The Crystal Structure of 9-Ethyl-8-hydroxyguanine

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Synopsis. Analysis of the crystal structure of 9-ethyl-8-hydroxyguanine using X-ray diffraction showed that it is in C-8 keto form. As compared with 9-ethylguanine, significant differences were observed in bond lengths and bond angles of the imidazole ring.

8-Hydroxyguanine (8-OH-Gua) in DNA, induced by oxygen radicals,[1] is a newly encountered form of DNA damage. Many mutagens or carcinogens which form oxygen radicals produce an 8-OH-Gua moiety in DNA in vitro. These agents include mutagenic reducing agents (ascorbic acid, hydrazine, hydroxylamine),[1] asbestos-H_2O_2,[2] polyphenol-H_2O_2-Fe^{3+},[3] and ionizing radiation.[4] The modified base, 8-OH-Gua, is also produced in cellular DNA by ionizing radiation,[5] and it is reasonable to speculate that it may be one of the forms of DNA damage responsible for mutation or carcinogenesis. In fact, misreading was observed during DNA replication in vitro at 8-OH-Gua residue and at adjacent residues.[6] To elucidate the mechanism of mispairing by the 8-OH-Gua residue in DNA, it is important to compare the molecular structure of 8-OH-Gua with that of Gua. It is also important to determine which tautomer of 8-OH-Gua, the 8-enol form or the 8-keto form, is favoured in solution, although the 8-keto form has been proposed for 8-hydroxyguanosine based on ^{13}C NMR shift.[7] This communication describes the analysis of the crystal structure of 9-ethyl-8-hydroxyguanine (9-Et-8-OH-Gua), a model compound of N-9 substituted 8-OH-Gua.

Experimental

9-Et-8-OH-Gua was prepared from *N*-9-ethylguanine (9-Et-Gua, SIGMA) by the treatment with 1 mM $FeSO_4$ (1 M=1 mol dm^{-3}), 5 mM EDTA, 10 mM ascorbic acid, 100 mM phosphate buffer (pH 6.8) in the presence of oxygen gas (Udenfriend system[8]). A 15% yield of pure 9-Et-8-OH-Gua was obtained after fractionation of the reaction mixture using high-performance liquid chromatography. Crystals of 9-Et-8-OH-Gua were then grown in an aqueous solution.

A crystal of approximate dimensions 0.6×0.1×0.1 mm was mounted on a Philips PW1100 diffractometer. The Setting angles for twenty-five high angle reflections and intensity data were measured using Cu $K\alpha$ monochromatic radiation reflected by a graphite plate. Crystal data: 9-Ethyl-8-hydroxyguanine monohydrate (9-Et-8-OH-Gua monohydrate), $C_7H_9O_2N_5\ H_2O$, MW=213.2. Triclinic, space group $P\bar{1}$, Z=2. Lattice constants, a=9.937(5), b=9.745(5), c=5.304(6) Å, α=99.95(5), β=92.52(4), γ=114.09(6)°, U=458.1 Å^3. Dcal=1.546 g cm^{-3}, μ for Cu $K\alpha$=1.54 cm^{-1}. Intensities were measured using a 2θ—ω scan method with a scan speed of 4° $2\theta s^{-1}$. The reflections having net intensities (I) above the $2\sigma(I)$ level were recorded as observed. Intensities of three standard reflections, whose values remained within ±2.5%, were monitored every 2 h. 1574 Independent structure factors, out of a theoretically possible 1879, were obtained within the 2θ range from 6—156°. The crystal structure was solved by the direct method, using the computer program MULTAN, and refined by the least-squares method with block-diagonal-matrix approximations. All eleven hydrogen atoms were located on a difference electron-density map and their positional and isotropic thermal parameters were included in the least-squares refinement. The final R value was 0.038 for the 1574 observed structure factors.

Results and Discussion

Analysis of the crystal structure of 9-Et-8-OH-Gua clearly shows it to be in the C-8 keto form, with a

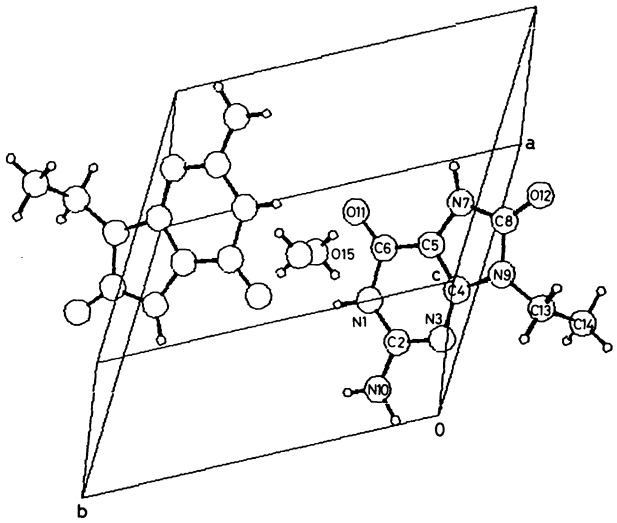

Fig. 1. Projection of the crystal structure of 9-Et-8-OH-Gua.

Table 1. Comparison of Bond Lengths (l/Å) in 9-Et-8-OH-Gua and 9-Et-Gua.

	9-Et-8-OH-Gua	9-Et-Gua	Deviation
	(±0.002)[a]	(±0.004)[a]	
N(1)-C(2)	1.369	1.370	−0.001
N(1)-C(6)	1.369	1.394	0.003
C(2)-N(3)	1.332	1.321	0.011
C(2)-N(10)	1.338	1.344	−0.006
N(3)-C(4)	1.350	1.350	−0.000
C(4)-C(5)	1.373	1.377	−0.004
C(4)-N(9)	1.376	1.375	0.001
C(5)-C(6)	1.395	1.406	0.013
C(5)-N(7)	1.395	1.384	0.005
C(6)-O(11)	1.249	1.238	0.011
N(7)-C(8)	1.358	1.312	0.046
C(8)-N(9)	1.391	1.369	0.022
C(8)-O(12)	1.237	—	—

a) Estimated SD.

Table 2. Comparison of Bond Angles (ϕ/°) in 9-Et-8-OH-Gua and 9-Et-Gua

	9-Et-8-OH-Gua	9-Et-Gua	Deviation
	(±0.2)[a]	(±0.5)[a]	
C(2)-N(1)-C(6)	125.0	124.8	0.2
N(3)-C(2)-N(1)	123.1	124.2	−1.1
N(3)-C(2)-N(10)	119.5	119.4	0.1
N(1)-C(2)-N(10)	117.4	116.4	1.0
C(4)-N(3)-C(2)	112.6	111.7	0.9
C(5)-C(4)-N(3)	127.5	128.5	−1.0
C(5)-C(4)-N(9)	107.4	105.7	1.7
N(3)-C(4)-N(9)	125.1	125.9	−0.8
C(6)-C(5)-C(4)	120.1	119.3	0.8
C(6)-C(5)-N(7)	132.1	130.1	2.1
C(4)-C(5)-N(7)	107.7	110.7	−3.0
O(11)-C(6)-N(1)	120.0	119.8	−0.2
O(11)-C(6)-N(5)	128.4	128.7	−0.3
N(1)-C(6)-C(5)	111.6	111.6	0
C(8)-N(7)-C(5)	108.9	104.4	4.5
N(9)-C(8)-N(7)	107.0	113.0	−6.0
N(9)-C(8)-O(12)	124.7	—	—
N(7)-C(8)-O(12)	128.3	—	—
N(13)-N(9)-C(4)	127.6	126.0	1.6
C(13)-N(9)-C(8)	123.5	127.7	−4.2
C(4)-N(9)-C(8)	109.0	106.4	2.6

a) Estimated SD.

proton attached to the N-7 position and with a C(8)-O(12) bond length of 1.237 Å (Fig. 1). Within the crystal, the molecules of 9-Et-8-OH-Gua are linked together and to water molecules by the seven kinds of hydrogen bonds. When the bond lengths and bond angles of 9-Et-8-OH-Gua were compared with those of 9-Et-Gua,[9] significant differences were observed in the imidazole ring due to the 8-keto structure (Tables 1 and 2). Bond lengths, C(8)-N(7) and C(8)-N(9) of 9-Et-8-OH-Gua are considerably longer than those of 9-Et-Gua, and there is a highly significant deviation in the bond angles C(8)-N(7)-C(5), N(9)-C(8)-N(7), and C(13)-N(9)-C(8) from the corresponding bond angles in 9-Et-Gua. In contrast, the structure of pyrimidine ring of 9-Et-8-OH-Gua is very similar to that of 9-Et-Gua. Based on ^{13}C NMR studies, it has been reported that 8-hydroxyguanosine favors the syn-conformation.[7] Our finding based on ^{1}H and ^{13}C NMR spectra suggest 8-OH-dG can also adopt the syn-conformation (unpublished results). From these results it is concluded that misreading by 8-OH-dG observed during DNA replication in vitro,[6] may be due either to formation of Hoogsteen type base-pairing by 8-OH-dG (6,8-diketo form and syn-conformation) or to disturbance of the tertially structure of DNA around 8-OH-dG residues. To elucidate the actual effect of the 8-OH-Gua residue in DNA, the tertiary structure of selfcomplementary oligodeoxynucleotides containing 8-OH-Gua at specific position need to be studied, and work on this line is now in progress.

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