Low-Energy, Low-Yield Photoionization, and Production of 8-Oxo-2′-deoxyguanosine and Guanine from 2′-Deoxyguanosine

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Experiments employing electron scavenging methods and high performance liquid chromatography with mass spectrometry detection indicate that electrons, formed via one-photon ionization, guanine (G) and small amounts of 8-oxo-2′-deoxyguanosine (8-oxo-dG) are formed during 254 nm irradiation of deaerated alkaline 2′-deoxyguanosine (dG) solutions containing N_2O. The G, 8-oxo-dG and electron yields vary in a similar way when the pH changes. At a dG concentration of 1.2×10^{-4} M, the 254 nm photoionization quantum yield is in the range 0.01 to 0.02 at pH 11.4. The low sensitivity with which photoionization can be monitored in electron scavenging experiments does not permit the direct measurement of dG photoionization at near neutral pH. At pH 6.3, the 254 nm photoionization quantum yield for dG is no larger than 0.003. At pH 11.4, the yields of G and 8-oxo-dG formed from 254 nm irradiation of 1.2×10^{-4} M dG for 40 min are more than 5× larger than that at pH 6.3. The similar pH dependence of the G, 8-oxo-dG and photoelectron yields, and earlier reports linking G and 8-oxo-dG formation to dG photoionization provide evidence that, under the present conditions, G and 8-oxo-dG quantum yields, which can be monitored with higher sensitivity than hydrated electrons in electron scavenging experiments, parallel the photoionization quantum yield. From this perspective, the wavelength dependence of G and 8-oxo-dG quantum yields indicates that dG ionization threshold wavelengths at pH 11.4 and 6.3 are 266 ± 16 and 260 ± 16 nm, respectively. When the 260 nm threshold of dG at pH 6.3 is adjusted to account for an approximately 0.5 eV lowering of the ionization energy associated with incorporation of dG into native B DNA sequences containing multiple stacked guanines (Sugiyama, H.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 7063−7068. Zhu, Q.; LeBreton, P. R. *J. Am. Chem. Soc.* **2000**, *122*, 12 824−12 834.), the present results provide evidence that low quantum-yield DNA photoionization occurs near the short-wavelength cutoff (290 nm) of the ground-level solar spectrum.

Introduction

The finding that UV radiation causes mutations and cancer, and that, at ground level, the solar spectrum contains 0.3% UV−B radiation in the range 280 to 320 nm has inspired interest in the photochemistry of DNA.[1,2] This is a rich chemistry in which nucleic acids participate in many photochemical pathways, including reactions that involve direct DNA photoexcitation and reactions that are mediated by photosensitizers.[3−5] Among carcinogenic DNA products associated with direct UV−B excitation, a great deal of research has examined cyclobutadipyrimidines and pyrimidine(6−4)pyrimidone photoadducts which are formed by reactions in the pyrimidine first excited singlet (S_1) and triplet (T_1) states.[1,4,5b] Another family of reactions generally believed to be associated with wavelengths below the UV−B range[4] is initiated by direct photoionization of DNA, and much work investigates the photoionization of guanine (G), the DNA base with the lowest ionization energy.[6−10] Earlier research focuses almost exclusively on products and mechanisms related to guanine photoionization.[3−5,11−13] There has been less experimental effort to evaluate threshold photon energies for DNA photoionization in physiological environments, or even in simple aqueous solution.[4,13,14]

Threshold measurements are hindered by a combination of factors, including the low photoionization quantum yields of DNA components at physiological pH and the low sensitivity with which aqueous photoionization is detected. In the photoionization of guanine, and guanine containing nucleosides and nucleotides with pulsed lasers, transient absorption spectroscopy has been used to monitor hydrated electrons.[4,13,15−18] Here, the high pulse power needed to detect low quantum-yield photoionization has restricted the laser measurements primarily to one or two-photon ionization at the easily accessible wavelengths of 266, 248, and 193 nm.[4] At neutral pH, the one-photon 193 nm ionization quantum yields of guanosine and 2′-deoxyguanosine (dG) are 0.073.[13] Experiments with continuous Xe and Hg arc lamps frequently employ electron scavengers to monitor photoionization.[19] However, the low sensitivity and inconvenience of electron scavenging experiments make it impractical to use these methods to measure thresholds of the low quantum yield associated with DNA photoionization.

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One product from UV damage of DNA is 8-oxo-2′-deoxyguanosine (8-oxo-dG), formed via photooxidation of dG.[11,12,20] 8-Oxo-dG causes miscoding by DNA polymerase in vitro,[21] is mutagenic in bacterial and mammalian cells,[22] and induces guanine → thymine transversions that occur frequently in mutated oncogenes and tumor suppressor genes,[23] resulting in the implication of 8-oxo-dG in carcinogenic mechanisms.[24] In addition to 8-oxo-dG, other products associated with the photooxidation of dG are 2,2-diamino-5-[2-deoxy-β-D-*erythro*-pentofuranosyl]amino-5(2H)-oxazolone, 4-hydroxy-8-oxo-4,8-dihydro-2′-deoxyguanosine and guanine.[5a,25,26] The relative yields of different dG photooxidation products depend on whether reaction is initiated by direct photoionization or by photosensitizers and whether O_2 is present.[25,26] With photosensitizers, dG oxidation proceeds via a dG photoionization mechanism or via a singlet O_2 ($^1\Delta_g$) mediated mechanism.[3,5,25,27−29] Interestingly, 5′-dGMP$^-$, itself, is a photosensitizer that produces O_2 ($^1\Delta_g$).[30] Under some reaction conditions, the same product is formed via more than one mechanism. For example, in DNA, 266 nm, two-photon laser experiments, 8-oxo-dG is formed via direct photoionization and via reaction with OH· radicals.[11] Relative yields of 2′-deoxyguanosine reactions initiated by direct photoionization and by photosensitizers also depend on structure. 8-Oxo-2′-deoxyguanosine yields from dG incorporated into DNA are larger than from dG in solution.[5a,11]

Guanine forms, with a low quantum yield (0.0002), when dry guanosine-5′-monophosphate (5′-GMP$^-$) is irradiated at 140 to 160 nm.[31] Guanine formation also occurs in dG, guanosine, and 2′-deoxyguanosine 5′-monophosphate (5′-dGMP$^-$) solutions irradiated at 254 nm.[26] and in calf thymus DNA irradiated at 193 nm.[32] Investigation of the mechanisms for G formation from nucleosides, 5′-dGMP$^-$ and DNA provides evidence that photoionization of the guanine moiety is an initiating event.[26,32] At 254 nm, the quantum yield for G formation from 75 μM guanosine was reported to decrease to a value of zero at pH 11.9[26] a surprising result in view of the fact that the photoionization quantum yields of dG, guanosine and 5′-GMP$^-$ increase with increasing pH.[16,18a]

At wavelengths longer than 193 nm, different experimental measurements of the photoionization quantum yields of guanosine and dG at neutral pH have yielded different results. For example, results from one transient-absorption, pulsed-laser experiment indicate that at pH 7.0 the 266 nm one-photon quantum yield for photoionization of 95 μM guanosine is 0.083.[15] However, in other laser experiments[16,18a] at 248 and 266 nm, no one-photon ionization of dG, guanosine or 5′-GMP$^-$ was observed near neutral pH for concentrations between 100 and 300 μM, whereas quantum yields between 0.01 and 0.07 were measured at pHs between 11.0 and 11.5. At neutral pH, support for small quantum yields, significantly less than 0.083, for photoionization between 248 and 266 nm is provided by results from a steady-state measurement using a 254 nm Hg arc lamp.[15] For guanosine, this experiment provides a one-photon quantum yield of 0.0058.

Gas-phase photoionization energies and conformations of nucleotide bases, base pairs, stacked bases, nucleosides, and mono-, di-, and oligonucleotides have been widely examined using photoelectron, mass spectrometry and theoretical methods.[7,10,33−36] Aqueous ionization energies have only been determined using complicated methods that involve the combined use of gas-phase photoelectron data, results from electronic structure calculations at the SCF and post-SCF levels, and theoretical evaluations of free energies of hydration.[6,37,38] For 5′-dGMP$^-$ at neutral pH, results from these investigations yield an aqueous photoionization threshold energy of 4.9 ± 0.5 eV.[37]

The main goal of the present investigation is to employ a tunable weak light source, with which one-photon photolysis predominates, and high performance liquid chromatography (LC) with mass spectrometric analysis to examine how changes in pH and wavelength influence the yields of 8-oxo-dG and G associated with dG photolysis. The experiments were carried out under anaerobic conditions to reduce the number of potential photolysis pathways by eliminating singlet O_2($^1\Delta_g$) photooxidation. The dependence of 8-oxo-dG and G yields on pH was compared to the dependence of the dG photoionization yield on pH determined in electron scavenging experiments. The dependence of the quantum yields for G and 8-oxo-dG formation on wavelength was compared to dG and dG$^-$ aqueous photoionization thresholds obtained using gas-phase data and the theoretical methods outlined above. These comparisons were made in order to examine the relationship between dG photoionization and mechanisms leading to the formation of 8-oxo-dG and G.

Experimental Section

Preparation of Solutions. 2′-Deoxyguanosine, G and 8-oxo-dG were obtained from Sigma (St. Louis, MO) and were at least 99% pure. Solutions were prepared in doubly distilled water provided by a Corning Mega Pure System MP-1. The concentration of all dG solutions used in photolysis experiments was 1.2×10^{-4} M as determined by UV absorption measurements with a Cary 14/OLIS spectrophotometer. Photolysis experiments at pH 6.3 were carried out with unbuffered dG solutions. For experiments at pH 11.4, 2 M NaOH was added dropwise. The solutions were deaerated on a glass vacuum line, where each solution was subjected to five freeze−pump−thaw cycles using liquid N_2.

In all experiments, N_2O (99.5% pure from AGA Gas, Hammond, IN) was equilibrated with the dG solutions at 25 °C. Prior to use, the N_2O was passed through an Oxy-trap (Alltech Associates, Deerfield, IL) and then further purified on the vacuum line by three liquid N_2 freeze−pump−thaw cycles. Purified N_2O was bubbled through the dG solutions, originally at the vapor pressure of water, 21.08 Torr,[39] until the final pressure was 740 Torr. To ensure N_2O saturation under these conditions, the solution and the gas were frozen to 77 K and then thawed to room temperature. This was repeated three times. Using previously reported data,[40] the concentration of the dissolved N_2O was estimated to be 0.02 M.

Irradiation of 2′-Deoxyguanosine Solutions. Steady-state irradiation of deaerated dG solutions containing N_2O was performed with an ILC Technology (Sunnyvale, CA) 1000 W Xe, Cermak-type lamp. The light was reflected on a concave, Al(MgF_2) coated mirror (100 mm focal length) from Oriel Instruments (Stratford, CT) and directed to a McPherson (Chelmsford, MA) Model 272, 0.2 m, f/2, grating monochromator. To obtain detectable signals, the monochromator slits were adjusted to provide a 32 nm bandwidth. The emerging light beam was directed onto the sample contained in a custom-made 8 mm × 12 mm o.d. Suprasil Quartz (Technical Glass Products, Mentor, OH) cylindrical cuvette mounted on the vacuum line. The irradiation area was 1.3×10^{-4} m^2. The power of the irradiating light on the sample was obtained from actinometry measurements[41] using 1.0 mL of potassium ferrioxalate in the sample cuvette. The power at different wavelengths is given in Table 1.

TABLE 1: Lamp Intensities in the Sample Cell

wavelength (nm)	photons/second	power (watts)
240	5.61×10^{13}	4.64×10^{-5}
254	7.85×10^{13}	6.13×10^{-5}
266	5.45×10^{14}	4.07×10^{-4}
280	1.05×10^{15}	7.45×10^{-4}

N_2 Analysis. In scavenging experiments, aqueous electrons, e^-_{aq}, formed from photoionization yielded N_2 via the reaction, $e^-_{aq} + N_2O + H_2O \rightarrow N_2 + OH^- + OH\cdot$.[19d] After irradiation, N_2 analysis of the gas above the dG solutions was carried out at room temperature with an Agilent Technologies (Palo Alto, CA) 6890 Series gas chromatograph equipped with a thermal conductivity detector. 19091P-MS4 HP−PLOT MoleSieve 5A and 19091J-413 HP-5 Cross-linked 5% PH ME siloxane columns were used.

Liquid Chromatography-Mass Spectrometry Analysis. On-line, high-performance liquid chromatography−mass spectrometry (LC-MS-MS), using positive ion electrospray ionization, was carried out with a Waters (Milford, MA) 2690 chromatograph, a Waters 2487 UV detector, and a Micromass (Manchester, UK) Quattro II triple quadrupole mass spectrometer. Nitrogen was used as a nebulizing gas with a flow rate of 0.8 L/min, and the electrospray source was maintained at 140 °C.

After irradiation, dG solutions at pH 11.4 were adjusted to pH 7.0 by adding 5% acetic acid. Solutions irradiated at pH 6.3 were analyzed without changing pH. Reverse-phase chromatographic separation of dG photolysis products was performed on a YMC (Wilmington, NC) YAQ C_{18} (2.0 × 250 mm) column. The mobile phase consisted of methanol (solvent A) and water (solvent B). The gradient varied linearly, and was 0−2 min 94−90% B, 2−14 min 90% B, 14−14.5 min 90−80% B, 14.5−23 min 80% B, 23−23.5 min 80−10% B and 23.5−26 min 10% B. The flow rate was 200 μL/min and the injection volume was 25 μL.

2′-Deoxyguanosine, which was the largest component, was detected by UV absorbance at 260 nm. Guanine was detected by mass spectrometry using selective ion recording at *m*/*z* 152.0 in the positive ion mode. Calibration curves for dG and G were obtained from standard aqueous solutions that were analyzed and detected by UV absorbance and MS, respectively. In chromatograms of samples prepared in the same manner but measured on different days the corresponding areas associated with the dG absorbance, and the G ion signal differed by less than 10%.

8-Oxo-2′-deoxyguanosine was monitored during HPLC by using tandem mass spectrometry with collision-induced dissociation (CID) and product ion scanning. The protonated molecule of *m*/*z* 284 was selected as the precursor ion and the product ion was *m*/*z* 168.[42] CID was carried out using Ar as the collision gas at a pressure 1.5×10^{-3} mTorr and a collision energy of 18 eV. 8-Oxo-2′-deoxyguanosine was quantified using an internal standard. 10.0 μL of isotopically labeled 8-oxo-dG ([$^{13}C_{10}$, $^{15}N_5$]-8-oxo-2′-deoxyguanosine, at a concentration of 70 ppb were added to 60.0 μL of each sample. The internal standard was synthesized using a previously reported procedure.[43] The equation relating the ratio between the areas of the ion signals associated with the labeled and unlabled 8-oxo-dG to the concentration of unlabeled 8-oxo-dG was determined in standardization experiments. These were carried out using solution conditions employed in the present investigations with known quantities of labeled and unlabled 8-oxo-dG. The resulting equation was the same as that reported earlier.[42]

Results

Figure 1 shows results from LC analysis of samples of 2′-deoxyguanosine irradiated at 254 nm for 40 min in 0.02 M N_2O. Results are given for experiments carried out at pH 11.4 (panel A) and 6.3 (panel B). The figure also contains results from samples that were not irradiated. At both pH values the figure only shows portions of the chromatograms where dG, guanine and 8-oxo-2′-deoxyguanosine elute. Retention times are given above each chromatographic peak, and concentrations reported in terms of mass ratios relative to dG are given in square brackets.

In panels A and B, the G, 8-oxo-dG and dG results from the irradiated solutions are given in the three chromatograms at the top. The results from the nonirradiated solutions are given in the three chromatograms on the bottom. Panel A demonstrates that at pH 11.4, irradiation at 254 nm for 40 min results in measurable yields of G and 8-oxo-dG. The G and 8-oxo-dG mass ratios relative to dG are larger than the corresponding mass ratios in the nonirradiated sample by 75 and 200 **%**, respectively. The mass ratio of 8-oxo-dG is at least 10 times smaller than that of G. The results in Panel B demonstrate that at pH 6.3 the G and 8-oxo-dG yields are much smaller than at pH 11.4. At pH 6.3, the G and 8-oxo-dG mass ratios for the irradiated sample differ from those in the nonirradiated sample by less than 1 **%.** The present results indicating that the yields of G and 8-oxo-dG at pH 11.4 are at least 5 times larger than at pH 6.3 are different from results obtained in earlier experiments[26] employing higher radiation levels and smaller dG concentrations. There it was found that the yield of G decreases with increasing pH.[44]

Figure 2 contains results from N_2O electron scavenging experiments carried out at 240 nm. The figure shows chromatograms from N_2 analysis of the gas above 1.2×10^{-4} M dG solutions containing N_2O (0.02 M) that were irradiated for 19 h. Each panel gives results from an irradiated sample and from a sample that was not irradiated. The amount of N_2 obtained in an experiment in which a sample of distilled water containing N_2O at pH 11.4 without dG was irradiated for 19 h at 240 nm was the same as that obtained from a sample that was not irradiated.

The left panel of Figure 2 shows data from N_2 analysis of a dG solution irradiated at pH 11.4. The right panel shows data from a solution irradiated at pH 6.3. The results indicate that the yield of hydrated electrons produced during photolysis of dG at pH 11.4, as indicated by the N_2 yield, is more than at pH 6.3. Furthermore, measurements of UV absorption spectra at 254 nm as a function of irradiation time indicate that the photodestruction of dG at pH 11.4 is greater than at pH 6.3, in agreement with earlier results.[15] For example, the absorbance at 254 nm decreases from 1.64 to 1.04 after 19 h of irradiation at pH 6.3, whereas at pH 11.4 the decrease is from 1.28 to 0.36**.** The increase in the 240 nm one-photon ionization quantum yield of dG that occurs with increasing pH, indicated by the results in Figure 2, is similar to that reported earlier from solvated electron transient absorption measurements obtained in 266 and 248 nm pulsed laser experiments on dG and other guanine derivatives.[16,18]

Figure 3 shows the mass concentration (ppm) of 8-oxo-dG and G produced when 1.2×10^{-4} dG solutions at pH 11.4 were irradiated at 254 nm for times between 0 and 18 h. The figure also shows how the dG mass concentration changes with time. After 9 h, the concentration of dG has decreased 19-fold. The maximum 8-oxo-dG concentration, which is more than 45× smaller than the maximum G concentration, occurs after 1 h of

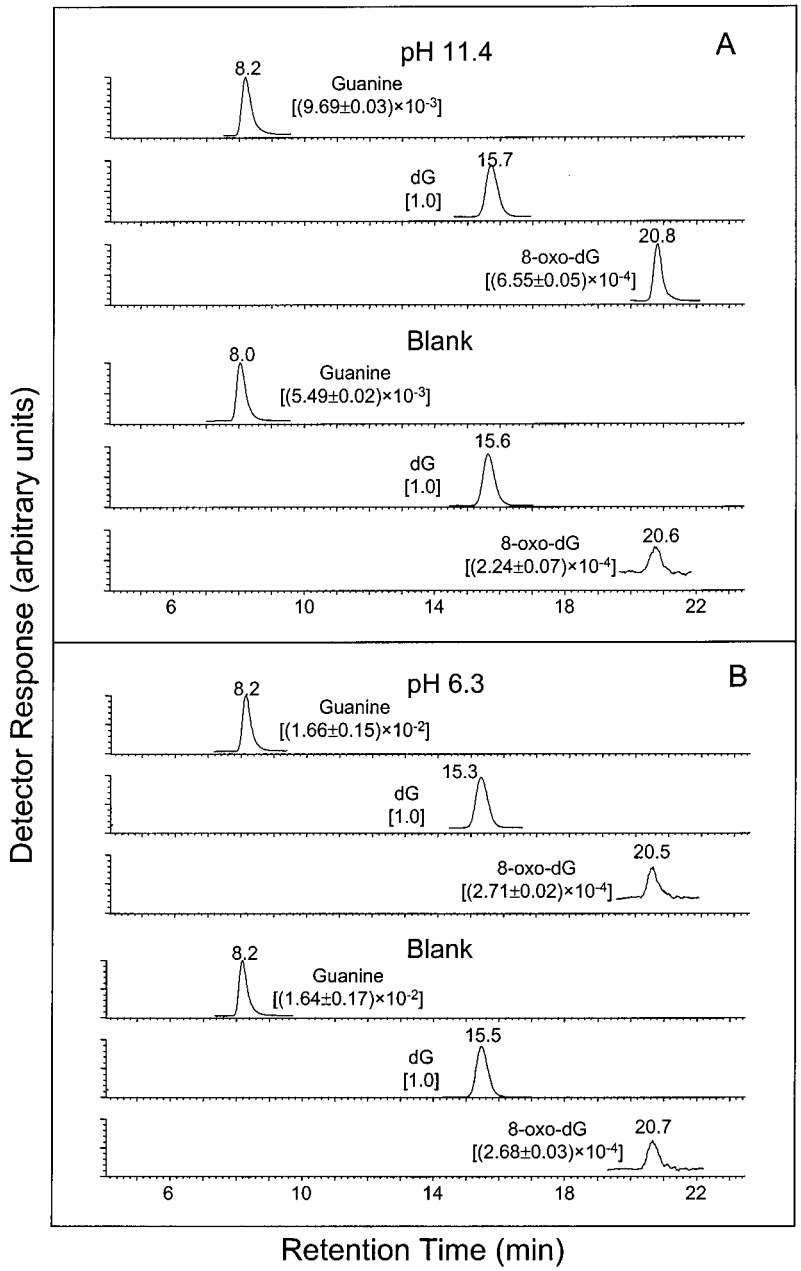


Figure 1. Chromatograms showing results from guanine (G), 2′-deoxyguanosine (dG) and 8-oxo-2′-deoxyguanosine (8-oxo-dG) analysis of deaerated samples of 1.2×10^{-4} M dG irradiated at 254 nm for 40 min in 0.02 M N_2O. Analytes were detected by UV absorbance, MS or MS/MS. See text. Panel A shows results from a dG sample irradiated at pH 11.4. Panel B show results at pH 6.3. Each of the panels show three chromatograms (above) obtained from an irradiated sample and three chromatograms (below) obtained from a blank, nonirradiated sample. The figure shows retention times, given above the chromatographic peaks, and mass ratios relative to dG, given in square brackets. Uncertainties in the mass ratios represent the standard deviations of results from nine analyses obtained from three irradiation experiments.

irradiation. In contrast, the maximum G concentration occurs after 6 h, indicating that, at pH 11.4, 8-oxo-dG undergoes 254 nm photodamage more rapidly than G. The inset in Figure 3 shows an N_2 chromatogram obtained after 254 nm irradiation for 9 h in an N_2O electron scavenging experiment carried out with 1.2×10^{-4} M dG. The results in the inset indicate that, over the time period between 0 and 9 h, when the dG concentration is highest, significant photoionization occurs.

Figure 4 shows quantum yields for formation of guanine and 8-oxo-2′-deoxyguanosine from irradiated samples of 1.2×10^{-4} M dG measured at different wavelengths. The ordinates on the left and right give quantum yields for formation of G and 8-oxo-dG, respectively. The upper and lower panels show results obtained at pH 11.4 and 6.3. At pH 11.4, data were obtained from samples irradiated for 40 min at wavelengths of 280, 266, and 254 nm.

As Figure 1 indicates, significant levels of G and 8-oxo-dG were not observed after 40 min of irradiation at pH 6.3. However, G and 8-oxo-dG were observed in solutions irradiated for longer times. The lower panel of Figure 4 shows data obtained at pH 6.3 from samples irradiated for 9 h at the same wavelengths as in the upper panel, and at 240 nm. For the different irradiation wavelengths used, the levels of dG depletion that occurred over 40 min in the pH 11.4 experiments, and over 9 h in the pH 6.3 experiments resulted in absorbance changes at the radiation wavelengths of less than 9 and 26%, respectively.[45] In Figure 4, the quantum yields reported at pH 6.3 are the average from the analysis of six samples obtained in two

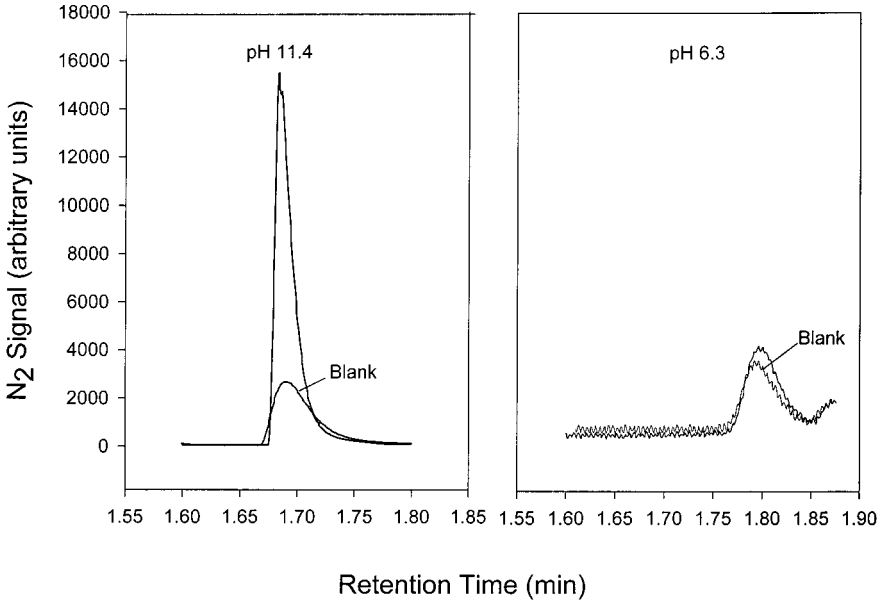


Figure 2. N_2 gas chromatograms measured after 240 nm irradiation of 2′-deoxyguanosine for 19 h in 0.02 M N_2O at pH 11.4 (left panel) and at pH 6.3 (right panel). The left and right panels also contain blank N_2 chromatograms obtained from samples containing 0.02 M N_2O without dG, irradiated for 19 h at pH 11.4 and 6.3, respectively. The small difference in the N_2 retention times in the two panels is due to a difference in the flow pressure of the He carrier gas.

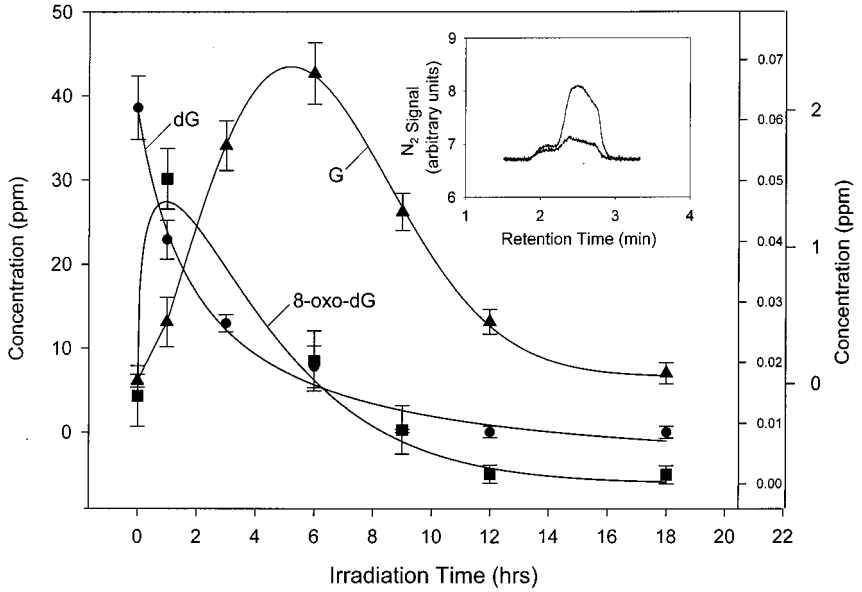


Figure 3. Concentrations of 2′-deoxyguanosine (●), 8-oxo-2′-deoxyguanosine (■) and guanine (▲) obtained after 254 nm irradiation of 1.2×10^{-4} M 2′-deoxyguanosine at pH 11.4 for varying times. The dG, G and 8-oxo-dG concentration scales are given on the left ordinate, on the right ordinate, and on the right ordinate inside the border, respectively. Concentrations of G and 8-oxo-dG associated in blank nonirradiated samples were subtracted from concentrations in irradiated samples. The inset shows an N_2 gas chromatogram measured after 9 h of 254 nm irradiation of 1.2×10^{-4} M dG in 0.02 M N_2O at pH 11.4. The inset also shows a chromatogram of a blank containing 0.02 M N_2O at pH 11.4 measured after 9 h of 254 nm irradiation. A similar blank chromatogram was obtained from a nonirradiated sample of dG in 0.02 M N_2O at pH 11.4.

irradiation experiments. Those reported at pH 11.4 are the average from nine samples obtained in three irradiation experiments. The error bars represent the standard deviations.

The inset in the upper panel of Figure 4 indicates how the absorbance (A) of dG before irradiation at pH 11.4 and the lamp intensity (B) change with wavelength. The quantum yields were obtained by dividing concentrations of 8-oxo-dG and G measured by the number of photons absorbed,[46] and have not been corrected for the dG or photoproduct damage that occurs during the course of an experiment.[45] The 8-oxo-dG and G product concentrations were determined by subtracting background mass concentrations measured in nonirradiated samples from mass concentrations measured in irradiated solutions.

Consideration was given to the possibility that the 8-oxo-dG and G formed in the wavelength region examined in these experiments was associated with the low monochomator resolution employed. For example, the low resolution might give rise to low-intensity, low-wavelength (<200 nm) photons that occur in the tail of the light distributions obtained with the monochomator settings employed in Figure 4. It might then be that these high-energy photons are responsible for the products observed. However, the observation that the total 8-oxo-dG signal decreases as the monochomator settings are reduced from 254 nm indicates that this is not the case for 8-oxo-dG production. At pH 6.3, the 8-oxo-d*G*/d*G* mass ratios after 9 h irradiation with 254 and 240 nm photons were 2.46×10^{-10}

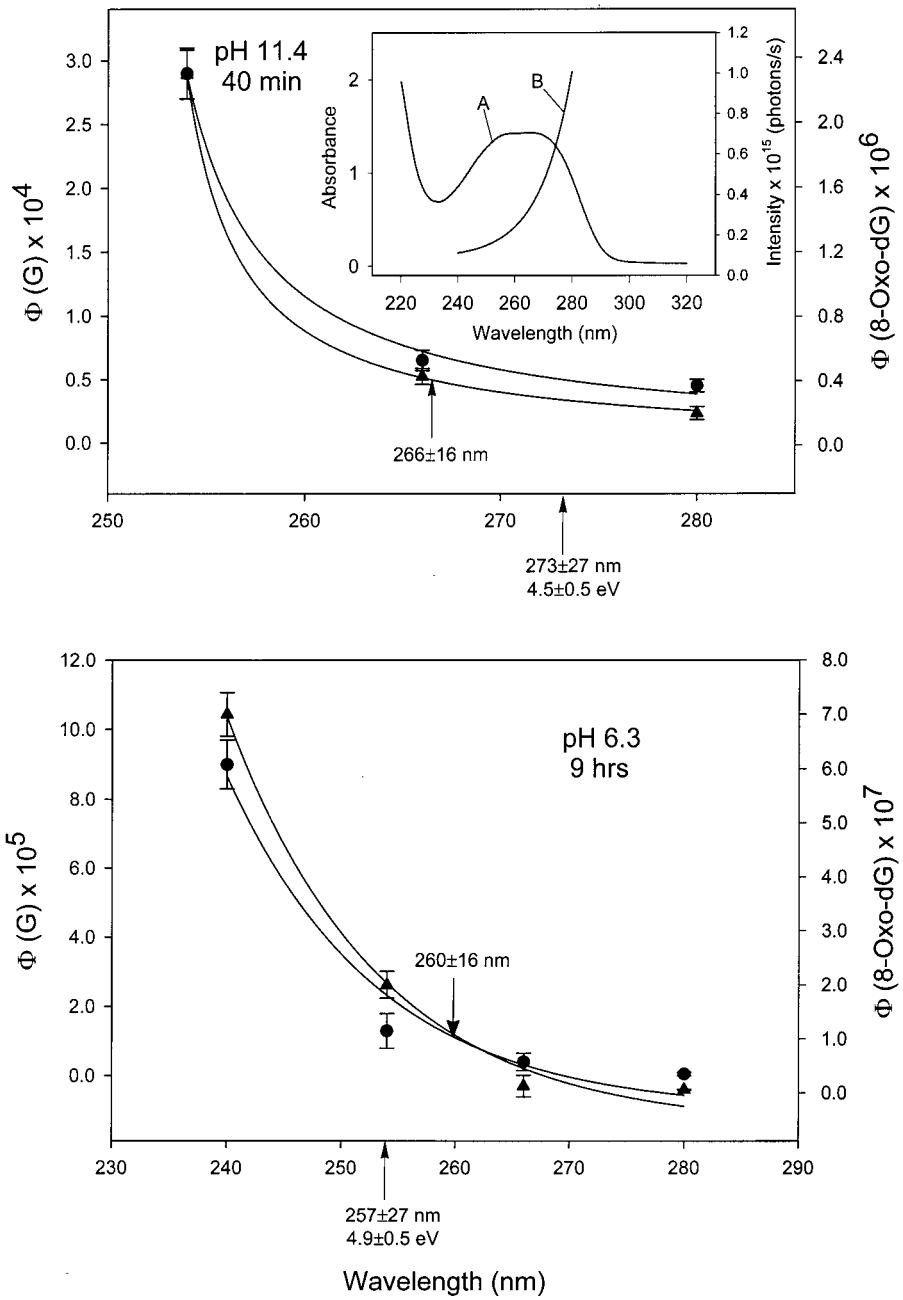


Figure 4. Average quantum yields of 8-oxo-2′-deoxyguanosine (●) and guanine (▲) formed from 1.2×10^{-4} M 2′-deoxyguanosine measured as a function of wavelength. The upper and lower panels show results obtained at pH 11.4 and 6.3, where the radiation times were 40 min and 9 h, respectively. Ordinates on the left give guanine quantum yields; ordinates on the right give 8-oxo-2′-deoxyguanosine quantum yields**.** The inset in the upper panel shows the absorption spectrum of dG measured at pH 11.4 (A) and the lamp intensity (B) as a function of wavelength. Arrows within the borders of each panel give estimates of experimental photoionization threshold energies of anionic and neutral dG obtained from the data. Arrows below the upper and lower panels give ionization energies of anionic and neutral dG obtained by employing a combination of gas-phase photoelectron data, and theoretically calculated gas-phase ionization potentials and aqueous solvation energies. See text and refs 37 and 38b.

and 1.66×10^{-10}, respectively. In Figure 4, the increase in the 8-oxo-dG quantum yield with decreasing wavelength is due to the decrease in light intensity that occurs as the wavelength decreases. See curve B of the inset. The results from N_2O electron scavenging experiments carried out at pH 11.4 also indicate that photoionization is not occurring exclusively from high-energy photons in the tail of the light distribution. Here, the amount of N_2 formed decreased more than 3-fold when the wavelength was reduced from 254 to 225 nm.

The results in both panels of Figure 4 show that at the longer wavelengths the quantum yields of 8-oxo-dG and G are near zero and constant. However, at both pH 11.4 and 6.3, the quantum yields increase significantly at wavelengths below 266 nm. These increases in the quantum yields provide evidence of the existence of thresholds for photochemical production of 8-oxo-dG and G at wavelengths in the region 280−250 nm.

Small quantum yields were measured at 280 nm for 8-oxo-dG and G formation at pH 11.4 and 6.3. However, these are negligible on the scale at which Figure 4 is drawn, and their occurrence may be associated with the large band-pass of the monochromator (±16 nm) that is needed to obtain measurable signals. The 254 nm quantum yield for G formation at pH 6.3, reported in Figure 4 ($6.02 \pm 0.08 \times 10^{-6}$), is similar to the 254 nm quantum yield (3×10^{-6}) for guanine release from double-stranded calf-thymus DNA in aerated solution at pH 7.[4] However, this value is smaller than expected, based on the

quantum yield ($5.05 \pm 0.09 \times 10^{-4}$) reported earlier from 1.05×10^{-4} M dG at neutral pH.[16] Although the source of the discrepancy between the present and earlier results is uncertain, it may be related to the different irradiation procedures employed. Mononchomator selected radiation with a wide bandwidth, and a lamp, which has sharply decreasing intensity between 270 and 240 nm were used in the present experiments. The 254 nm line from an unfiltered low-pressure Hg lamp was used in the earlier experiments. [26, 15]

The long radiation times required to obtain the results in Figure 4, especially at pH 6.3, and the low levels of G and 8-oxo-dG observed, which are near the detection limit, made it impossible to carry out experiments at a sufficient number of wavelengths to determine the thresholds with high precision. Within this limitation, the results provide similar estimated thresholds for G and 8-oxo-dG with values of 266 ± 16, and 260 ± 16 nm at pH 11.4 and 6.3, respectively. These estimates are indicated by arrows within the borders of the upper and lower panels of the figure.

Discussion

Figure 1 shows that guanine and 8-oxo-dG are easily observed products formed at pH 11.4 when dG (1.2×10^{-4} M) is irradiated for 40 min at 254 nm. At pH 6.3, the 8-oxo-dG and G occurring after 40 min of irradiation are near background levels. While 8-oxo-dG and G, formed at pH 6.3, are observed at longer radiation times (9 h, Figure 4), the results in Figure 1 indicate that their quantum yields are small near physiological pH, but increase significantly with increasing pH.

The low sensitivity of N_2O electron scavenging experiments does not permit the detection of photoionization products at the short radiation time (40 min) used to obtain the results in Figure 1; however, the results in Figure 2, obtained after 19 h of radiation, exhibit pH dependence similar to the 8-oxo-dG and G yields. These results demonstrate that the 240 nm photoionization yield at pH 6.3 is more than 4 times smaller than that at pH 11.4. The results in the inset in Figure 3 obtained at 254 nm also demonstrate the occurrence of one-photon ionization at high pH. When the 254 nm experiment in the inset in Figure 3 was carried out at pH 6.3 the result was the same at that in Figure 1. Nitrogen formation was negligible. Consideration of results from laser experiments at 248 and 266 nm with dG[16] and guanosine[18a] at concentrations between 1.2 and 1.4×10^{-4} M and at pH 11.0 to 11.5 indicates that, at pH 11.4, the 254 nm photoionization quantum yield of dG is in the range 0.01 to 0.02. On the basis of this observation, the present electron scavenging results indicate that, at pH 6.3, the 254 nm quantum yield for dG photoionization is smaller than 0.003.

The increase in the dG photoionization quantum yield with increasing pH correlates with the decrease in the dG rate constant for electron scavenging. At natural pH, the dG scavenging rate constant ($k = 6.0 \times 10^9$ M^{-1} s^{-1}) is similar to the N_2O rate constant ($k = 8.7 \times 10^9$ M^{-1} s^{-1}).[47] If dG behaves like G, the rate constant at pH 11.4 is significantly smaller. At pH 7.0 and 11.0 the rate constants for G electron scavenging are 1.2×10^{10} and 2×10^9 M^{-1} s^{-1}, respectively.[47] The large guanosine and dG electron scavenging rate constants at natural pH may account, in part, for the large difference in photoionization quantum yields reported from different 266 nm laser experiments[15,16] that were carried out at different concentrations (0.75 versus 1.2×10^{-4} M). Because of guanosine and dG electron scavenging, the apparent quantum yields are expected to decrease with increasing concentration both in transient absorption experiments and in N_2O electron scavenging experiments.

It is important to acknowledge that over the 19 h irradiation time required to obtain the electron scavenging results in Figure 2, or the 9 h used to obtain the data in the inset of Figure 3, photoionization can occur not only from dG but from dG photoproducts such as G and 8-oxo-dG. Nevertheless, the results in Figures 1 and 2, as well as those from pulsed-laser experiments,[16,18] support the conclusion that the quantum yield for photoionization of dG, as well as the quantum yields for G and 8-oxo-dG production increase with increasing pH.

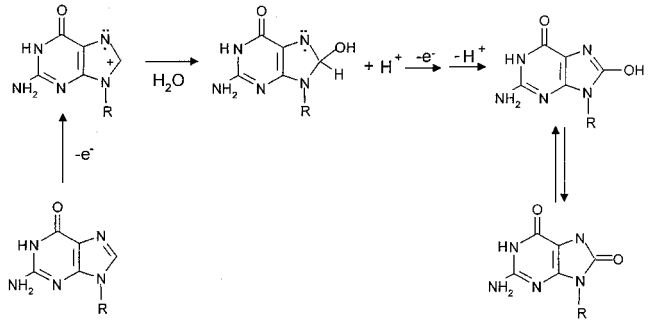
Detailed photochemical mechanisms for the formation of 8-oxo-dG and G from dG and guanosine are not yet available. For G release from guanosine[26] and from calf thymus DNA,[32] it is proposed that the principal mechanisms are initiated by photoionization. Results from investigations of G formation from guanosine demonstrate that the relative importance of different mechanisms initiated by photoionization changes at different pH's. For guanosine at neutral pH without N_2O, the finding that the addition of electron scavengers reduces the yield of G supports the conclusion that the mechanism is initiated by capture of an electron by neutral guanosine[26]

$$\text{Guanosine} + h\nu \rightarrow \text{Guanosine}^{+\cdot} + e^-_{aq}$$

$$\text{Guanosine} + e^-_{aq} \rightarrow \text{Guanosine}^{-\cdot} \rightarrow\rightarrow \text{G}$$

With N_2O, the proposed mechanism for G formation from guanosine and DNA involves reaction of ground-state, closed-shell guanosine or DNA with OH· radicals formed via N_2O scavenging of guanosine or DNA photoelectrons.[26,32]

Reactions of hydroxyl radicals formed via N_2O scavenging of dG photoelectrons may also account for the formation of 8-oxo-dG.[25,48] Alternatively, 8-oxo-dG may be produced via the mechanism shown below that occurs through hydration of the radical cation formed from direct photoionization of dG.[11,12] Although there is consensus that in DNA photolysis the formation of 8-oxo-dG proceeds in this way,[5,11,12,27,28,49] there are different views about the significance of this mechanism in the photolysis of free dG in solution.[11−13,27,50,51] Assessment of the mechanism in free dG is made difficult by the small 8-oxo-dG yield compared to that in native DNA[11]



Doubt about the involvement of the dG radical cation in the formation of 8-oxo-dG from free dG is provided by guanosine pulsed radiolysis experiments employing a high energy γ source and analysis involving LC with electrochemical detection.[50] In pulsed radiolysis of 1 mM guanosine, where the guanosine radical cation was formed by employing the dibromide radical or thallium (II), no evidence of the hydration reaction was observed. An upper limit of the rate constant for hydration of the radical cation or of the deprotonated cation was estimated to be 0.1 s^{-1}.[50]

Differences between reports about the importance of the dG radical cation for 8-oxo-dG formation have been attributed, in

part, to different wavelengths used in different UV photolysis experiments, to different photooxidation conditions in UV photolysis versus pulsed radiolysis experiments, and to the ease with which 8-oxo-dG undergoes secondary oxidation.[12] Support for an important role of the radical cation in formation of 8-oxo-dG is provided by the observation that, in two-photon 248 nm laser experiments carried out at 77 K in $NaClO_4$ glasses, 8-oxo-dG production from DNA and dG exhibit similar dose dependence.[12] On the other hand, two-photon 266 nm experiments at room temperature indicate that the 8-oxo-dG yield from native DNA in solution is approximately 100 times larger than that from free dG.[11] Nevertheless, in the two-photon 266 nm solution experiments, 8-oxo-dG formation from free dG persists in the presence of an OH· scavenger, supporting the conclusion that approximately 25% of the total 8-oxo-dG yield from free dG is formed via hydration of the radical cation.[11]

Uncertainty in the detailed mechanisms associated with G and 8-oxo-dG formation from dG in the present experiments adds uncertainty to interpretation of the results in Figure 4. Nevertheless, the similar pH dependence of the quantum yields for photoionization, and for G and 8-oxo-dG formation (Figures 1 and 2), and the strong implication, from earlier work, that photoionization initiates G and 8-oxo-dG formation provide evidence that the threshold behavior exhibited in Figure 4 represents the threshold of the dG photoionization quantum yield. This reasoning leads to the conclusion that, under the present experimental conditions, G and 8-oxo-dG formation are photoionization markers even when, as in the case of measurements at pH 6.3, the low sensitivity of N_2O scavenging prevents the direct detection of electrons.

In Figure 4, the experimental thresholds for 8-oxo-dG and G formation at pH 11.4 (4.7 ± 0.3 eV) and pH 6.3 (4.8 ± 0.3 eV) are similar to aqueous photoionization threshold energies of anionic dG^- at pH 11.4 (4.5 ± 0.5 eV) and of neutral dG at pH 6.3 (4.9 ± 0.5 eV) obtained via a previously described combination of gas-phase data and theoretical methods.[37,38b] The theoretical thresholds are given by the arrows below the horizontal axes in Figure 4. The finding that the theoretical dG^- and dG ionization thresholds are within the range of uncertainty associated with the experimental thresholds for 8-oxo-dG and G formation provides further evidence that, under the experimental conditions employed here, 8-oxo-dG and G are markers of dG photoionization.

If the 8-oxo-dG and G results in Figure 4 represent thresholds for dG photoionization, the results lead to an interesting conclusion about the energetics of guanine photoionization in native double-stranded DNA. The data in Figure 4 indicate that the photoionization threshold of free dG occurs at approximately 260 nm at pH 6.3, and that photoionization of free dG is not an environmentally significant event induced by solar radiation because the solar spectrum at ground level has no contribution from wavelengths shorter than 290 nm.[2] However, in native DNA the situation may be different. Here, recent computational investigations[9,10,33] indicate that hydrogen-bonding and base-stacking interactions, which occur in sequences containing multiple repeating guanines (G runs), have threshold ionization energies that are 0.5 to 0.7 eV smaller than threshold energies of free guanine. Applying this lowering of the ionization energy to the present data for free dG, leads to a threshold wavelength of 290 nm or longer for guanine photoionization in G runs, within the UV−B range occurring in the solar spectrum. The present data surprisingly indicate that low quantum-yield DNA photoionization may be a ubiquitous environmentally induced event that will become more important if or when ground-level solar UV intensities increase.

Conclusions

The main conclusions of this investigation are as follows:

1. The 254 nm irradiation of deaerated 2′-deoxyguanosine solutions containing N_2O at pH 11.4 gives rise to easily monitored photoionization, as well as to the formation of guanine and 8-oxo-2′-deoxyguanosine, all of which occur via one-photon processes. 8-Oxo-2′-deoxyguanosine is a minor photoproduct which has a yield that is at least 10× smaller than that of G.

2. The yields of formation of 8-oxo-dG and G, as well as the quantum yield for dG photoionization increase with increasing pH. At pH 11.4, yields of G and 8-oxo-dG and the quantum yield for photoionization are 4 or more times larger than at pH 6.3.

3. The quantum yields for G and 8-oxo-dG exhibit threshold behavior with estimated thresholds of 266 ± 16 and 260 ± 16 nm at pH 11.4 and 6.3, respectively. These threshold values are similar to ionization energies of dG^- and dG estimated from gas-phase photoelectron data and theoretical calculations.

4. The similarity between the pH dependence of 8-oxo-dG, G and photoelectron yields, as well as the observance of thresholds for 8-oxo-dG and G, near those expected for dG photoionization support the conclusion that, under the present experimental conditions, 8-oxo-dG and G formation is initiated by dG photoionization. A consideration of the lowering of the guanine ionization energy by approximately 0.5 eV that occurs in G runs in DNA indicates that in physiological environments, low quantum-yield ionization of guanine occurs at wavelengths near the UV cutoff of the ground-level solar spectrum.

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(44) Differences between results presented here and in refs 15 and 26 may be related to the lower fluence used in the present experiments. Here, the longer path length and the monochromator give rise to a 254 nm intensity of 7.85×10^{13} photons/second. In a 40 min experiment, the fluence is 1132 J m^{-2}. The experiments of refs 15 and 26 employed a low-pressure Hg lamp located 1 cm from the sample. Evidence of the higher fluence employed in the earlier experiments is indicated by an examination of the dG absorption spectra of samples measured before and after irradiation. In results in Figure 1 of ref 15 obtained from experiments carried out at natural pH, the UV absorption spectrum of dG exhibits more change after 28 min of 254-nm irradiation than after 9 h of irradiation in the present experiments. Furthermore, examination of the pH dependence in ref 26 was carried out at a smaller dG concentration (7.5×10^{-5} M) than the present experiments (1.2×10^{-4} M), and in an N_2 saturated solution without N_2O.
(45) For experiments at pH 11.4, the initial absorbances at 280, 266 and 254 nm were 0.94, 1.43 and 1.38. The final absorbances were 0.94, 1.31 and 1.34, respectively. For experiments at pH 6.3, the initial absorbances at 280, 266, 254 and 240 nm were 1.03, 1.27, 1.64, and 1.18. The final absorbances were 1.02, 1.26, 1.22 and 0.91.
(46) The number of absorbed photons was approximately evaluated by employing the absorbance at the irradiation wavelength of the dG sample at the beginning of an experiment, and the lamp intensity obtained from actinometry measurements.
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