hg(Ad\text{-}N\text{-}O)Cl_2$ was prepared by adding 0.5 mmol of $HgCl_2$ (0.136 g) to a suspension of 0.5 mmol (0.075 g) of adenine N(1)-oxide in 10 ml of dimethyl sulphoxide (DMSO). Adenine N(1)-oxide is not soluble in DMSO at ambient temperature, but slowly goes into solution on heating. The reaction mixture was then refluxed for about 48 h and the clear solution was maintained at room temperature. Pale pink tabular crystals were obtained after about 15 days.

Adenine N(1)-oxide was obtained from Sigma Chemical Company and mercuric chloride from BDH.

Collection and Reduction of the X-ray Intensity Data

Preliminary oscillation and Weissemberg photographs showed the crystal system to be monoclinic and the space group to be $P2_1/n$. Unit cell dimensions and their associated standard deviations were derived from a least-squares fit to the setting angles for 21 carefully selected and centered reflections on a CAD-4 automated diffractometer. Complete crystal data are collected in Table I. The intensities of 2539 reflections ($2\theta \leqslant 60°$) were measured on the Nonius CAD-4 diffractometer employing graphite monochromatized Mo-Kα radiation. The crystal used for data collection was mounted approximately parallel to the crystallographic a axis and its dimensions and face assignments are given in Table I. Intensity data were collected in the $\omega/2\theta$ scan mode with a constant scan speed of 1°/min. Data collection parameters are given in Table I. the intensities of two standards were monitored after every 3000 s and showed no systematic variation over the duration of the experiment. The intensities and their standard deviations were corrected for Lorentz and polarization effects. An absorption correction was applied on the basis of the dimensions and face assignments. An approximation to the absolute scale factor was derived by the method of Wilson.

TABLE I. Crystal Data.

Molecular formula	$Hg(C_5H_5N_5O)Cl_2$
Crystal system	Monoclinic
Space group	$P2_1/n$
a, Å	6.685(1)
b, Å	11.798(2)
c, Å	10.155(1)
β, deg.	100.22(1)
V, Å^3	906.04
Z	4
D_c, g cm^{-3}	3.09
M	422.49
Crystal dimensions, mm	(100)–($\bar{1}$00), 0.65
	(010)–(0$\bar{1}$0), 0.1
	(001)–(00$\bar{1}$), 0.225
F(000), electrons	800
Wavelength, λ, Å	MoKα, 0.7107
μ(MoKα), cm^{-1}	178.9
2θ upper limit, deg.	60
No. of reflections measured	2539
No. of reflections used	
($I \geqslant 3\ \sigma(I)$)	1912
R	0.074
R_w	0.077

Solution and Refinement of the Structure

The positional parameters of the Hg atom, chlorine atoms and those of the adenine N(1)-oxide ligand were obtained from a three-dimensional Patterson synthesis followed by two difference Fourier syntheses. Several cycles of isotropic refinement gave a conventional R value of 0.122. A difference Fourier computed at this stage revealed the position of all the hydrogen atoms. The temperature factors of hydrogen atoms were set equal to 1.5 Å^2 + B of the heavy atom to which they were bonded. Three cycles of anisotropic refinement holding the hydrogen atom parameters fixed led to convergence. The final conventional R and R_w values are 0.074 and 0.077 respectively*. The weighting scheme used was $w = 1/\sigma(F_o)^2 + 0.0027|F_o|^2$. A final difference Fourier synthesis was essentially featureless (maximum and minimum peaks less than ±0.5 e/Å^3), with the exception of a peak of about 3 e/Å^3 near mercury.

*The major computations in this work were made on a DEC-10 computer using the programs listed in reference 16. Other calculations were performed with locally written programs.

TABLE II. Final Fractional Parametrs ($\times 10^4$). e.s.d.s are in parentheses.

Atom	*x*	*y*	*z*
Hg	3499(1)	5069(1)	3035(1)
Cl(1)	2270(5)	3291(3)	2468(3)
Cl(2)	5646(5)	6324(3)	4051(3)
N(1)	9202(17)	5182(9)	2686(10)
O(1)	487(12)	5881(8)	3248(8)
C(2)	8607(21)	4337(12)	3389(14)
N(3)	7491(15)	3556(10)	2896(10)
C(4)	6934(17)	3672(9)	1608(12)
C(5)	7403(15)	4485(9)	741(10)
C(6)	8574(18)	5319(10)	1330(11)
N(6)	9216(15)	6188(9)	720(10)
N(7)	6544(14)	4297(9)	−563(10)
C(8)	5575(19)	3371(10)	−460(12)
N(9)	5731(15)	2968(9)	809(10)
H(C2)	9181	4264	4414
H(C8)	5102	2928	−1397
H(N9)	5353	2198	947
H′(N6)	8616	6381	−302
H′(N6)	9309	6998	673

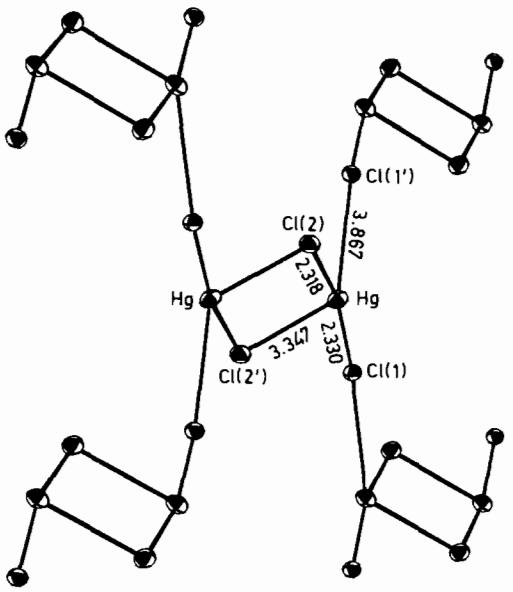

Fig. 1. A view of the mercury–chlorine network in the (101) plane. Cl(1′) and Cl(2′) represent the symmetry-related positions of Cl(1) and Cl(2) respectively. Note the three types of mercury–chlorine interactions.

Neutral scattering factor curves for the nonhydrogen [17] and hydrogen [18] atoms were used. Anomalous dispersion corrections were applied to the scattering curves for all the nonhydrogen atoms. Final atomic coordinates are listed in Table II. Bond lengths and bond angles are given in Table III. Tables of thermal parameters, least-squares planes and observed and calculated structure factors are available as supplementary material.

TABLE III. Bond Lengths and Bond Angles. e.s.d.s are in parentheses.

Bond	A	Bond	Angle, deg.
Hg–Cl(1)	2.330(3)	Cl(1)–Hg–Cl(2)	155.5(1)
Hg–Cl(2)	2.318(3)	Cl(1)–Hg–O(1)	91.4(2)
Hg–Cl(2′)	3.347(3)	Cl(1)–Hg–Cl(2′)	77.5(1)
Hg–Cl(1′)	3.867(3)	Cl(1)–Hg–N(7)	94.9(2)
Hg–O(1)	2.55(1)	Cl(2)–Hg–Cl(2′)	84.7(1)
Hg–N(7)	2.61(1)	Cl(2)–Hg–O(1)	107.9(2)
N(1)–O(1)	1.33(1)	Cl(2)–Hg–N(7)	97.6(2)
N(1)–C(2)	1.35(2)	O(1)–Hg–Cl(2′)	98.5(2)
C(2)–N(3)	1.30(2)	O(1)–Hg–N(7)	96.7(8)
N(3)–C(4)	1.31(2)	Cl(2′)–Hg–N(7)	163.1(2)
C(4)–C(5)	1.39(2)	N(1)–O(1)–Hg	110.3(8)
C(5)–C(6)	1.40(2)	Hg–N(7)–C(8)	113.4(8)
C(6)–N(6)	1.34(2)	Hg–N(7)–C(5)	142.5(8)
C(6)–N(1)	1.39(2)	O(1)–N(1)–C(2)	122(1)
C(5)–N(7)	1.39(1)	O(1)–N(1)–C(6)	117(1)
N(7)–C(8)	1.34(2)	C(2)–N(1)–C(6)	121(1)
C(8)–N(9)	1.36(2)	N(1)–C(2)–N(3)	126(1)
N(9)–C(4)	1.39(2)	C(2)–N(3)–C(4)	113(1)
C(2)–H	1.06	N(3)–C(4)–C(5)	129(1)
C(8)–H	1.09	N(1)–C(6)–N(6)	117(1)
N(9)–H	0.97	C(4)–C(5)–C(6)	116(1)
N(6)–H	1.08	C(5)–C(6)–N(1)	115(1)
N(6)–H′	0.96	C(5)–C(6)–N(6)	127(1)
		C(4)–C(5)–N(7)	111(1)
		C(5)–N(7)–C(8)	103(1)
		N(7)–C(8)–N(9)	114(1)
		C(8)–N(9)–C(4)	106(1)
		N(9)–C(4)–C(5)	105(1)
		N(9)–C(4)–N(3)	126(1)
		N(7)–C(5)–C(6)	133(1)

Results and Discussion

While the structure determination of the complex was undertaken for a variety of reasons, the principal interest was in the mode of coordination of the mercury atom to the adenine N(1)-oxide ligand. Each mercury ion has two strong and two weak Hg–Cl bonds. The chlorines Cl(1) and Cl(2) are nearer to Hg(II) at distances of 2.33 Å and 2.32 Å respectively (Fig. 1). The Hg–Cl distances observed for a variety of complexes are shown in Table IV. The chlorine Cl(2′), centrosymmetrically related to Cl(2), is at a distance of 3.35 Å from Hg(II). This bridging interaction is even weaker than that observed in the complex, $Hg(Py\text{-}N\text{-}O)Cl_2$ (Hg–Cl = 3.32 Å) [24], but is within the sum of van der Waals radii (1.73 + 1.8 = 3.53 Å) for Hg and Cl [29]. The chlorine Cl(1) is also involved in bridg-

TABLE IV. Binding Sites and Coordinating Distances in Some Mercury Complexes.

Compound[a]	Binding sites on the base	Hg–N	Hg–O	Hg–Cl	Ref.
$Hg(8\text{-}AAd)_2Cl_2$	N(3)	2.73		2.39, 2.92	19
$Hg(8\text{-}AHX^-)_2(H_2O)_4$	N(9)	2.04	2.68, 2.80		19
$Hg(Guo)Cl_2$	N(7)	2.16		2.34, 2.66, 2.76	20
$Hg(1\text{-}MeCyd)Cl_2$	N(3), O(2)	2.17	2.84	2.32, 2.72, 2.75	21
$Hg(Ura)Cl_2$	O(4)		2.71	2.30, 3.07	22
$Hg(Dihydro\ Ura)Cl_2$	O(4)		2.88	2.28, 3.05	22
$Hg(1\text{-}Methy)_2$	N(3)	2.04			23
$Hg(Py\text{-}N\text{-}O)Cl_2$			2.59	2.32, 3.19	24
			2.60	2.34, 3.32	
$Hg(Nap)_3ClO_4^+$		2.14, 2.87			
		2.20, 2.84	2.93		25
		2.30, 2.64			
$Hg(CH_3)(9\text{-}MeGua)^+$	N(7)	2.09	2.75, 2.99		26
$(Hg(CH_3))_2(Ade)^+$	N(7), N(9)	2.09, 2.08			27
$(Hg(CH_3))_2(9\text{-}MeAde)^+$	N(1), N(6)	2.09, 2.08			27
$Hg(AdH)Cl_3$				2.34, 2.38, 2.76	28
				3.26, 3.25, 2.82	
$Hg(Ad\text{-}N\text{-}O)Cl_2$	N(7), O(I)	2.61	2.54	2.33, 2.32	Present work
				3.35, 3.87	

[a]Abbreviations: 8-AAd, 8-azaadenine; 8-AHX, 8-azahypoxanthine; Guo, guanosine; 1-MeCyd, 1-methylcytosine; Ura, uracil; Dihydro Ura, dihydrouracil; I-MeThy, 1-methylthymine; Py-N-O, pyridine N-oxide; Nap, 1,8-naphthyridine; 9-MeGua, 9-methylguanine; Ade, adenine; 9-MeAde, 9-methyladenine; AdH, adeninium cation; Ad-N-O, adenine N(1)-oxide.

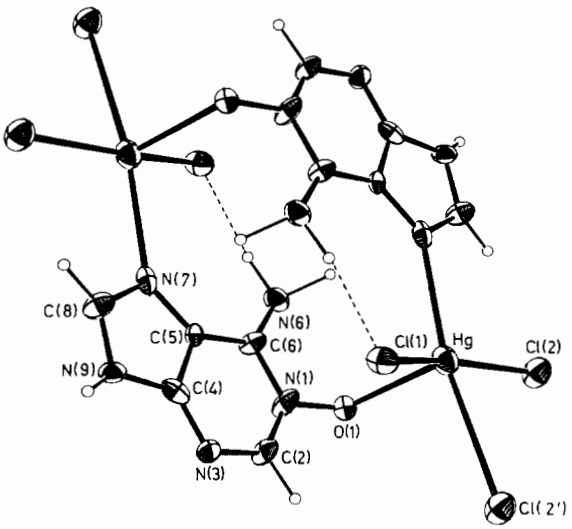

Fig. 2. A perspective view of the dimeric unit in the structure, displaying the coordination polyhedron of mercury. Broken lines indicate intramolecular hydrogen bonds.

ing the glide-related mercury ion, at a distance of 3.87 Å. This is the longest among the values reported for a variety of mercury structures and also is longer than the sum of van der Waals radii. The mercury-chlorine interaction in the present case appears to be similar to that observed in the structure of $HgCl_2$ [30], where mercury is bonded to three pairs of chlorine atoms at distances of 2.25 Å, 3.34 Å and 3.63 Å respectively.

The adenine N(1)-oxide acts as a briding bidentate ligand, coordinating through O(1) and N(7). The molecular structure consists basically of a dimeric unit with a centre of symmetry, involving two $HgCl_2$ moieties and two adenine N(1)-oxide ligands as shown in Fig. 2. The neighbouring dimers along [101] are linked by chlorine bridges and this arrangement extends infinitely forming a chain. The bridging chlorines, Cl(2), Cl(2′) and the two nearest mercury atoms form a four-membered ring with a centre of symmetry. The glide-related molecules form a similar chain and are linked to the former through weak chlorine bridges. The chlorine Cl(1) at a distance of 3.87 Å from Hg(II) takes part in bridging the glide-related molecules. A polymeric structure results due to the bridging interactions of the chlorines.

The coordination geometry around mercury is distorted. The coordination polyhedron cannot be clearly defined when the longest Hg–Cl(1) distance is included. If this bond is not considered, the polyhedron can be described as a distorted square pyramid (Fig. 2). Three chlorine atoms Cl(1), Cl(2),

Cl(2′) and N(7) of the base define the square plane, while O(1) occupies the axial position. The *cis* bond angles in the plane range from 77.5° to 97.6° and the two *trans* angles are 155.5° and 163.2°. The Hg–O(1) bond is nearly perpendicular to the square plane with the angles ranging from 91.4° to 107.9°.

The least-squares plane passing through the four atoms shows that Hg(II) is deviated by 0.40 Å towards O(1). The Hg–O(1) bond (2.55 Å) is similar to those listed in Table IV. The Hg–N(7) bond length, 2.61 Å, is significantly longer than most Hg–N separations collected in the table and is comparable to the Hg–N distance observed in the case of Hg-(8-AAd)$_2$Cl$_2$ [19] and the secondary Hg–N interactions in the case of [Hg(Nap)$_3$ClO$_4$]$^+$ [25].

An interesting feature in the structure is that mercury binds as $HgCl_2$ rather than Hg^{2+}. The $HgCl_2$ moiety lies approximately in the plane of the ligand. The dihedral angle between the coordination plane and the plane of the purine ring is 6.5°. The angle Cl(1)–Hg–Cl(2) measured on the side away from N(7) is 155.5° indicating that the linear Cl–Hg–Cl moiety has experienced considerable distortion due to the effect of the bulky adenine N(1)-oxide ligand coordinating at N(7). A strong Hg–N interaction is not possible because the amino substituent at the C(6) position, which lies in the plane of the base, prevents a closer approach of $HgCl_2$. The large difference of 29.1° in the external angles at N(7) (Hg–N(7)–C(8) = 113.4°; Hg–N(7)–C(5) = 142.5°) is also a consequence of these geometrical constraints. This situation can be contrasted with the case of [(HgCH$_3$)$_2$Ade]$^+$ [27] where the Hg–C bond is collinear with Hg–N(7). Consequently there are no steric effects as observed in the present case, thus resulting in a strong Hg–N(7) bond (2.09 Å) and more symmetric angles at N(7) (121° and 134°).

The bond distances and bond angles for the adenine N(1)-oxide ligand are listed in Table III. The e.s.d.s (0.01–0.02 Å in bond lengths and 1° in bond angles) are comparatively large in view of the presence of a heavy mercury atom in the structure. Thus a detailed discussion of these values is not justified. However, they agree well within the limits of experimental error with the reported values for the adenine N(1)-oxide molecule in the structure of the adenine N(1)-oxide-sulphuric acid complex [31]. The adenine N(1)-oxide molecule is approximately planar, the average deviation for the nine-atom frame-work being 0.01 Å. The atom O(1) deviates significantly out of the plane by 0.16 Å towards the mercury ion. The pyrimidine and imidazole planes are inclined to each other by 1.3°, a value normally observed in the purines.

The centrosymmetrically related bases in neighbouring chains overlap partially as shown in Fig. 3 and are perfectly parallel with a separation of 3.2 Å.

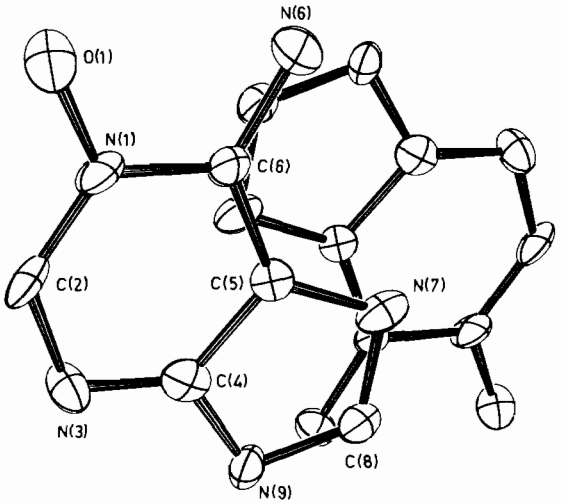

Fig. 3. Intermolecular stacking of two adenine N(1)-oxide groups.

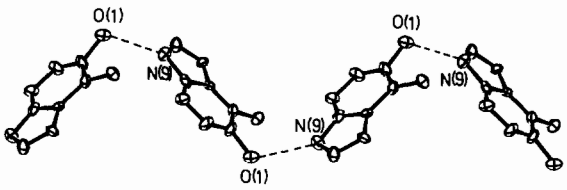

Fig. 4. Zigzag array of N(9)–H····O(1) hydrogen bonded adenine N(1)-oxide molecules. The chain extends through glide symmetry and unit cell translation along *b*.

The adenine N(1)-oxide molecule is linked to its glide-related equivalent by a N(9)–H····O(1) hydrogen bond. The hydrogen bonded molecules are oriented antiparallel and they are inclined by 109.7°. The unit cell translation extends this linkage infinitely along *b* giving rise to zigzag chains (Fig. 4). The positions of all the hydrogens were identified in the final difference map. The amino group donates a proton to chlorine, resulting in the formation of a N–H····Cl hydrogen bond, the N(6)····Cl(1) distance being 3.29 Å. The O(1) atom which coordinates to Hg(II) accepts a proton from the N(9) atom of the glide-related molecule (N(9)·····O(1) = 2.86 Å). The packing of the molecules is shown in Fig. 5.

X-ray crystallographic studies show that in purine complexes, it is a ring nitrogen which is favoured for metal binding. Adenine has the largest number of deprotonated endocyclic donor atoms, N(1), N(3) and N(7). However, the N(9) position is deprotonated easily and is the most commonly observed site for metal binding, both as a unidentate site and as part of a brdging system together with N(3). When N(9) is blocked, *e.g.*, when it is attached to a CH_3 group or a sugar, it is found that N(7) is the preferred site over N(1) or N(3). In the case of adenine N(1)-oxide, N(1) is covalently bonded to O(1)

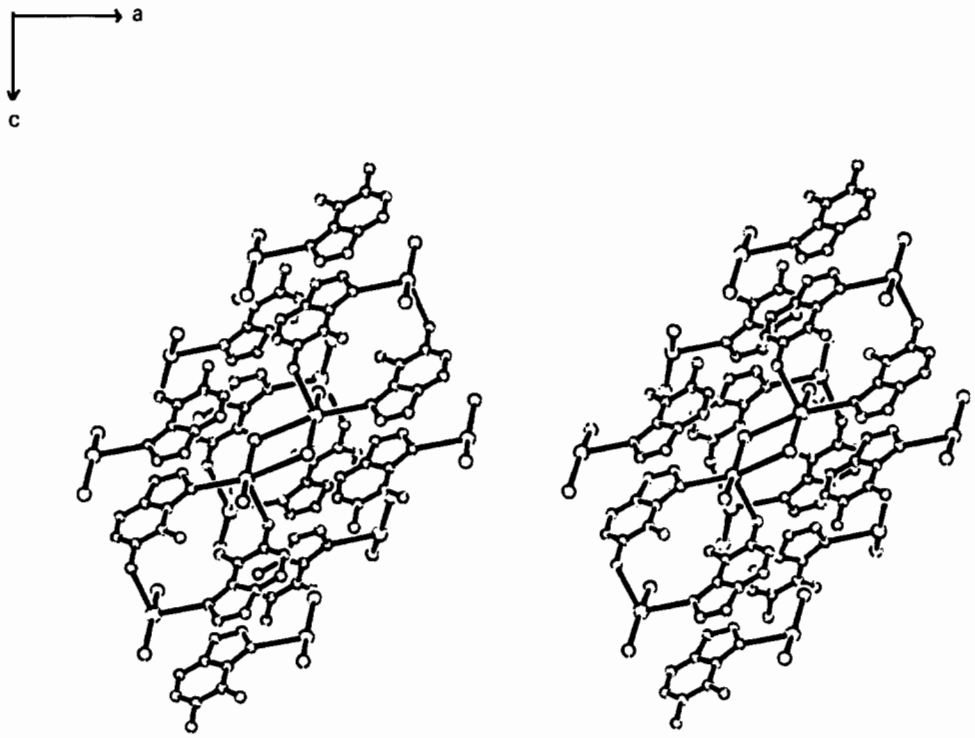

Fig. 5. A stereoview of the crystal packing. The long Hg–Cl(1′) distance is not marked.

and this oxygen is also a potential binding site in the ligand. A number of studies of adenine N(1)-oxide metal complexes [32, 33] have shown that for the first row transition metal ions (Mn through Zn), the neutral or the deprotonated ligand functions as bidentate, coordinating through NH_2 (or NH^- in the deprotonated form) and the N(7) atom without the involvement of the O(1) in coordination. On the other hand, the corresponding nucleoside, adenosine N(1)-oxide, has shown a tendency to chelate through O(1) and the amino nitrogen N(6) [33–38]. In the case of the nucleotide, adenosine 5′-monophosphate N(1)-oxide, the metal ion can coordinate to two different binding sites, the phosphate moiety and the O-amino N(1)-oxide group, which cannot complex simultaneously to the same metal ion [34]. A similar conclusion was reached for adenosine 5′-diphosphate N(1)-oxide. On the other hand, with adenosine 5′-triphosphate N(1)-oxide the formation of a macrochelate seems to be possible. The pH dependence of metal ion coordination to these ambivalent ligands has been discussed at length by Sigel [39].

Recently a series of adenine N(1)-oxide complexes with 3d-metal perchlorates [40, 41] have been investigated. The studies have shown significant differences in ligand binding sites with metal ion variation and it was concluded that the ligand binds exclusively through the imidazole nitrogen (most probably N(7)) when functioning as a unidentate terminal. Also it has been suggested that the ligand may act as bidentate, either bridging through O(1) and N(N(7) or N(9)), or chelating through O(1) and the amino group. However, there is no crystallographic evidence for any of the structures proposed for the coordination behaviour of this ligand.The present study shows that the adenine N(1)-oxide acts as a bidentate ligand involving O(1) and N(7) in coordination. The N(9) position, the commonly observed site for metal interaction in the parent adenine base is protonated in the present case. A comparison of the two coordinating distances shows that between the two binding sites mercury interaction with O(1) appears to be stronger. The significant deviation of O(1) from the plane of the purine ring in the direction of mercury further supports this deduction.

Structural Relevance to the Hg(II)–DNA Interaction

Studies on Hg(II)–DNA interaction have predicted different possible binding sites for Hg(II) both in thymidine and adenosine of the AT base pair of DNA. Davidson and coworkers [5, 11], on the basis of solution studies suggested a structure in which Hg(II) binds to the N(3) of thymidine and the NH_2 group of adenosine by displacing protons, thus cross-linking the original complementary base pair. This proposed model explains the observed

proton release upon complex formation according to equation (1).

$$HgCl_2 + H_2B_2 \overset{pH=9}{\rightleftharpoons} HgB_2 + 2H^+ + 2Cl^- \quad (1)$$

where H_2B_2 represents one base pair of DNA and B is a deprotonated base.

Later, Carrabine and Sundaralingam [22], explaining the structures of complexes of $HgCl_2$ with uracil and dihydrouracil argued that Hg(II) binding at N(3) of thymidine, a well-protected site in the ordered helix, does not fully explain the ease with which the complex forms with DNA. On the basis of structural observations, they proposed a model for Hg-DNA interaction, where Hg(II) binds to the O(4) position on thymidine. The proton release during Hg-DNA complex formation was attributed to the hydrolysis of $HgCl_2$, as shown in eqn. (3), and not to the deprotonation of the nitrogen base as suggested by Davidson and coworkers:

$$HgCl_2 + H_2B_2 \rightleftharpoons H_2B_2HgCl_2 \quad (2)$$

(complex formation)

$$H_2B_2HgCl_2 + 2H_2O \rightleftharpoons H_2B_2Hg[OH]_2 + 2H^+ + 2Cl^- \quad (3)$$

(hydrolysis of complex)

However, it should be pointed out that earlier studies have concluded that mercury binds to the nitrogen atoms of the heterocyclic base and the Hg–nitrogen bonding is stronger than the Hg–oxygen bonding. On the other hand, the preparation of both the uracil and dihydrouracil complexes was carried out at pH ~ 4.2, under which conditions the formation of a Hg-nitrogen bond is unlikely. Therefore the extension of these results to explain Hg-DNA interaction (pH = 9.0) does not appear to be convincing.

The present study involves mercury interaction with adenine N(1)-oxide, an analogue of the adenine base, and not with the base itself. Accordingly, it offers no unequivocal evidence for the nature of Hg-interaction with DNA. However, the present structural observations allow some reasonable inferences to be drawn. Hg binding at the O(1) position, observed in the present structure, may not have any significance, as this atom is not present in the parent adenine base and N(1) to which this atom is connected is a protected site in the AT base pair. An important observation in the structure is the formation of a loose chelate by Hg(II) through direct bonding to N(7) and indirect interaction with N(6) through the coordinated chlorine via Cl····H–N bonding (Fig. 2). It was shown (eqn. (1)) by Nandi *et al.* [11] that the chlorine ions are displaced from

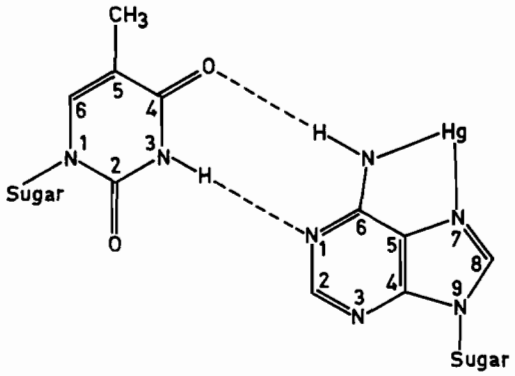

Fig. 6. Schematic representation of the proposed interaction between Hg(II) and the AT base pair of DNA.

the coordination sites of Hg at pH 9.0. Thus it is reasonable to expect that at higher pH the hydrogen bonding scheme is disturbed and Hg may then interact directly with N(6) by replacing the proton (Fig. 6). Hg binding to the primary amine nitrogen of purines and pyrimidines by displacing hydrogens is well established [8, 9]. The hydrogen bond interaction with N(6) appears to be the primary attack in order to bring mercury closer to the lone pair of nitrogen after deprotonation at higher pH. Furthermore, the deprotonation of the amino group and a direct Hg–N(6) interaction will overcome the steric strain observed in the presence of hydrogen-bonded interaction and favour a stronger Hg–N(7) bond and more symmetric angles at N(7). The second chloride ion may be replaced by OH^- through hydrolysis of the complex formed (Fig. 6) resulting in the release of another proton. The release of two protons in the process of Hg–DNA interaction observed by Nandi *et al.* at pH 9 may be the result of the deprotonation of the adenine NH_2 group and hydrolysis of the $HgCl^+$ moiety as described above.

Davidson and coworkers [5] also proposed an identical structure (Fig. 6) involving chelate formation to explain the observed proton release. However, they ruled this out on the following considerations. According to them, the normal tendency of mercury is to adopt a linear X–Hg–X configuration, which is stereochemically not possible when mercury forms a chelate involving N(6) and N(7). They also suggested that Hg complexes have a low degree of chelate stability compared to other metal complexes. However, in favour of the chelate structure, it should be argued that even tetrahedral configurations have been established and chelate stability does occur with mercury [8]. For example, the mercury atom has a tetrahedral coordination in the structure of the $HgCl_2$ complex of 1,6-dithiacyclodeca-*cis*-3-*cis*-8 diene [42]. In the complex, Hg(1-MeCyd)Cl_2, mercury forms a chelate with N(3) and O(2) of 1-

methyl cytosine [21]. Thus, it is reasonable to predict that Hg bound to the N(7) position can interact directly with N(6) of adenosine to form a chelate. Again it has to be stressed here that N(7) of adenosine is a preferred binding site for Hg in the AT base pair compared to N(3) of thymidine or N(1) of adenosine. The latter two sites are well protected in the ordered helix and Hg binding at these sites would, therefore, disrupt the hydrogen bonds between AT base pairs.

Moreover, since the $HgCl_2$ group is expected to be coplanar with the bases, the linear $HgCl_2$ moiety would experience more steric hindrance at N(1) of adenosine or N(3) of thymidine due to substituents at neighbouring atoms. The results discussed here are based on the structural study of a base, but not a nucleoside or nucleotide. Structural studies with nucleosides or nucleotides will be useful in throwing more light on Hg–DNA interaction.

Note added in proof: Recently a paper describing the crystal structure of a Cu(II) complex of doubly deprotonated adenine N(1)-oxide has appeared [E. Sletten, T. Marthinsen and J. Sletten, *Inorg. Chim. Acta, 93,* 37 (1984)], which shows metal binding to O(1) and deprotonated N(6) of the ligand resulting in a chelate.

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