The Crystal and Molecular Structure of a 2:1 Complex of 1-Methylthymine–Mercury(II)†

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ABSTRACT: Single crystals of Hg(II)–1-methylthymine were prepared by crystallizing the compound from an aqueous solution containing 1 equiv of HgO and 2 equiv of 1-methylthymine. The compound, $Hg(C_6H_7N_2O_2)_2$, crystallizes in space group $P2_1/a$ with unit cell parameters, $a = 13.325 \pm 0.003$ Å, $b = 11.783 \pm 0.003$ Å, $c = 4.423 \pm 0.001$ Å, and $\beta = 91.59 \pm 0.02°$. The structure was determined with a sharpened and origin removed Patterson map. All non-hydrogen atoms were located and refined by the method of full-matrix least squares to a weighted R of 0.077. The structure consists of a N(3)–Hg–N(3′) bond which links the two thymine moieties together; the N–Hg bond length is 2.04 Å. The bonding supports a structure previously proposed for Hg(II) binding to DNA and dA-T alternating copolymer.

The reversible binding of Hg(II) to DNA has been an area of study since the phenomenon was first reported by Katz (1952). The binding sites are known to be the purine and pyrimidine bases (Thomas, 1954; Yamane and Davidson, 1961; Simpson, 1964). By addition of Cl^- or CN^- to the Hg(II)DNA complex, the original DNA is recovered; biological activity is not lost by this reversible process (Dove and Yamane, 1960; Nandi *et al.*, 1965). The binding is favored by AT-rich DNAs (Yamane and Davidson, 1961). The addition of Hg^{2+} to DNA releases protons, so that the equilibrium of binding is pH dependent. The mercury complexes of DNA have been successfully fractionated in a Cs_2SO_4 density gradient (Davidson *et al.*, 1965; Nandi *et al.*, 1965).

There has been considerable speculation on the structure of the Hg(II) complexes wittith DNA, or polynucleotides such as dA-T (desoxyadenosylthymidine alternating copolymer). In particular, Katz (1963) proposed a chain slippage mechanism for the binding of Hg^{2+} to dA-T whereby the Hg^{2+} forms a bond between two thymine moieties on opposite strands of the polymer. The two protons released per Hg^{2+} bound are attributed to the imino N–H bond in each thymidine.

The crystal and molecular structures of complexes of uracil–mercuric chloride have recently been reported (Carrabine and Sundaralingam, 1971). The binding of $HgCl_2$ was to the oxygen atom in both structures. The authors also proposed a $HgCl_2$ complex to the keto oxygen of thymidine in the A-T pair of DNA; proton release was ascribed to hydrolysis of the $HgCl_2$ *complex*, where the Cl^- were replaced with OH^- from water. It is important to point out, however, that the preparation of both the uracil and dihydrouracil complexes were at a sufficiently low pH (≤4.2) that formation of a mercury to nitrogen bond is almost certainly unlikely. Most of the Hg^{+2}–DNA binding studies have been carried out at higher pH's. The work reported by Nandi *et al.* (1965) was done at pH 9. In an effort to shed some light on these structural proposals, we have synthesized a 2:1 1-methylthymine–Hg(II) complex and have determined its crystal and molecular structure.

Experimental Section

Preparation of Complex and Crystal Data. In order to avoid possible interference from undesirable anions and to ensure basic conditions, 1 mmol of solid HgO was added to a 100 ml of aqueous solution of 2 mmol of 1-methylthymine. The mixture was stirred and then heated to speed up the reaction. White crystalline needles formed in about 10 min. In a similar way, a complex with thymidine was also made, only heat was not applied. In this case the mixture was stirred for 24 hr. The HgO had indeed reacted, but upon evaporation the Hg–thymidine formed a glass. Attempts to crystallize the material from several alcohols were unsuccessful.

The Hg–1-methylthymine complex was separated by filtration and then recrystallized from an ethanol–water mixture. The crystals were prismatic with well-developed faces [110] and c parallel to the needle axis. Cleavage along [001] could easily be done with a razor blade. The density was measured by flotation in a mixture of methyl iodide and cyclohexane. Oscillation and Weissenberg photographs established the crystals as monoclinic, space group $P2_1/a$. In addition, the diffraction intensities for which $h + k = 2n + 1$ were very weak and this indicated pseudo-C centering in the cell. These data, in addition to the density measurements, fix mercury at the origin of the cell. Accurate cell parameters were determined from 24 2θ measurements on a Picker diffractometer with Mo K$\bar{\alpha}$ radiation. Pertinent crystal data are given in Table I.

The bulk of the three-dimensional diffraction data were determined from crystal number 1, the dimensions of which are given in Table I. A second crystal was used for collection of higher order diffraction data. The dimensions are given in Table I. Details of data collection, of data reduction, of the structure solution and of the refinement of atomic parameters, and a list of observed and calculated structure factors are provided in the supplementary material. The final atomic positions and thermal parameters are given in Table II. Hydrogen atom positions could not be located from these diffraction data.

Description of the Structure. The molecular structure of the Hg(II) complex with 1-methylthymine is shown in Figure 1. Notice that the Hg atom is at a center of symmetry for this 2:1

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TABLE I: Crystal Data for Hg(II)–(1-Methylthymine)$_2$.

Formula	$Hg(C_6H_7N_2O_2)_2$
Crystal habit	Needles elongated along *c*. Prominent forms {110}, {001}
Crystal system	Monoclinic
Systematic absences	*h*0*l*, $h = 2n + 1$ 0*k*0, $k = 2n + 1$
Space group	$P2_1/a$
a (Å)	13.325 ± 0.003
b (Å)	11.783 ± 0.003
c (Å)	4.423 ± 0.001
β (deg)	91.59 ± 0.02
λ(Mo K$\bar{\alpha}$) (Å)	0.71069
Volume (Å^3)	694.2
Molecular Weight	478.9
Z	2
ρ_{micro}	2.29
ρ_{macro}	2.35, by flotation in CH_2I_2–cyclohexane mixture
μ (cm^{-1})	113 (linear absorption coefficient for Mo K$\bar{\alpha}$ radiation)
Dimensions (mm)	{110}–{1$\bar{1}$0}{$\bar{1}$10}–{1$\bar{1}$0}{001}–{00$\bar{1}$}
Crystal 1	0.11 0.14 0.24
Crystal 2	0.06 0.09 0.19

complex. The bond lengths and angles shown in Figure 1 have estimated standard deviations of 0.02 Å and 1–2°, respectively. The 1-methylthymine structure is comparable to the free moiety determined by Hoogsteen (1963). Although the precision in the geometry for the Hg(II)–1-methylthymine complex is considerably less than the uncomplexed 1-methylthymine, it appears that the Hg binding to the N(3) atom does not drastically alter the valence angles or bond lengths in the thymine ring. The C(2)–O(2) and C(4)–O(4) bond lengths (1.25 and 1.20 Å) compared to N(1)–C(9) and C(5)–C(10) (1.49 and 1.48 Å) are compelling evidence that Hg(II) is bound to N(3) and not to C(6). As discussed in the supplementary material, we believe the assignment of N(1) and C(5) (as opposed to C(5) and N(1)) is correct as judged from atomic thermal parameters. The Hg–N(3) bond of 2.04 Å is rather short, but we were unable to find any precedent of a Hg(II)–N(aromatic) bond in the literature. In the crystal structure of $HgCl_2$–collidine ($HgCl_2NC_8H_{11}$) a Hg–N bond of 2.181 ± 0.018 Å has been reported (Kulpe, 1967), but in this case Hg is also participating in bonding with Cl atoms.

The least-squares plane for the 1-methylthymine moiety in this structure is given by $-3.504x + 7.249y + 3.318z = -0.299$ Å, where *x*, *y*, and *z* are fractions of the unit cell axes. (The ten light atoms were used to define the plane.) The two oxygen atoms, O(2) and O(4), are significantly out of the plane. This may be due to the long Hg–O bonds in the intermolecular packing of the molecules (*vide infra*). The methyl group, C(9), is also significantly out of the plane and this is presumably due to crystal packing forces. The Hg atom is 0.30 Å above this plane and the angle between the plane of 1-methylthymine and the N(3)–Hg–N(3′) bond is 172°. The least-squares plane for the complex Hg(II)–(1-MT)$_2$ is given by $-2.712x + 7.807y + 3.212z = 0$. (Because of the center of symmetry in the molecule, the deviation for N(1′), for example, is just the negative of that for N(1).) This 2:1 complex of Hg(II) is crudely planar where, of the pyrimidine ring atoms, N(3) and C(2) have the largest deviation. The external atoms

TABLE II: Atomic Positions and Thermal Parameters and Their Estimated Standard Deviations for Hg(II)–(1-Methylthymine)$_2$.

Atom	*x*	*y*	*z*	U_{11}[a]	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
Hg	0	0	0	0.0198 (3)	0.0316 (4)	0.0291 (3)	−0.0082 (5)	0.0036 (2)	0.0054 (5)
N(1)	0.1715 (12)	0.2469 (13)	−0.4485 (35)	0.043 (8)	0.034 (8)	0.040 (8)	−0.019 (7)	−0.003 (6)	−0.009 (6)
C(2)	0.0933 (10)	0.1727 (13)	−0.3718 (35)	0.018 (6)	0.029 (7)	0.031 (7)	−0.009 (5)	0.004 (5)	−0.004 (6)
N(3)	0.1156 (10)	0.0927 (12)	−0.1623 (29)	0.029 (6)	0.031 (6)	0.024 (6)	−0.012 (5)	0.010 (5)	0.002 (5)
C(4)	0.2091 (10)	0.0733 (13)	−0.0229 (36)	0.015 (6)	0.027 (7)	0.035 (8)	−0.003 (5)	−0.002 (5)	−0.002 (6)
C(5)	0.2871 (12)	0.1472 (15)	−0.1247 (36)	0.030 (7)	0.036 (8)	0.025 (7)	−0.009 (7)	0.008 (6)	−0.006 (6)
C(6)	0.2645 (12)	0.2320 (15)	−0.3245 (35)	0.028 (7)	0.039 (8)	0.025 (7)	−0.012 (7)	0.004 (6)	−0.002 (6)
O(2)	0.0087 (12)	0.1771 (12)	−0.5027 (45)	0.026 (6)	0.060 (8)	0.052 (7)	0.001 (7)	0.006 (5)	0.005 (9)
O(4)	0.2182 (9)	0.0006 (16)	0.1674 (29)	0.050 (7)	0.055 (8)	0.049 (7)	0.029 (9)	0.010 (6)	0.020 (9)
C(9)	0.1481 (15)	0.3454 (20)	−0.6513 (44)	0.045 (10)	0.056 (12)	0.035 (9)	−0.013 (10)	0.003 (8)	0.012 (9)
C(10)	0.3889 (12)	0.1333 (20)	0.0115 (44)	0.020 (7)	0.073 (14)	0.040 (9)	−0.012 (8)	−0.003 (7)	0.002 (10)

[a] U_{ij}'s (in units of Å^2) are elements in the tensor **U** from the temperature factor expression: $T = \exp[-\frac{1}{2}H'\mathbf{U}H]$ where $H = 2\pi(ha^*,kb^*,lc^*)$.

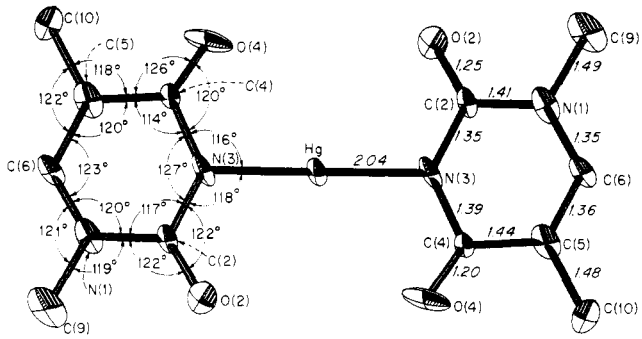

FIGURE 1: The molecular structure of Hg(II)-(1-methylthymine)$_2$ projected into its least-squares plane. The Hg atom is at a center of symmetry.

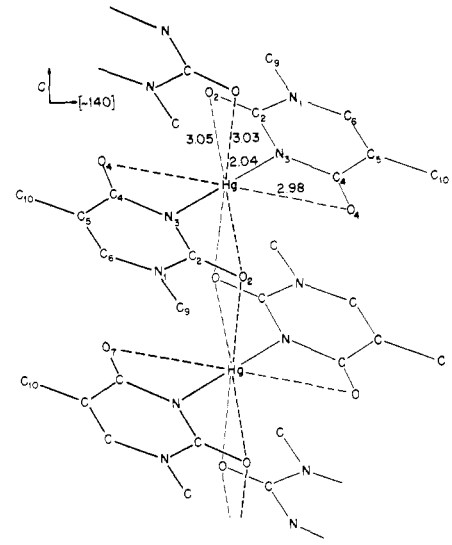

FIGURE 2: View along a vector about 12.5° from the *b* axis toward *a* of the Hg(II)-(1-MT)$_2$ crystal structure showing the staking of the complex along the *c* axis and the highly distorted octahedral arrangement of N(3), O(2), and O(4) about the Hg atoms.

O(2) and C(9) are canted out of plane the most (−0.26 and 0.20 Å, respectively) but O(4) (and also O(4′)) is essentially in this least-squares plane.

The packing of the molecules in the unit cell effects a herringbone pattern along the *c* axis. One chain along *c* is shown in Figure 2. The "plane" of the complex is tilted at an angle of 43° with respect to the *c* axis. The glide related chain completes the herringbone pattern. Note that O(2) forms two long bonds of 3.05 and 3.03 Å which form angles of 43.2 and 43.6°, respectively, with the *c* axis. The O(4) atom, on the other hand, forms a long bond of 2.98 Å which is virtually in the plane of the complex. Roughly speaking then, the Hg atom is in a highly distorted octahedral complex where two of the linkages are the covalent and linear N-Hg-N bonds of 2.04 Å and the other six are very long Hg-O contact distances of 3 Å. By contrast, these Hg-O distances are much longer than the results found for the $HgCl_2$-uracil complex (2.71 Å) or the $HgCl_2$-dihydrouracil complex (2.88 Å) (Carrabine and Sundaralingam, 1971).

Conclusion

The Hg(II)-(1-methylthymine)$_2$ complex reported in this work is now a documented case of the Hg(II)-nitrogen bonding of thymine as proposed by Katz (1963). The structure here does provide some geometrical constraints on models that employ a similar binding for Hg(II) to double-stranded DNA or the alternating copolymer, dA-T. The N-Hg(II)-N bond is linear and has a N-N distance of 4.08 Å. From the coordinates of the nucleotides for the B-form of DNA (Arnott *et al*, 1969), the N(3)-N′(3) distance for adjacent thymines is 5.47 Å. If the hydrogen bonds to the complementary adenines are broken, and the thymine bases are tilted from the planar configuration by 23°, then the N(3)-N′(3) distance becomes 4.68 Å. This tilt can be approximately accomplished by a single rotation about the N(1)-C′(1) glycosyl bond. Then if each strand near the adjacent thymine moieties moves into the helical axis by 0.3 Å, the Hg(II) complex would conform to the geometry of the Hg(II)-(1-methylthymine)$_2$ reported here. Base tilting and contraction of complimentary strands is essentially Katz's (1963) chain slippage mechanism. In any event it would be desirable to obtain a structure of Hg(II)-thymine complexes with the desoxyribose and phosphate groups attached in order to determine the detailed conformation of the sugar-phosphate backbone.

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Supplementary Material Available

The X-ray diffraction data collection and reduction as well as refinement details and list of calculated structure factors will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24X reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number BIO-74-3949.

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Separation of Newly Synthesized Nucleohistone by Equilibrium Centrifugation in Cesium Chloride

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ABSTRACT: Nucleohistone, fixed by formaldehyde using previous methods, does not permit a separation of density-labeled, newly synthesized nucleoprotein. The reasons for this behavior lie in inter- or intrastrand cross-linking when fixation is performed at intermediate ionic strength (0.01–0.2) and also involve nucleoprotein aggregation in solutions containing elevated concentrations of CsCl. If fixation is performed at low ionic strength, and the equilibrium density centrifugation is performed in the presence of denaturing solvents, discrete separation of newly synthesized, density-labeled nucleoprotein is possible. Techniques for an effective reversal of nucleoprotein fixation are described. Histones can be recovered unchanged.

A great deal of valuable information has been obtained concerning DNA replication by exploiting the incorporation of a density label and performing a subsequent analysis by buoyant density equilibrium centrifugation in cesium chloride solutions. This approach has been particularly fruitful in work with prokaryotes. The more complex eukaryotic chromosome has been studied, on occasion, in a similar way, though an early step in such an analysis is to remove the proteins which constitute more than 50% of the chromosome material (Kidwell and Mueller, 1969).

A better understanding of chromosome synthesis in higher organisms requires that in addition to studying the mode of DNA replication, we also learn how and when the proteins become associated with DNA during replication. In principle much can be learned by utilizing the techniques of density labeling; however, the elevated ionic strengths required for density separation are such that almost all of the chromosomal proteins are dissociated by the conditions required for the analysis of the experiments (Ohlenbush *et al.*, 1967).

It is possible to avoid the problem of dissociation if the proteins are chemically bound to the RNA or DNA using formaldehyde (Brutlag *et al.*, 1969; Spirin *et al.*, 1965). However, we will show that if the chromosomal material is labeled by a short *in vivo* pulse of a density label, bromodeoxyuridine, then analysis of the chemically fixed nucleoprotein on a CsCl density gradient reveals that the density label is uniformly distributed throughout the nucleoprotein peak rather than asymmetrically organized on the denser side. Since the incorporation of such a density label has been used to identify and separate newly synthesized DNA, the system as presently used does not permit an identification and separation of newly synthesized nucleoprotein.

The reasons for the failure to separate density-labeled nucleoproteins have been studied. They lie in cross-linking during fixing and in the additional short range interactions among the material itself. Procedures are described which avoid these problems and lead to a partially successful resolution of newly synthesized, density-labeled chromosomal nucleoproteins.

Materials and Methods

Density Labeling. Hepatoma tissue culture (HTC)[1] cells were grown in Swims 77 medium supplemented with 5% calf and 5% fetal calf serum. Exponentially growing cells (200 ml, 5×10^5/ml) were labeled with 100 μCi [^{3}H]thymidine (Nuclear Dynamics, Inc., specific activity 24 Ci/mmol) and 1.0×10^{-5} M bromodeoxyuridine (Sigma Chemical) for 2 hr at 37°. The cells were collected by centrifugation at 2000*g* for 5 min, frozen, and homogenized directly in 25 ml of 10 mM $MgCl_2$, 10 mM Tris, 50 mM $NaHSO_3$, 0.25 M sucrose, and 1% Triton X-100 (pH 7.0) using a Potter-Elvejheim homogenizer. Nuclei were isolated by centrifugation at 1000*g* for 10 min and the pellet was washed three times by successive suspension and centrifugation from the above buffer. The nuclei were washed once with 10 mM Tris-HCl–12.5 mM EDTA (pH 8.0) and twice with glass-distilled water to generate the typical chromatin gel. The gel was vigorously sheared (VirTis Model 45 homogenizer) at 80 V in two bursts of 45 sec. The resulting solution which has lost the gel-like nature of chromatin is operationally defined as nucleohistone though it is known to contain contaminating membrane fragments. A large fraction of the membrane was removed from the nucleohistone by centrifugation at 27,000*g* for 30 min. The supernatant contains the nucleohistone used for the following experiments. For some of the experiments to be described Ehrlich ascites tumor cells were used as the source of nucleohistone.

Fixation of Nucleohistone and Centrifugation in CsCl. Nucleohistone was treated with formaldehyde of required concentration as defined in the text. The formaldehyde (Fisher Scien-

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[1] Abbreviations used are: HTC cells, hepatoma tissue culture cells; brUdRib, bromodeoxyuridine.