A novel photoproduct of 2'-deoxyguanosine induced by acetone photosensitization: 8-(2,3,4-trihydroxybutyl)guanine

Narain D.Sharma, R. Jeremy H.Davies*, Dennis R.Phillips¹ and James A.McCloskey¹

Biochemistry Department, Medical Biology Centre, Queen's University, Belfast BT9 7BL, Northern Ireland and ¹Departments of Medicinal Chemistry and Biochemistry, University of Utah, Salt Lake City, UT 84112, USA

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ABSTRACT

Acetone photosensitisation of 2'-deoxyguanosine in deaerated aqueous solution gives 8-(2,3,4-trihydroxybutyl)guanine as a major photoproduct. Its structure and that of its tetraacetate have been determined primarily by high resolution ¹H NMR and mass spectrometry; a di-isopropylidene derivative has also been prepared. Mechanistic aspects of this novel photochemical transformation are discussed, particularly in relation to the alkaline cleavage of acetone photosensitised DNA at the sites of guanine bases.

INTRODUCTION

Besides confirming the preponderance of pyrimidine photoproducts, the application of gel sequencing methods to the detection of photolesions in DNA¹⁻³ has provided compelling evidence that its constituent purine bases can also undergo modification by ultraviolet (UV) light³⁻⁵. An interesting example is the discovery by Umlas *et al.*⁶ that when DNA is irradiated at 313 nm, in the presence of acetone as triplet photosensitiser, subsequent heating with piperidine causes specific cleavage at the sites of guanine residues. Triplet photosensitisation of DNA by acetone is generally reckoned to induce almost exclusive formation of cyclobutane pyrimidine photodimers^{7,8}, though it has been noted by Patrick and Snow⁹ that a significant proportion of the resulting photodamage cannot be reversed by photoreactivation. In principle, the accompanying modification of guanine bases in DNA might be related to their known susceptibility to photoalkylation at C(8) by exogeneous free radical species^{10,11} and/or photosensitised oxidation^{11,12}. To assess whether these or other types of photochemical processes are responsible for the observed cleavage at guanine we investigated the behaviour of 2'-deoxyguanosine (1) when it is irradiated at wavelengths >290 nm in deaerated 20% aqueous acetone. This paper describes the isolation of the principal UV-absorbing photoproduct whose

structure has been determined as 8-(2,3,4-trihydroxybutyl)guanine (2). It arises by a novel photochemical transformation that results in cleavage of the glycosidic bond and the attachment of a four-carbon fragment of the deoxyribose moiety to C(8) of the guanine nucleus. No analogous photoreaction has been reported previously for any nucleic acid derivative and the photoproduct is not among the known γ -radiolysis products¹³ of deoxyguanosine in oxygen-free aqueous solution. It remains to be established whether formation of the photoproduct can occur at the polynucleotide level in DNA. If so, it is likely to be associated with either direct chain breaks or the production of alkali-labile cleavage sites.

EXPERIMENTAL

Materials and Methods

2'-Deoxyguanosine was purchased from Sigma and from BDH. Other chemicals were obtained from the following sources: acetone (A.C.S. reagent grade) and 2,2-dimethoxypropane from Aldrich; methanol (HPLC grade) from Lab-Scan; 2'-deoxyadenosine, adenosine, guanosine, trifluoroacetic acid and bis(p-nitrophenyl) hydrogen phosphate from Sigma.

High performance liquid chromatography (HPLC) was carried out at ambient temperature with equipment described elsewhere¹⁴. Separations were effected, at a flow rate of 0.8 ml min⁻¹, on columns (300 x 3.9 mm) of μ Bondapak C₁₈ (Waters Associates) by using isocratic elution with 0.05% trifluoroacetic acid (TFA) for 5 min followed by a linear gradient to 0.05% TFA containing 30% methanol after 15 min; elution profiles were monitored at 254 nm. Analytical thin layer chromatography (TLC) was performed on precoated (0.2 mm) silica gel 60 F₂₅₄ plastic sheets (Merck) in a solvent containing 1-butanol/water/acetic acid (5:3:2, by vol.).

UV spectra were recorded on a Perkin-Elmer lambda 5 spectrophotometer; optical rotations were measured with a Perkin-Elmer model 241 polarimeter.¹ ¹H NMR spectra were recorded at 250 and 400 MHz with Bruker WM 250 and WH

400 instruments, or at 300 MHz with a Nicolet QE-300 spectrometer. The instrumentation used to acquire fast atom bombardment (FAB) mass spectra of the photoproduct (2) and electron ionization (EI) mass spectra has been described elsewhere¹⁵. High resolution FAB mass spectra of compounds (3) and (4) were obtained through the SERC Mass Spectrometry Centre, Swansea. To silylate the deoxyguanosine photoproduct a sample of ~20 µg was vacuum dried and then heated at 100°C, for 1 h, in a sealed capillary tube with 10 µl of pyridine, 20 µl of N,O-bis(trimethylsilyl)-acetamide containing 1% chlorotrimethylsilane and 1 µl of N,N-dimethylformamide.

Photosensitised irradiation of deoxyguanosine

2'-Deoxyguanosine was dissolved at a concentration of 0.1 mg/ml (0.37 mM) in 20% aqueous acetone and was irradiated at wavelengths >290 nm with a 100 W medium pressure mercury lamp (Hanovia) surrounded by a tubular Pyrex glass cooling jacket through which water was continuously circulated. For small scale experiments, the solution of deoxyguanosine (~50 ml) was held in a cylindrical sleeve which fitted closely round the lamp and water jacket assembly; for preparative purposes, the lamp and water jacket assembly was placed in a photoreactor vessel with a capacity of 1 litre. In both cases, oxygen-free nitrogen was bubbled through the solution during irradiation and it was stirred continuously with a magnetic follower.

To monitor the course of the photoreaction small aliquots of solution were withdrawn periodically, evaporated to dryness in a vacuum concentrator, redissolved in water, and then analysed by HPLC. The results of a typical experiment are shown in Fig. 1. Apart from guanine, only one UV-absorbing photoproduct is formed in significant amounts. No photoreaction occurred in the absence of acetone.

Isolation and properties of the photoproduct.

8-(2,3,4-trihydroxybutyl)guanine (2)

Small quantities of spectroscopically pure photoproduct were isolated by successive paper chromatography and HPLC. The irradiated solution of deoxyguanosine was evaporated to dryness and the residue then subjected to descending chromatography on Whatman 3 MM paper employing 1-propanol/water (7.3; by vol.) as solvent. The chromatogram was viewed under UV light and the dark band corresponding to the photoproduct, which migrates more slowly than residual deoxyguanosine, was excised and extracted with warm water. The material obtained after concentration of the aqueous extract was further purified by HPLC. Eluent fractions comprising the photoproduct peak were pooled and evaporated to dryness to yield the photoproduct as a white

amorphous solid which was also homogeneous on analytical TLC (R_f 0.52).

To prepare the photoproduct on a larger scale eight 1 litre batches of solution, containing a total of 800 mg of deoxyguanosine, were each irradiated for 9 h and then combined and evaporated to dryness. The residue was dissolved in hot water (20 ml), treated with charcoal, and filtered. On standing at 4°C the filtrate deposited the crude photoproduct as a brownish solid and a further crop of the material separated on concentrating the mother liquor to 10 ml. In all, 102 mg of crude product was recovered which was shown by TLC to contain a small proportion of guanine. Further recrystallisation of the material from water and decolorization with charcoal gave the photoproduct as an off-white microcrystalline solid but failed to remove all the contaminating guanine whose content was estimated as 5% by HPLC. When required, the photoproduct could be completely freed of guanine by fractionating it on HPLC or by paper chromatography.

The chromatographically homogeneous photoproduct decomposed on heating above 250°C. Found (for a sample dried in vacuo at 60°C for 6h): C, 40.2; H, 5.4; N, 25.9. Calc. for $C_9H_{13}N_5O_4 \cdot 3/4 H_2O$: C, 40.2; H, 5.4; N, 26.1%. UV: λ_{max} (pH 1) 250, 276 nm (ϵ 12200, 7800); (pH 7) 248, 277 nm (ϵ 10900, 8000); (pH 13) 276 nm (ϵ 9100). Optical rotation: $[\alpha]_D^{68} (H_2O) = -24^\circ$. Mass spectra: FAB (positive ion, glycerol/thioglycerol (1:1) matrix) m/z 256.1044, $[M+H]^+$, calc. for $C_9H_{14}N_5O_4$ m/z 256.1046; the EI mass spectrum of the trimethylsilylated photoproduct is shown in Fig. 2. The 400 MHz 1H NMR spectrum of the photoproduct in D_2O was recorded with suppression of the HOD signal and is reproduced in Fig. 3. It was calibrated by setting the chemical shift of HOD at δ 4.80. Signals for the non-exchangeable protons (which are designated as in Fig. 3) have been assigned as follows: δ 2.82, dd, H_f ; 3.08, dd, H_e ; 3.58, m, H_b and H_c ; 3.72, m, H_a ; 3.98, m, H_d . The extracted coupling constants are: $J_{ab} = -11.0$, $J_{ac} = 2.5$, $J_{bc} = 6.7$, $J_{cd} = 6.3$, $J_{de} = 3.5$, $J_{df} = 9.4$, $J_{ef} = -15.0$ Hz.

Preparation of N²-acetyl-8-(2,3,4-triacetoxybutyl)guanine (3)

A suspension of the deoxyguanosine photoproduct (8 mg) in anhydrous pyridine (0.5 ml) containing an excess of acetic anhydride (0.1 ml) was heated in a sealed glass tube at 100°C for 90 min. The light yellow solution of the product mixture was evaporated to dryness at room temperature in a Gyrovap vacuum concentrator (Uniscience) and the residue then purified by preparative TLC on silica gel 60 PF_{254 + 366} (from Merck) using chloroform/methanol (23:2, by vol.) as solvent. The tetraacetate

migrated as a dark UV absorbing band with R_f 0.5. After extraction in chloroform/methanol and evaporation, it was recovered as a colourless glassy solid (6 mg) which was homogeneous on analytical TLC (R_f 0.72). UV: $\lambda_{\max}$ (MeOH) 262, 280(sh) nm. Mass spectrum: FAB (positive ion, glycerol matrix) m/z 424.1455, $[M+H]^+$, calc. for $C_{17}H_{22}N_5O_8$ m/z 424.1468. The 300 MHz 1H NMR spectrum (in $CDCl_3$ containing a few drops of C_5D_5N with tetramethylsilane as internal reference) showed 3 singlets, each integrating for 3 protons, at δ 1.85, 1.93 and 2.01 assigned to O-acetyl groups, and another 3-proton singlet at δ 2.24 assigned to an N-acetyl group. Signals for the side-chain protons, designated as in Fig. 3, were assigned as follows: δ 3.32, dd, H_f ; 3.41, dd, H_e ; 4.30, dd, H_b ; 4.43, dd, H_a ; 5.47, m, H_c ; 5.76, m, H_d . The estimated coupling constants are: $J_{ab} = -12.2$, $J_{ac} = 3.7$, $J_{bc} = 6.1$, $J_{cd} = 5.1$, $J_{de} = 5.0$, $J_{df} = 8.5$, $J_{ef} = -14.8$ Hz. A separate 1H NMR spectrum, run at 250 MHz in $CDCl_3$, showed broad singlet absorptions, centered at δ 9.85, 10.28 and 11.14, for the three D_2O -exchangeable N-H protons in the molecule.

Di-isopropylidene derivative of the photoproduct

Following the method of Hampton¹⁶, 2,2-dimethoxypropane (70 μ l) and bis(p-nitrophenyl) hydrogen phosphate (23 mg) were added to a magnetically stirred suspension of the deoxyguanosine photoproduct (12 mg) in anhydrous acetone (0.7 ml). The reaction mixture was stirred for 3 h at ambient temperature under anhydrous conditions and the resulting clear yellow solution then diluted with water. The buff coloured precipitate that formed was collected by centrifugation, washed successively with dilute ammonia and water, and finally dried under vacuum. This gave the isopropylidene derivative as a cream coloured powder (10 mg) that behaved as a single component on reversed-phase HPLC when eluted isocratically with 50% aqueous methanol. m.p. $>250^\circ C$. UV: $\lambda_{\max}$ (MeOH) 254, 276(sh) nm. Mass spectrum: FAB (positive ion, glycerol matrix) m/z 336.1655, $[M+H]^+$, calc. for $C_{15}H_{22}N_5O_4$ m/z 336.1672. EI m/z 335, M^+ ; 320 $[M-CH_3]^+$; 234 $[M-C_5H_9O_2]^+$.

RESULTS AND DISCUSSION

In comparison with their pyrimidine counterparts, guanine nucleosides are very resistant to photochemical alteration by short wavelength UV radiation. When irradiated at 254 nm in aerated aqueous solution, 2'-deoxyguanosine undergoes photodecomposition to a variety of products¹⁷ but the overall quantum yield is only $\sim 1.5 \times 10^{-4}$. Guanine is, however, the

Fig. 1. Time course (0 to 60 min) of the acetone-sensitised photoreaction of 2'-deoxyguanosine (dG) monitored by HPLC. Conditions used for UV irradiation and for HPLC are given in the text. The samples analysed were derived from equal volumes of the irradiated solution, containing initially 8 nmol of dG. The elution positions of guanine (G) and the principal photoproduct (P) are arrowed.

most sensitive of the nucleobases towards photodynamic destruction induced by exposure to near-UV or visible light in the presence of photosensitising agents and oxygen. Several degradation products have been characterised that are formed from deoxyguanosine and its 3',5'-di-O-acetylated derivative by type I and type II photooxidation processes¹². Here we report the effect of irradiating deoxyguanosine with the triplet photosensitiser acetone in deaerated aqueous solution. Under these conditions, photodynamic breakdown of the guanine nucleus should be suppressed.

It is evident from the HPLC profiles (Fig. 1) depicting the course of the reaction that when deoxyguanosine is irradiated at wavelengths >290 nm,

Fig. 2. Electron ionization mass spectrum of the trimethylsilylated deoxyguanosine photoproduct. The insert shows the proposed structures and origin of major fragment ions from the two components. Note that the B^a and B^b moieties simply define the derivatised base residue in each case; they do not represent expected fragment ions.

in 20% aqueous acetone under nitrogen, only one major photoproduct is formed possessing strong absorption at 254 nm. The photoproduct has a retention time of 10.4 min; a much smaller adjacent peak, with a retention time of 9.3 min, is attributable to guanine produced by photocleavage of the glycosidic bond. Allowing for unchanged deoxyguanosine, the overall yield of the major photoproduct is ~15%. Apart from trace entities, the remaining photoproducts do not absorb appreciably at 254 nm and must have suffered loss of the conjugated chromophore associated with the guanine ring system. Irradiation of guanosine under the same conditions also caused extensive photodegradation but there was no evidence from HPLC analysis for the formation of a comparable major UV-absorbing photoproduct. In the case of 2'-deoxyadenosine and adenosine very little decomposition occurred at doses sufficient to completely degrade deoxyguanosine. Hence, among the four common purine nucleosides the photoreaction under discussion appears to be confined to 2'-deoxyguanosine. The photoreaction was, however, undetectable when deoxyguanosine was irradiated in air-saturated aqueous acetone, possibly owing to triplet quenching by oxygen and/or concomitant photooxidation of the guanine base moiety.

The photoproduct was isolated as described above (Experimental) and its structure elucidated as follows. High resolution mass spectrometry established its elemental composition as $C_9H_{13}N_5O_4$. The presence of an intact guanine base could be inferred from the photoproduct's UV absorption spectrum which very closely resembles that of guanine at acid, neutral and alkaline pH. The similarity of the UV spectra further implied that the remainder of the molecule must comprise a non-conjugated substituent attached to C(8) of the guanine nucleus; from its composition, $C_4H_9O_3$, it could not contain rings or a double bond. Substitution at C(8) was confirmed by the photoproduct's 1H NMR spectrum which showed no signals attributable to either an aromatic proton or a glycosidic H(1') proton. In addition, it was shown by FAB mass spectrometry that no stable incorporation of deuterium occurred when the photoproduct was heated in D_2O at $100^\circ C$ for 1 h. Hydrogen atoms bonded to C(8) of a guanine ring exchange with deuterium under these conditions¹⁸ as observed for guanosine which was used as an internal standard in this experiment.

Detailed analysis of the electron ionization mass spectrum of the trimethylsilylated photoproduct enabled the side chain to be identified with reasonable certainty as a 2,3,4-trihydroxybutyl function. The spectrum and a schematic representation of the major fragmentation pathways is shown in Fig. 2. The sample comprised a mixture of the pentakis- and hexakis-trimethylsilyl derivatives which gave rise to molecular ions at m/z 615 and 687 respectively. Their assignment as molecular ions was confirmed by the characteristic¹⁹ loss of CH_3 (m/z 600 and 672) and $TMSOH$ ($M-90$ ions). An intense m/z 103 peak for $^+CH_2OTMS$ suggested a terminal $-CH_2OH$ group¹⁹. The m/z 309 and 381 ions were assigned to silylated $B_aH-CH_2^+$ and $B_bH-CH_2^+$ ruling out the possibility of an ether linkage between the side chain and the guanine base. Further supporting the proposed structure, ions with m/z 410 and 482 correlated to the fragments $B_a-CH_2CHOTMS^+$ and $B_b-CH_2CHOTMS^+$ and an ion with m/z 205 to the fragment $^+CHOTMS-CH_2OTMS$. Although the m/z 307 ion was present, it was not intense enough to be distinguished from the background. The absence of B_aH and B_bH fragment ions in the mass spectrum is consistent with the photoproduct being a C-nucleoside analogue^{20,21}. Ions of mass 147 and 217 are common to TMS ethers of polyols such as nucleosides¹⁹ and confer no structural information in the present case.

Confirmation of the structure of the side chain was obtained from the

Fig. 3. The 400 MHz ^1H NMR spectrum of the deoxyguanosine photoproduct in D_2O . Multiplets have been assigned to the non-exchangeable protons in the side chain as indicated (see text).

400 MHz ^1H -NMR spectrum of the photoproduct in D_2O . The pattern of signals arising from the six non-exchangeable and non-equivalent protons was assigned as indicated in Fig. 3. Spin coupled protons were identified by two-dimensional homonuclear shift correlation spectroscopy (2D COSY) and the relevant coupling constants are given under Experimental. A notable feature is the large value (~ 15 Hz) of the geminal coupling constant, J_{ef} , which is characteristic of protons attached to a carbon atom adjacent to a π -system.

Acetylation of the photoproduct with acetic anhydride in pyridine at 100°C gave the tetraacetate N^2 -acetyl-8-(2,3,4-triacetoxybutyl)guanine (3) whose spectroscopic properties (Experimental) are fully in accord with this structure. The formation of this derivative provided unequivocal evidence for the presence of three hydroxyl groups in the side chain. Monoacetylation of the exocyclic amino group of guanine has been observed previously²²

under similar reaction conditions. Based on the disposition of its three hydroxyl groups, the photoproduct would be expected to form two distinct mono-isopropylidene derivatives. However, reaction with dimethoxypropane in the presence of bis(p-nitrophenyl) hydrogen phosphate afforded a single product which was highly insoluble in all common solvents (thus precluding NMR measurements). Its elemental composition, determined by high resolution FAB mass spectrometry, indicated that it was a di-isopropylidene derivative. Presumably, this is formed by cyclisation involving N(7) or N(9) of the guanine ring and the hydroxyl group on the 2' position of the side chain to give a stable six-membered ring, as illustrated for the N(9) adduct in structure (4); the other isopropylidene group is then incorporated by reaction of the distal 3' and 4' hydroxyl functions on the side chain. Supporting this, the EI mass spectrum exhibited an intense fragment ion at m/z 234 attributable to loss of the terminal 1,3-dioxole ring. Owing to the presence of vicinal diol groupings, the photoproduct is susceptible to cleavage by periodate; although its degradation by periodate was demonstrated by HPLC no attempt was made to isolate the oxidation products.

The photoproduct is optically active and possesses two chiral carbon atoms within its side chain. Its homogeneity on HPLC, and its ^1H NMR spectrum, indicate that it constitutes a single stereoisomer but the configuration of the central carbon atoms in the trihydroxybutyl group cannot be realistically assigned until the mechanism of the photoreaction is understood. In this regard, it is worth emphasising that the photoreaction, which occurs in the absence of oxygen, is clearly unrelated to the dye-sensitised photooxidation reactions of deoxyguanosine that have been widely investigated¹² because guanine is the main target for photodynamic damage to DNA. On forming the photoproduct (2) from deoxyguanosine there is a net loss of one carbon atom; this might well result from a combination of

water addition and the loss of formaldehyde. In principle, the methylene group attached directly to the purine ring might be derived from C(2') or C(5') of the original deoxyribose residue. Both of these atoms are close to the C(8) position of guanine when 2'-deoxyguanosine adopts the favoured anti conformation and 8,5'-cyclo-2',5'-dideoxyguanosine is a known²³, but chemically stable, γ -radiolysis product of deoxyguanosine. A definitive mechanism must also accommodate the observation that, as judged by ¹H NMR and FAB mass spectrometry, there is no stable incorporation of deuterium into the photoproduct when it is prepared by irradiating deoxyguanosine with acetone in D₂O solution.

Charlier and Helene²⁴ have shown that triplet state molecules of acetophenone and benzophenone can abstract hydrogen atoms from purine derivatives with C(8) being the most likely site of attack. This process, which would generate an 8-guaninyl free radical, might therefore initiate the acetone photosensitised reaction under discussion. However, an important difference between acetone and the aromatic ketones is that its triplet state energy is higher than that of the guanine chromophore^{8,25}. Accordingly, when acetone is used as a photosensitiser, energy transfer to produce triplet state deoxyguanosine molecules as the primary reactive species is a plausible alternative. Photoalkylation^{10,11} of deoxyguanosine by free radical species derived from acetone can be excluded in view of the structure of the side chain which is only compatible with it originating from deoxyribose; furthermore, simple C(8) substitution of the guanine nucleus should not cause cleavage of the glycosidic bond. Additional work will be necessary to elucidate the mechanism of this unusual photoreaction but we speculate that following initial bond formation between C(8) of the guanine base and either C(2') or C(5') of the deoxyribose moiety the elements of water are incorporated through a hydrolytic fission step that generates the photoproduct bearing a labile formyl group on N(9). The instability of N(9) acyl purines towards hydrolysis in aqueous solution has been noted²⁶.

Photosensitisation with aromatic ketones and acetone has been widely used to induce efficient, and largely exclusive, photodimerisation of the pyrimidine bases in DNA. However, it has been remarked that this treatment also creates single strand chain breaks^{8,9} and, in the case of acetone, alkali-labile cleavage sites at the positions of guanine⁶. Experiments with radiolabelled acetone have revealed⁹ that there is no significant covalent binding of the photosensitiser to DNA upon UV irradiation. In this context,

it will be important to determine whether the deoxyguanosine photoproduct (2) contributes to photosensitised damage in DNA. It is reasonable to assume that, depending on the mechanism, its formation in DNA would cause either direct rupture of the deoxyribose-phosphate backbone or the induction of alkali-labile cleavage sites. In particular, if the continuity of the DNA backbone were maintained through phosphodiester linkages involving any two of the three hydroxyl functions in the photoproduct's side chain it would be susceptible to base catalysed cleavage by neighbouring group participation of a vicinal hydroxyl group as occurs in RNA. Detection of the photoproduct in irradiated samples of DNA and other polynucleotides should be facilitated by its stability under conditions of acid hydrolysis (1 M HCl, 100°C, 3 h) and by its retention, relative to the normal nucleobases, on phenylboronate agarose gel at pH 8.6 (shown in preliminary experiments).

Finally, from a biological standpoint, it is noteworthy that most of the mutagenic base sequence changes induced in the *cI* gene of bacteriophage λ by ketonic photosensitisation are transversions originating from G.C base pairs²⁷. The new deoxyguanosine photoproduct might be pertinent to this phenomenon if, as has been suggested²⁷, it results from the photochemical modification of guanine.

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*To whom correspondence should be addressed

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