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Structural studies of O^6-methyldeoxyguanosine and related compounds: a promutagenic DNA lesion by methylating carcinogens

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ABSTRACT

O^6-Methylation of guanine residues in DNA can induce mutations by formation of base mispairing due to the deprotonation of N(1). The electronic, geometric and conformational properties of three N(9)-Substituted O^6-methylguanine derivatives, O^6-methyldeoxyguanosine (O^6mdGuo), O^6-methylguanosine (O^6mGuo) and O^6,9-dimethylguanine (O^6mGua), were investigated by X-ray and/or NMR studies. O^6mdGuo crystallizes in the monoclinic space group $P2_1$ with cell parameters a=5.267(1), b=19.109(2), c=12.330(2) Å, β=92.45(1)°, V=1239.8(3) Å^3, Z=4 (two nucleosides per asymmetric unit), and O^6mGua in the monoclinic space group $P2_1/n$ with cell parameters a=10.729(2), b=7.640(1), c=10.216(1) Å, β=92.17(2)°, V=836.7(2) Å^3, Z=4. The geometry and conformation of O^6-methylguanine moieties observed in both crystals are very similar. Furthermore, the molecular dimensions of the O^6methylguanine residue resemble more closely those of adenine than those of guanine. The methoxy group is coplanar with the purine ring, the methyl group being cis to N(1). The conformation of O^6-methylguanine nucleosides is variable. The glycosidic conformation of O^6mdGuo is anti for molecule (a) and high-anti for molecule (b) in the crystal, while that of O^6mGuo is syn [Parthasarathy, R & Fridey, S. M. (1986) Carcinogenesis 7, 221-227]. The sugar ring pucker of O^6mdGuo is C(2')-endo for molecule (a) and C(1')-exo for molecule (b). The C(4')-C(5') exocyclic bond conformation in O^6mdGuo is gauche$^-$ for molecule (a) but trans for molecule (b), in contrast with gauche$^+$ for O^6mGuo. The hydrogen bonds exhibited by O^6-methylguanine derivatives differ from those in guanine derivatives; the amino N(2) and ring N(3) and N(7) atoms of O^6-methylguanine residues are involved in hydrogen bonding. ^{1}H-NMR data for O^6mdGuo and O^6mGuo reveal the predominance of a C(2')-endo type sugar puckering. In O^6mdGuo, however, a contribution of a C(1')-exo sugar puckering is significant. The NOE data also indicate that O^6mdGuo molecules exist with nearly equal population for anti (including high anti) and syn glycosidic conformations. These observations and their biological implications are discussed.

INTRODUCTION

Carcinogenic and/or mutagenic alkylating agents are known to modify cellular DNA when cells are treated with them (1,2). Among the modified DNA constituents, O^6-alkylation of guanine residues in DNA is considered to be one of the most detrimental DNA-modifications that initiate the mutagenicity or carcinogenicity (3,4). The molecular mechanism of mutation is due to the

abnormal base-paring resulting from the deprotonation of N(1) of guanine. O^6-Methylguanine-DNA methyltransferase, the repair enzyme of this DNA damage, was found by Lindahl et al.(5) and its primary structure and function were reported recently (6,7). Although the investigation of this repair enzyme proceeded well, few studies have been done about the chemical and stereochemical characteristics of the substrate, O^6-methylguanine. In the present work we report X-ray analyses of O^6-methyldeoxyguanosine (O^6mdGuo) and O^6,9-dimethylguanine (O^6mGua), and NMR analyses of O^6mdGuo and O^6-methylguanosine (O^6mGuo) for which the X-ray analysis has been reported by Parthasarathy and Fridey (8). The formation of O^6-methylguanine-thymine/uracil mispairing requires the alkyl group to be directed toward the imidazole ring of the purine ring and deprotonation of N(1). However, our X-ray data show that the O^6-methyl group is directed away from the imidazole ring. The orientation of the O^6-methyl group and the mode of base mispairings are studied by means of molecular orbital calculation on the basis of our X-ray results. The implication of the results for the mechanism of the removal of the O^6-methyl group by O^6-methylguanine-DNA methyltransferase is also discussed.

MATERIALS AND PROCEDURES

O^6mdGuo (9), O^6mGuo (10) and O^6mGua (11) were prepared by the procedures reported. O^6mdGuo was recrystallized from MeOH or MeOH-H_2O, O^6mGuo from MeOH or H_2O, and O^6mGua from EtOH. The crystals of O^6mGuo obtained by recrystallization from MeOH are the same as those obtained by recrystallization from hot water by Parthasarathy and Fridey (8).

Crystallographic data involving data collection and refinement parameters of O^6mdGuo and O^6mGua are given in Table I. Unit-cell parameters were obtained by the least-squares fit of the angular settings of 25 reflections for both crystals. Intensity data were corrected for Lp effects. The structures were solved by direct methods using the program MULTAN 78 (12) and refined by block-diagonal least-squares with anisotropic temperature factors for non-hydrogen atoms. All hydrogen atoms were located on difference Fourier maps and refined isotropically. The scattering factors used were those in International Tables for X-ray Crystallography (1974) (13). All numerical calculations were carried out on an ACOS 850 computer at the Crystallographic Research Center, Institute for Protein Research, Osaka University, by using the programs of The Universal Crystallographic Computing System (1979) (14) and their modifications. The atomic parameters and geometrical data for

Table I. Crystal, Data Collection and Refinement Parameters

	O^6mdGuo	O^6mGua
formula	$C_{11}H_{15}N_5O_4$	$C_7H_9N_5O$
crystal system	monoclinic	monoclinic
space group	$P2_1$	$P2_1/n$
a Å	5.267(1)	10.729(2)
b Å	19.109(2)	7.640(1)
c A	12.330(2)	10.216(1)
β°	92.45(1)	92.17(2)
V $Å^3$	1239.8(3)	836.7(2)
Z	4	4
F(000)	592	376
μ mm^{-1}	0.948	0.822
Dx Mg m^{-3}	1.507	1.422
color	colorless	colorless
size mm	0.3x0.08x0.04	0.3x0.25x0.25
temp K	280	280
diffractometer	Rigaku AFC-5	same
radiation	CuKα	same
monochromator	graphite	same
scan limits deg	$2\theta<120°$	$2\theta<120°$
scan method	$2\theta-\omega$	$2\theta-\omega$
unique data	1899	1322
observed data ($F_o \neq 0$)	1835	1210
decay %	<0.6	<0.4
R, wR %	4.7,5.8	4.2,5.3
weight	$1/(\sigma^2+0.0227\|F_o\|)$	$1/\sigma^2$
GOF	0.71	3.04
No/Nv	3.8	7.8
$\Delta\rho_{max}$ $eÅ^{-3}$	0.18	0.14
$(\Delta/\sigma)_{max}$	0.30	0.25

O^6mdGuo and O^6mGua are deposited at the Cambridge Crystallographic Data Centre, Lensfield Road, Cambridge, CB2 1EW, England.

^1H NMR spectra of O^6mdGuo and O^6mGuo were recorded on a JEOL-GX 500 spectrometer. Each sample was dissolved in sufficient D_2O to adjust the final concentration to 10 mM. Chemical shifts were measured by using tBuOH as an internal standard (1.230 ppm). The resonance peaks were assigned with the method of homonuclear decoupling. The results are given in Table II.

Molecular orbital calculations were performed with the intermediate neglect of differential overlap (INDO) method (15). The coordinates for the O^6-methyguanine residue were derived from our X-ray data.

RESULTS AND DISCUSSION

ORTEP drawings (16) of the two independent molecules of O^6mdGuo are represented in Figure 1. The molecular packings and hydrogen bondings of

Table II. ^{1}H Chemical Shifts (δ in ppm) and Coupling Constants (J in Hz) for O^6mdGuo and O^6mGuo in Aqueous Solution

δ	O^6mdGuo	O^6mGuo	J	O^6mdGuo	O^6mGuo
8	8.034	8.063	1'2'	7.7	6.1
1'	6.319	5.936	1'2"	6.1	-
2'	2.796	in HDO	2'3'	6.1	5.2
2"	2.486	-	2"3'	3.1	-
3'	4.616	4.405	3'4'	3.3	3.4
4'	4.131	4.246	4'5'	3.4	3.0
5'	3.813	3.885	4'5"	4.6	3.7
5"	3.737	3.809	2'2"	-17.0	-
CH_3	4.054	4.074	5'5"	-12.5	-12.8

O^6mdGuo and O^6mGua are illustrated in Figure 2.

O^6-Methylguanine Base Moiety

The corresponding bond lengths and angles found in three O^6-methylguanine derivatives are in good agreement. The standard bond lengths and angles for O^6-methylguanine residue were obtained with the averaged values of four kinds of O^6-methylguanine moieties (17) and the values are compared with those of guanine and adenine(18) (Table III). The O^6-methylation of guanine produces the enol form of the purine ring resulting in a tautomer with no proton on N(1). The change from the keto to enol form makes significant alteration on the geometric and electronic properties of the pyrimidine moiety as compared with those of normal guanine (18). The C(6)-O(6) bond is longer by ca 0.10 Å than that in guanine, whereas the N(1)-C(6) bond is shorter by ca 0.08 Å. Also, the N(1)-C(6)-C(5) angle is larger by 9° than that in guanine, while the O(6)-C(6)-C(5) and C(6)-N(1)-C(2) angles are smaller by 10° and 7°,

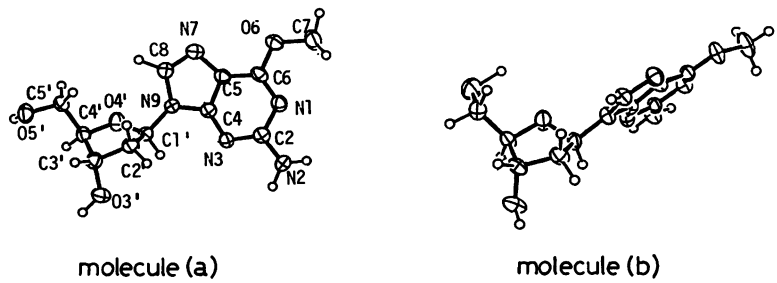

Figure 1 ORTEP drawings of the independent molecules (a) and (b) of O^6mdGuo and numbering system. Both drawings are projected down normal to the plane formed by the C(4'), C(1') and N(9) atoms.

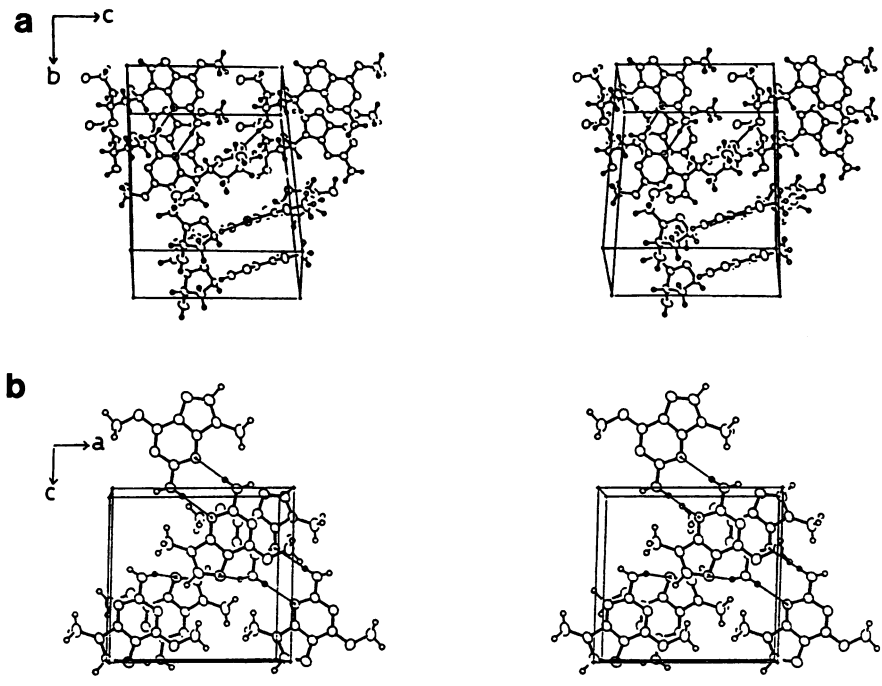

Figure 2 Stereoview of the molecular arrangement in a unit-cell. Thin lines indicate hydrogen bonds. (a); O^6-mdGuo, (b); ^{6}mGua.

respectively. Similar deviations for the bond lengths and angles are observed between O^4-alkylthymidine/uridine and thymidine/uridine (19-21). As shown in Table III, the overall geometry of the pyrimidine moiety in O^6-methylguanine resembles that in adenine rather than that in guanine, in the same way as the geometry of O^4-alkylthymidine resembles that of cytidine rather than that of thymidine. However, the differences from geometry of adenine are also significant. As compared to adenine, the shortening of the N(1)-C(6) bond and the lengthening of the N(1)-C(2) bond suggest the localization of electrons in the O(6)-C(6)=N(1)-C(2) fragment of O^6-methylguanine, whereas they are dellocalized in the adenine N(6)=C(6)-N(1)=C(2) fragment. On the other hand, the geometry of the imidazol moiety is in good agreement with standard values in guanine.

Each purine ring of the O^6-methylguanine moiety is almost planar. The orientation of the methoxy group in O^6mdGuo and O^6mGua is identical. It is

Table III. Comparison of Mean Values of Bond Lengths (Å) and Angles (°) in O^6-Methylguanine, Guanine and Adenine Residues of their Nucleosides

	O^6-methylguanine[a]	guanine[b]	adenine[b]
N(1)-C(2)	1.362(7)	1.375(3)	1.338(3)
C(2)-N(3)	1.346(5)	1.327(2)	1.332(3)
N(3)-C(4)	1.338(14)	1.355(2)	1.342(2)
C(4)-C(5)	1.381(7)	1.377(2)	1.382(2)
C(5)-C(6)	1.396(5)	1.415(5)	1.409(1)
C(6)-N(1)	1.317(9)	1.393(2)	1.349(2)
C(6)-O(6)[N(6)]	1.337(3)	1.239(5)	1.337(3)
C(2)-N(2)	1.356(7)	1.341(3)	
O(6)-C(7)	1.445(6)		
C(5)-N(7)	1.393(5)	1.389(3)	1.385(2)
N(7)-C(8)	1.307(5)	1.304(3)	1.312(2)
C(8)-N(9)	1.376(6)	1.374(4)	1.367(4)
N(9)-C(4)	1.376(7)	1.377(2)	1.376(2)
C(2)-N(1)-C(6)	118.2(3)	124.9(2)	118.8(2)
N(1)-C(2)-N(3)	127.0(3)	124.0(2)	129.0(1)
C(2)-N(3)-C(4)	111.5(5)	111.8(1)	110.8(1)
N(3)-C(4)-C(5)	127.4(3)	128.4(2)	126.9(2)
C(4)-C(5)-C(6)	115.1(5)	119.1(1)	116.9(1)
C(5)-C(6)-N(1)	120.8(2)	111.7(2)	117.6(1)
C(4)-C(5)-N(7)	110.9(4)	110.8(2)	110.7(1)
C(5)-N(7)-C(8)	103.9(7)	104.2(3)	103.9(2)
N(7)-C(8)-N(9)	113.6(8)	113.5(4)	113.8(2)
C(8)-N(9)-C(4)	106.2(6)	106.0(2)	105.9(1)
N(9)-C(4)-C(5)	105.5(4)	105.6(1)	105.7(1)
N(3)-C(4)-N(9)	127.1(5)	126.0(2)	127.4(1)
C(6)-C(5)-N(7)	134.0(8)	130.1(2)	132.3(2)
N(1)-C(6)-O(6)[N(6)]	121.0(7)	120.0(2)	119.0(2)
C(5)-C(6)-O(6)[N(6)]	118.2(8)	128.3(2)	123.4(2)
N(1)-C(2)-N(2)	115.8(5)	116.3(2)	
N(3)-C(2)-N(2)	117.2(3)	119.7(2)	
C(6)-O(6)-C(7)	117.7(9)		

[a] ref. 17, [b] ref. 18

almost coplanar with the purine plane and directed away from the imidazole moiety of the purine base. The C(7)-O(6)-C(6)-N(1) torsion angles are -2.0(7)∿ 6.3(2)° and in the syn periplanar range with respect to the N(1) atom. The same conformation was found in O^6mGuo (8) and in the two independent molecules of O^6-methylinosine (22). To date, the anti periplaner conformation [N(1)-C(6)-O(6)-C(7)≈180°] with the methyl group directed towards the imidazole ring has not been observed in any O^6-alkylpurine moieties (23).

Glycosidic Bond and Sugar Moiety

The conformations around the glycosidic bond are different for the two independent O^6-mdGuo molecules. The torsion angle O(4')-C(1')-N(9)-C(4) is -

145.1(4)° for molecule (a), and -81.4(5)° for molecule (b), in contrast to 59.6(5)° for O^6mGuo; they are in the anti(-180∿-90°), high-anti (-90∿-60°) and syn (30∿80°) ranges, respectively. Many crystallographic, spectroscopic and theoretical studies on β-purine nucleosides have shown that the rotational barrier about the glycosidic bond is relatively small and a variety of conformations around the glycosidic bonds such as anti, high-anti and syn are possible (24). Therefore, this result may reflect a rotational flexibility about the glycosidic bond rather than a difference of the sugar ring, ie., deoxyribose or ribose. Interestingly, the crystals of N-methylpurine nucleosides, that is, 1-methyladenosine (25) and the complex of 7-methylguanosine and its iodide (26), also have two independent molecules in the asymmetric unit with different conformations around the glycosidic bond. In both the crystals, one molecule is in anti and the other is in syn conformation. In spite of this apparent flexibility, the glycosidic bond lengths [1.456(6)-1.461(6) Å] are comparable with those of unmodified purine nucleosides (27).

In this work, the sugar conformations of molecules (a) and (b) of O^6mdGuo belong to the S-type conformer. The deoxyribose of molecule (b) is C(1')-exo ($P=131.1°, \tau_m=39.9°$) which deviates somewhat from the usual pseudorotational angles (P=144-180°) for the S-type. On the other hand, the deoxyribose of molecule (a) and ribose of O^6mGuo (8) are in the usual C(2')-endo,C(3')-exo ($P=171.0°$, $\tau_m=38.5°$) and C(2')-endo ($P=158.2°$, $\tau_m=38.1°$) conformations, respectively. De Leeuw et al. (28) classfied 178 nucleosides obtained from X-ray analyses by the types of conformer and calculated the typical conformational parameters P, χ and ψ. Their results indicate that S-type and high anti conformations are found in 6 nucleosides and their sugar conformations tend to shift from a C2'-endo puckering to a C(1')-exo puckering, as actually observed for molecule (b) of O^6mdGuo which has a high anti and C(1')-exo conformation. Kitamura et al. (29) have shown that in nucleosides with anti conformation there are high angular correlations between χ' and τ_0 ($\chi'=29°-1.61\tau_0$) for the adenine nucleoside group, and between χ' and τ_4($\chi'=107°-4.61\tau_4$) for the guanine nucleoside group, by means of circular correlation analyses using 127 known angular data in the crystal structures of nucleosides. In our molecules (a) and (b) of O^6mdGuo, there is a correlation of $\chi'-\tau_0$ as found in adenine nucleosides rather than of $\chi'-\tau_4$ as in guanine nucleosides [(a), $\chi'=39.7(6)°$, $\tau_0=-18.0(5)°$, $\tau_4=-6.8(5)°$; (b), $\chi'=101.7(5)°$, $\tau_0=-37.6(4)°$, $\tau_4=20.0(5)°$]. In the two molecules of O^6-mGuo, the differences found in the bond angles of C(2')-C(3')-O(3') [(a), 107.2(4)°;

(b), 112.4(4)°] and C(4')-C(3')-O(3') [(a), 112.0(4)°; (b), 107.4(4)°] may probably be due to the differences in sugar conformations of the two molecules.

There are, also, differences in the conformation of the exocyclic C(4')-C(5') bond between the two nucleosides, i.e., it is gauche$^-$ [ψ=-72.7(5)°] for molecule (a) which is rarely observed, and trans [ψ=-178.0(4)°] for molecule (b), while O^6mGuo exhibits the most preferred gauche$^+$ conformation.

Hydrogen Bonding and Base Stacking

Understanding of the hydrogen bonding modes in O^6-methylguanine derivatives is fundamental for consideration on mutation mechanism induced by unusual base-pairings. In fact, there are some important features of the hydrogen bonding found in the crystal structures. In O^6mdGuo, self-association occurs between the independent molecules (a) and (b) [N(2)-H--N(3), 3.267(6) Å, 3.107(6) Å], and in O^6mGua, it is found between the molecules related by the center of symmetry [N(2)-H--N(3), 3.123(2) Å]. The N(2)-H---N(3) base pairing scheme between guanine bases is not unusual (30,31), but it is rarely found in guanine nucleosides or nucleotides (32). Second, the N(7) atom is used as a hydrogen bond acceptor in all O^6-methylguanine derivatives, but neither N(1) nor O(6) participate in hydrogen bonds. In a DNA double helix, N(1) and O(6) of guanine can participate in base pairing with cytosine as hydrogen bond donor and acceptor, respectively. In O^6-methylguanine, both N(1) and O(6) can act as acceptors, but the hydrogen bonding abilities of N(1) must be decreased by the steric hindrance by the methoxy and amino groups. Third, the hydroxy groups (O(3')-H and O(5')-H) of the deoxyribose moiety participate in hydrogen bonding.

Base stacking is also important in the molecular packing of both crystals. These stacking patterns are in agreement with those already found in many crytal structures of base derivatives, nucleosides, nucleotides (33): polar substituents like $-NH_2$ and =N- of one base tend to overlap with the adjacent base plane.

NMR Analysis

The observed coupling constants of O^6mdGuo and O^6mGuo correspond well to those of the parent nucleosides deoxyguanosine and guanosine (34). This indicates that the O^6-methylation of guanine nucleosides has little influence on the sugar ring conformation in aqueous solution. The conformation of the sugar ring may be assessed by making the assumption of a C(2')-endo C(3')-endo equilibrium. Their populations in the equilibrium mixture can be estimated from the comparison of the observed vicinal J values and those calculated for

Table IV. Observed and Calculated Vicinal Coupling Constants (Hz)

	Jobs	Jcalc				
O^6mdGuo		^3E	^2E	$_1E$	26:74	26:37:37
1'2'	7.7	0.3	10.0	10.6	7.5	7.7
1'2"	6.1	6.8	6.6	6.0	6.7	6.4
2'3'	6.1	5.5	5.2	6.7	5.2	5.8
2"3'	3.1	11.3	0.5	0.0	3.3	3.2
3'4'	3.3	10.5	0.0	2.4	2.7	3.6
O^6mGuo	Jobs	Jcalc				
		^3E	^2E	35:65		
1'2'	6.1	0.0	9.7	6.3		
2'3'	5.2	5.2	4.9	5.0		
3'4'	3.4	10.5	0.0	3.6		

[a] the following Karplus equations were used in calculations:
$J=10.5\cos^2 -1.2\cos +0.3$ for O^6mdGuo, except for 3'4'
$J=10.5\cos^2 -1.2\cos$ for O^6mGuo and 3'4' of O^6mdGuo

C(2')- and C(3')-endo conformers. The J values for C(2')-endo conformer are calculated from our averaged HH torsion angles by using the modified Karplus equation (35) and the J values for C(3')-endo conformer are those cited in previous paper (Table IV). In O^6-mGuo, a 65:35 ratio for C(2')- and C(3')-endo conformers is estimated and the J values calculated are in agreement with the observed ones. On the other hand, in O^6mdGuo, the data fit best for 74% C(2')-endo and 26% C(3')-endo conformers. However, the calculated J values are somewhat different from the observed ones. Therefore, we assumed a contribution of the C(1')-exo conformer as in molecule (b) of crystalline O^6mdGuo. If half of the population (74%) of the C(2')-endo conformer is actually C(1')-exo, the observed J values are more consistent with the calculated ones than those estimated only from the C(2')-endo$\rightleftharpoons$C(3')-endo equilibrium.

The conformation around the glycosidic bond can be estimated by using the intramolecular nuclear Overhauser effect (NOE) data. In O^6mdGuo, the ratio of the NOE enhancements of H(1') and H(2') resonances upon saturating the H(8) resonance is 2:1. Assuming that the ratio of the NOE enhancements is almost equal to the inverse ratio of the involved internuclear distances to the sixth power, the ratio of weighted average distances with the populations for

various conformations between H(8) and H(1') and between H(8) and H(2') will be 0.89:1.0. When nucleosides have an equal population of anti and high-anti conformations with the sugar puckering of 37 % C(2')-endo, 37 % C(1')-exo and 26 % C(3')-endo, the weighted average internuclear distances of H(8)-H(1') and H(8)-H(2') are 3.72 and 2.76 Å, respectively, while in a syn conformer, they are 2.40 and 4.22 Å. Therefore, the almost equal population of anti (including high-anti) and syn conformers is consistent with the ratio of the weighted average distances [3.06 and 3.49 Å (0.88:1.0)]. Since in O^6mGuo, the H(2') resonance was covered by a large water proton resonance, we could not estimate the population of anti/syn conformation about the glycosidic bond as in the case of O^6mdGuo. If the NOE enhancement of H(1') resonance upon saturating H(8) resonance in O^6mGuo correlates to that in O^6mdGuo, that is, each correlation time and spin-lattice relaxation rate in both nucleosides are almost equal, the more intense H(1') NOE enhancement found in O^6mGuo compared to that in O^6mdGuo suggests that O^6mGuo has a preference for the syn conformer as found in the solid state.

Base Mispairing and Orientaion of Methoxy Group

The orientation of the methoxy group in O^6-methylguanine is very important for consideration of the base pairing modes. In particular, the base pairing between O^6-methylguanine and thymine/uracil, which was postulated by Loveless (3) and has been supported from in vitro and in vivo biological experiments (36,37), might be expected only when the N(1)-C(6)-O(6)-C(7) torsion angle is anti periplanar. However, all the crystal structures of O^6-methyl substituted guanine derivatives show that the preferred orientation of the methyl group is syn periplanar. Some energy calculations for rotation of the O^6-methyl group

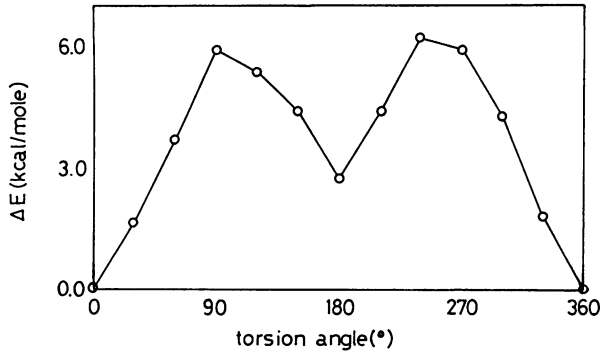

Figure 3 Variation of total energy with torsion angle N(1)-C(6)-O(6)-C(7).

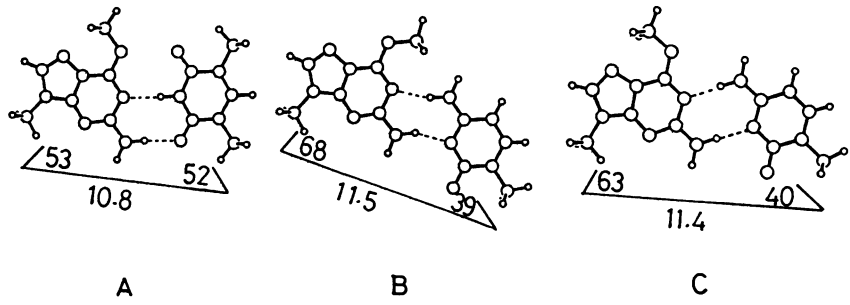

Figure 4 Some possible base-pairs involving O^6-methylguanine. A; O^6-methylguanine(anti):thymine, B; O^6-methylguanine(syn):cytosine, C; O^6-methylguanine(anti):cytosine. Horizontal lines indicate the separations between two C(1') atoms of both strands with lengths denoted in A. The angles (°) are defined as the angle between the C(1')---C(1') vector and the glycosidic bond C(1')-N(9).

and for base-pairs involving O^6-methyguanine have been performed under the lock of crystallographic data of the O^6-methylguanine moiety (38,39). In order to investigate more accurately whether the anti-periplanar conformer for the O^6-methylguanine residue is possible, we carried out molecular orbital calculations on the basis of X-ray structures by using the INDO method and estimated an energy variation of O^6-methylguanine when rotating the O^6-methyl group. The plot displayed in Figure 3 shows that the energy for the anti periplanar conformer is about 2.7 kcal/mol higher than that for the syn periplanar conformer. If this destabilizing energy is compensated by hydrogen bond formation, O^6-methylguanine can form a base-pair with thymine/uracil. Some models for base pairing including O^6-methylguanine were proposed by using the standard base geometry obtained from X-ray data (18,40) (Figure 4) and the energy difference between monomer and pase-paired dimer was calculated (Table

Table V. Monomer to Dimer Transition Energies

base pair[a]	E(monomer1)[b] (A.U.)	E(monomer2)[c] (A.U.)	E(dimer12)[d] (A.U.)	E(transition)[e] (A.U.)	(kcal/mol)
A	-128.29909	-104.71925	-233.02844	-0.01010	-6.3
B	-128.29909	-90.58594	-218.90492	-0.01989	-12.5
C	-128.29909	-90.58594	-218.90975	-0.02472	-15.1

[a] see Figure 5. [b]E(monomer1) indicates a total energy with the stable syn form for O^6-methylguanine. [c]E(monomer2) indicates a total energy of the base paired with O^6-methyguanine. [d]E(dimer12) indicates a total energy of the base paired dimer. [e]E(transition)=E(dimer)-E(monomer1)+E(monomer2) .

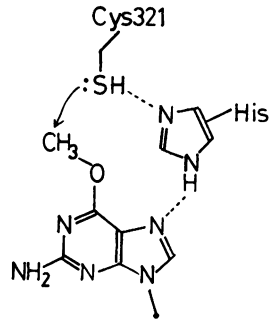

Figure 5 Proposed repair mechanism of O^6-methylguanine by O^6-methylguanine DNA methyltransferase.

V). These calculations reveal that the N(1)-C(6)-O(6)-C(7) torsion angle of O^6methylguanine can adopt the anti periplanar range in the case of the hydrogen bond formation, and the wobble O^6-methylguanine-cytosine base pair is more stable than the Watson-Crick type base pairing of O^6-methylguanine-thymine. These results are consistent with the fact that self-complementary oligonucleotides involving O^6-methylguanine-cytosine pairs have much higher Tm values than oligonucleotides with O^6-methylguanine-thymine pairs (41). In contrast, dTTP or UTP, but not dCTP or CTP is incorporated preferentially opposite the O^6-methylguanine residue in co-polymers when polymerization is achieved using E. coli DNA polymerase I(36) or RNA polymerase (37), respectively. The wobble O^6-methylguanine-cytosine base pair reveals the asymmetrical angles between the glycosidic N-C bonds and the sugar C(1')-C(1') vectors (Figure 4). On the other hand, the mismatched O^6-methylguanine-thymine base pair has the pseudo-2-fold symmetry with respect to the sugar-phosphate backbone as usually observed in Watson-Crick base pairs. The asymmetrical O^6-methylguanine-cytosine base pair may be recognized during proofreading by DNA or RNA polymerase. As pointed out by Hunter et al., the relative efficiencies of repair remakably correlate to the degree of asymmetry of the mismatched base pairs compared with Watson-Crick base pairs (42). On the other hand, the symmetrical O^6-methylguanine-thymine base pair may be regarded as correct base-pair formation by the polymerases, causing a G-A transition.

Repair of O^6-methylated guanine

The major mutagenic DNA lesion with incorporation of O^6-methylguanine by alkylating agents can be repaired by O^6-methylguanine DNA methyltransferase. In situ, the methyl group of O^6-methylguanine is transferred to a cysteine

residue of the repair enzyme itself (5). The hydrogen bonding at N(7) found in every crystal structure of O^6-methylguanine derivatives is thought to play an important role during the enzymatic repair of O^6-methylguanine residues in DNA. The active site of E. coli O^6-methylguanine-DNA methyltransferase involves Pro320-Cys321-His322 where the SH group of the cysteine residue accepts the methyl group from O^6mGuo, and the iminohydrogen of the histidine residue may form a hydrogen bond with N(7) of the O^6-methylguanine base. The significance of N(7) as an acceptor of hydrogen bond is also suggested by the chemical reaction where treatment of O^6mGuo with CH_3I in DMF gives O^6,7-dimethylguanosine (11). Chemically, the demethylation of CH_3-O-R is accelerated by pulling out the electrons from the O atom. Therefore, the demethylation of O^6methylguanine occurs more easily in the positively charged O^6-methylguanine than in the neutral form. The requirement for the strong methyl acceptor is the strong electron pushing effect of the reacting group. This means that $-S^-$ is a stronger methyl acceptor than -SH. These considerations could provide a model of the repair mechanism as shown in Figure 5. The activation of an active SH group of the cysteine residue by a histidine residue has been reported for the catalytic mechanisms of papain and glyceraldehyde-3-phosphate dehydrogenase (43).

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REFERENCES

1. Singer, B. and Kusmierek, J. T. (1982) Annu. Rev. Biochem. 51, 655-693.
2. Vogl, E, and Natarajan, A. T. (1982) Chemical Mutagens, Vol. 7, de Serres, F. J. and Hollaeder, A., Eds., New York and London, pp295-336.
3. Loveless, A. (1969) Nature, 223, 206-207.
4. Drake, J. W. and Baltz, R. H. (1976) Annu, Rev. Biol. 45, 11-37.
5. Olson, M. and Lindahl, T. (1980) J. Biol. Chem. 255, 10569-10571.
6. Nakabeppu, Y., Kondo, H., Kawabata, S., Iwanaga, S., and Sekiguchi, M. J. (1985) Biol. Chem. 260, 7281-7288.
7. Dample, B., Sedgwick, B., Robbins, P., Totty, N., Waterfield, M. D. and Lindahl, T. (1985) Proc. Natl. Acad. Soc. USA, 82, 2688-2692.
8. Parthasarathy, R. and Fridey, S. M. (1986) Carcinogenesis, 7, 221-227.
9. Farmer, P. B., Foster, A. B., Jarman, M. and Fisdale, J. (1973) Biochem. J. 135, 203-213.

10. Gerchman, L. L., Dombrowski, J. and Ludlum, D. B. (1972) Biochim. Biophys. Acta, 272, 672-675.
11. Kohda, K., Baba, K. and Kawazoe, Y. (1987) Tetrahedron Letters,28,6285-6288.
12. Main, P., Hull, S. E., Lessinger, L., Germain, G., Declercq, J. -P. and Woolfson, M. M. (1978) MULTAN 78, Univs. of York, England, and Louvain, Belgium.
13. Ibers, J. A. and Hamilton, W. C., Eds., (1974) International Tables for X-ray Crystallography, Vol. IV., Kynoch Press: Birmingham, England.
14. Ashida, T. and Yasuoka, N., Eds., (1979) The Universal Crystallographic Computing System, Library of Programs, Computation Center, Osaka Univ., Japan.
15. Pople, J. A. and Segal, G. A. (1966) J. Chem. Phys. 44, 3289-3296.
16. Johnson, C. K. (1976) ORTEP II REport ORNL-5138, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA.
17. Four O^6-methylguanine derivatives used in the calculation are molecules (a) and (b) of O^6mdGuo, O^6mGua and O^6mGuo. Structure of O^6mGuo was independently determined for the crystal recrystallized from MeOH.
18. Taylor, R. and Kennard, O. (1982) J. Am. Chem. Soc. 104,3209-3212.
19. Brennan, R. G., Prive, G. G., Blonski, W. J. P., Hruska, F. E. and Sundaralingam, M. (1983) J. Am. Chem. Soc. 105, 7737-7742.
20. Birnbaum, G. I., Sadana, K. L., Blonski, W. J. P. and Hruska, F. E. (1986) J. Am. Chem. Soc. 108, 1671-1675.
21. Brennan, R. G., Pyzalska, D., Blonski, W. J. P., Hruska, F. E. and Sundalalingam, M. (1986) Biochemistry, 25, 1181-1185.
22. Cook, W. J., Gartland, G. L. and Bugg, C. E. (1980) Acta Cryst. B36, 2467-2470.
23. Srikrishnan, T,, Parthasarathy, R., De, N. C. and Chheda, G. B. (1983) Acta Cryst. C39, 1441-1445.
24. Saenger, W. (1984) Principles of Nucleic Acid Structure, Springer-Verlag, New York.
25. Yamagata, Y. and Tomita, K. (1987) Acta Cryst. C43, 2117-2120.
26. Yamagata, Y., Fukumoto, S., Hamada, K., Fujiwara, T. and Tomita, K. (1983) Nucleic Acids Res. 11, 6475-6486.
27. Sundaralingam, M. (1973) Conformations of Biological Molecules and Polymers, Bergmann, E. D. and Pullman, B., Eds., pp417-456, The Israel Academy Sciences and Humanities, Jerusalem.
28. de Leeuw, H. P. M., Haasnoot, C. A. G. and Altona, C. (1980) Isr, J. Chem. 20, 108-126.
29. Kitamura, K., Wakahara, A., Mizuno, H., Baba, Y. and Tomita, K. (1981) J. Am. Chem. Soc. 103, 3899-3904. The designation of torsion angles χ' is O(4')-C(1')-N(9)-C(8) and it is different from that of χ being O(4')-C(1')-N(9)-C(4).
30. Shibata, M., Takenaka, A. and Sasada, Y. (1985) Acta Cryst. C41, 1501-1503.
31. Mandel, G. S. and Marsh, R.E. (1975) Acta Cryst. B31, 2862-2867.
32. We computed the intermolecular distances in 110 crystal structures of guanine derivatives which were obtained from Cambridge Crystallographic Database, October, 1986. The dimer formed by the base pairing with the N(3)--H-N(2) hydrogen bonds as found in only one crystal of deoxycytidylyl-(3'-5')-deoxyguanosine (Cruse, W. B. T., Egert, E., Kennard, O., Sala, G. B., Saliobury, S. A., Viswamitra, M. A. Biochemistry 1983, 22, 1833-1839.) among the crystal structures of guanine nucleosides and nucleotide.
33. Bugg, C. E., Thomas, J. M., Sundaralingam, M. and Rao, S. T. (1971) Biopolymers, 12, 175-219.
34. The data were based on our experimental results.
35. Altona, C. and Sundaralingam, M. (1973) J. Am. Chem. Soc. 95, 2333-2344.

36. Snow, E. T., Foote, R. S. and Mitra, S. (1984) J. Biol. Chem. 259, 8095-8100.
37. Mehta, J. R. and Ludlum, D. B. (1978) Biochim. Biophys. Acta, 562, 770-778.
38. Psoda, A., Kierdaszuk, B., Pohorille, A. and Geller, M. (1981) Int. J. Quantum Chem. XX, 543-554.
39. Caldwell, J. W. and Kollman, P. A. (1985) J. Biomol. Struct. Dyn. 3, 57-66.
40. Yamagata, Y., Miyashiro, H., Tomita, K., Ishida, T. and Inoue, M. (1985) Chem. Pharm. Bull. 33, 455-463.
41. Gaffney, B. L., Marky, L. A. and Jones, R. A. (1984) Biochemistry, 23, 5686-5691.
42. Hunter, W. H., Brown, J., Anand, N. N. and Kennard, O. (1986) Nature, 320, 552-555.
43. Schulz, G. E. and Schirmer, R. H. (1979) Principles of Protein Structure, pp234-241, Springer-Verlag, New York.