INTERMEDIATES AND QUANTUM YIELDS IN THE PHOTOLYSIS OF GUANINE AND ITS DERIVATIVES IN NEUTRAL GLASSES AT 77 K

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Summary

A comparative study on the photochemical properties of guanine (Gua), guanosine (Guo) and guanosine monophosphate (GMP), based on the detection and characterization of the reactive intermediates and on the determination of the photodestruction and intersystem crossing yields, has been undertaken. UV irradiation of Gua, Guo or GMP in neutral aqueous glasses at 77 K produces intermediates such as trapped electrons, base radical cations and anions, hydrogen addition radicals and solvent radicals. These have been studied and characterized by electron paramagnetic resonance and UV–visible spectroscopy. Photodestruction yields are similar for the three Gua derivatives within experimental error while photoionization decreases in the order Guo (0.0009) = GMP (0.0009) > Gua (0.0008). Photoionization occurs through a biphotonic mechanism involving the triplet state, for which yields of 0.15, 0.31 and 0.29 have been determined for Gua, Guo and GMP.

1. Introduction

The damaging action of UV radiation on the nucleic acids has been largely accounted for by damage to the pyrimidine bases. Guanine (Gua) and its derivatives absorb more strongly than other deoxyribonucleic acid (DNA) components at wavelengths above 295 nm. The importance of purine bases and in particular Gua and its derivatives in the photochemistry of nucleic acids has been suggested in several studies.

Morgan and Callis [1, 2] studied the photochemistry of Gua at low temperature in ethylene glycol–water (6:4 by volume) mixtures. For guanosine monophosphate (GMP), an increase in the irradiation time produces a decrease in its fluorescence, while a new band increases with a fluorescence maximum at 418 nm. Photodestruction quantum yields of 0.02 and 0.04 were found for excitation at 248 nm and 290 nm respectively. Only one fluorescent photoproduct was observed. This photoproduct showed an absorption maximum at 305 nm and was stable at low temperatures (140 K), reverting to the parent compound when the temperature was raised to 298 K. For all the Gua-containing dinucleosides the same photoprocess was

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observed but with different yields, guanylyl-(3′-5′)-adenosine (GpA) and 2′-deoxyguanylyl-(3′-5′)-thymidine) (dpGpT) having the higher values. The presence of the Gua triplet state was suggested as a precursor for photoproduct formation.

UV radiation damage to bases and nucleotides has been studied using inelastic electron tunnelling spectroscopy to monitor direct bond damage [3]. The damage rate on UV irradiation at 77 K follows the order UMP > TMP > CMP > GMP > AMP (UMP, uridine-5′-phosphate; TMP, thymidine-5′-phosphate; CMP, cytidine-5′-phosphate).

Irradiation of frozen aqueous solutions of guanosine (Guo) at 77 K ionizes the solute through a biphotonic process involving the purine base triplet state [4]. No quantitative studies of the trapped intermediates were reported.

Golikov *et al.* [5] and Heléne *et al.* [4] found that, in alcoholic solutions at 77 K, UV irradiation of purine bases photosensitized the formation of alcohol free radicals. Gua, Guo and deoxyguanosine (dGuo) were found to be the most effective sensitizers among the purine bases and were about 100 times more effective than pyrimidine bases [5]. A biphotonic mechanism was proposed for the process [5].

Graslund *et al.* [6] studied the UV production of radicals in oriented DNA samples containing 30 wt.% water at 77 K using electron paramagnetic resonance (EPR). At low UV doses the induced free radicals are thymine radical anions and Gua radical cations.

Radical cations of nucleotides, dinucleoside phosphates and DNA have been produced by photoionization of these in 8 M $NaClO_4$ glasses at 77 K [7]. In both neutral and basic glasses, mixed dinucleoside phosphates containing Gua showed the preferred formation of the Gua radical cation. A similar result was found for DNA, where the main contribution to the EPR signal was due to the Gua radical cation.

In this paper we present results on the identification of reactive intermediates by UV–visible and EPR spectroscopy and quantum yields for these intermediates, and we compare the photoreactivity of Gua, Guo and GMP in neutral aqueous glasses on the basis of the yields of the reactive intermediates in an effort to obtain a better understanding of the mechanism of photochemical nucleic acid damage.

2. Experimental details

2.1. Chemicals and sample preparation

Gua (Aldrich; purity, 99%), Guo (Sigma; purity, Sigma grade), GMP (Sigma; purity, Sigma grade), LiCl (Fischer; certified reagent), galvinoxyl and 2,2-diphenyl-1-picrylhydroxyl (DPPH) free radicals (Aldrich) were used as received. Ethylene glycol (Matheson; Chromato quality) was fractionally distilled. Aqueous glasses were formed by adding a high concentration of the inorganic salts or ethylene glycol to triply distilled water and cooling to 77 K.

Because of the low solubility of Gua in neutral solvents, solutions were prepared by adding 0.003 g of Gua to the appropriate solvent and stirring overnight to obtain a saturated solution of concentration 6.6×10^{-4} M, as determined from absorbance measurements and the known molar extinction coefficient. Solutions of Guo and GMP of concentrations between 2.5×10^{-4} and 3.2×10^{-2} M were prepared using standard weighing and volumetric techniques. Samples were degassed and vacuum sealed following procedures described previously [8 - 10].

2.2. Irradiation conditions, actinometry and quantum yield determinations

The solutions used had absorbance values between 0.7 and 0.9 at the maximum absorption wavelength. The irradiation procedures and conditions as well as the methods followed to determine the incident light intensity, radical concentrations from EPR data and quantum yield calculations have been described previously [8 - 10].

3. Results and discussion

3.1. Intermediates in the photodestruction mechanism

UV irradiation of frozen 12 M LiCl Gua solutions produces a blue coloration of the samples, characteristic of trapped electrons [11]. The observed EPR signal for irradiated samples (Fig. 1) consists of an unresolved singlet with two small bands at one side and a doublet 500 G apart. The small bands in the central region of the EPR spectrum with $g = 2.0043$ are very similar to the low field spectrum of Cl_2^- ions previously observed in

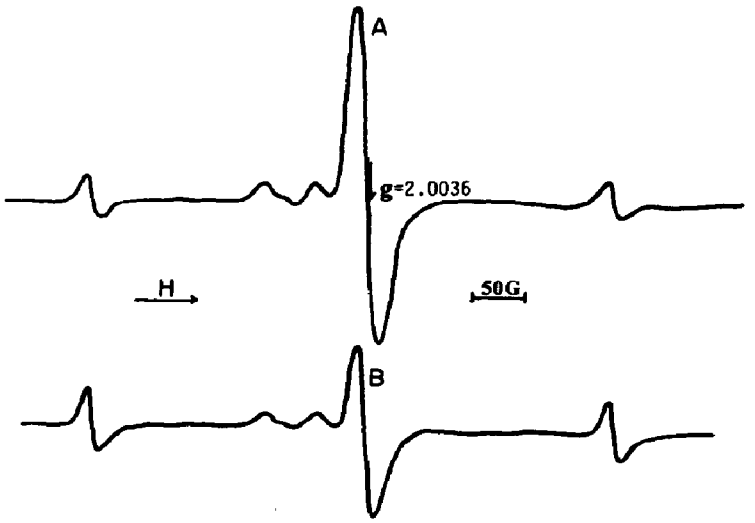

Fig. 1. EPR spectrum of the species produced during 254 nm irradiation of a saturated Gua solution in a 12 M LiCl glass (gain, 1.5×10^5; microwave power, 1 mW; modulation amplitude, 10 G): spectrum A, after 60 s UV irradiation; spectrum B, after 60 s UV irradiation followed by 5 min bleaching with visible light.

this laboratory in the photolysis of adenine [10] or purine free base [9] in 12 M LiCl and by Mohan and Kaalhus [12] on the X-ray irradiation of 9 M HCl glasses. The doublet with the 500 G separation has been assigned to trapped hydrogen atoms produced from the reaction of the photoejected electrons, resulting from the photoionization of the purine base, with hydronium ions present in the matrix. Thus, the central part of the EPR spectrum for UV-irradiated Gua samples in 12 M LiCl glasses has been assigned to the overlap of the EPR spectra of trapped electrons, Gua radical cations and anions and Cl_2^- ions, while the outer doublet has been assigned to trapped hydrogen atoms. Evidence for the presence of Gua radical cations and anions is presented later.

The UV–visible absorption spectrum of an irradiated sample shows a decrease in the intensity of the Gua absorption band (Fig. 2), indicating destruction of the base. A new absorption band appears in the 300 - 390 nm region with maximum absorption at 348 nm. No isosbestic points are present on the spectra. This band is characteristic of the Cl_2^- radical ion [9, 10, 13] and confirms the assignment of the shoulders in the central part of the EPR spectrum to that species. Formation of absorption bands in this wavelength region owing to photoproducts was not observed. However, their existence cannot be ruled out, as their absorption band could be hidden under the strong Cl_2^- absorption. A photoproduct with maximum absorption at 305 nm was reported by Morgan and Callis [1] in the photolysis of Gua in ethylene glycol–water mixtures at low temperatures. Throughout this work irradiation of Gua, Guo or GMP in 12 M LiCl at 77 K did not show evidence of formation of a photoproduct with a 305 nm absorption maximum. The difference between these two low temperature UV irradiation

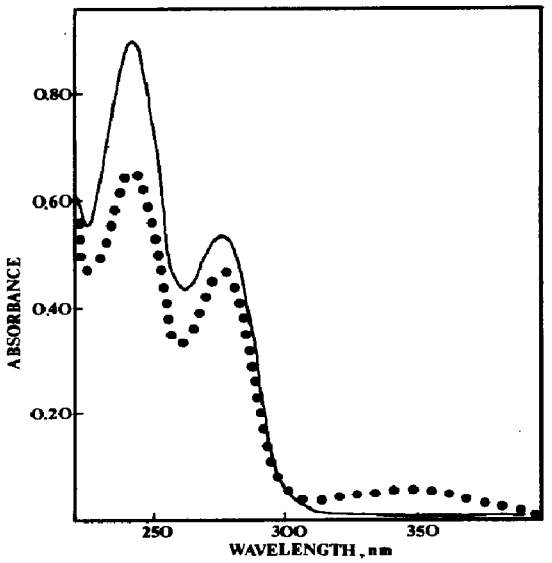

Fig. 2. Optical absorption spectra of a 3×10^{-4} M Gua solution in a 12 M LiCl glass (optical path length, 0.3 cm): ——, prior to irradiation; ● ● ●, irradiation for 60 s with 254 nm radiation.

studies on Gua is the presence of ethylene glycol; thus we postulate that the band observed by Morgan and Callis [1] results from an intermediate species in the formation of a Gua–alcohol adduct [14]. In the visible region, a broad absorption band appears with its maximum at 580 nm, characteristic of trapped electrons [11].

Irradiation of already UV-photolyzed samples with visible light for 5 min produces a decrease of 76% in the intensity of the central EPR peak, while the two small components at low field due to Cl_2^- ions remain constant (Fig. 1). The signal assigned to trapped hydrogen atoms increases in size. Simultaneously, the following changes are observed in the UV–visible absorption spectrum: the disappearance of the blue color of the sample and the corresponding absorption band with a maximum at 580 nm assigned to the trapped electron, an 80% regeneration of the Gua absorption band and no change in the band assigned to Cl_2^- ions. The latter indicates that if the Gua radical cation band is hidden under the strong Cl_2^- absorption it has a very low intensity. All these observations can be explained in terms of detrapping of electrons which can then recombine with the geminate radical cations to regenerate the parent molecule or react with protons in the matrix to yield hydrogen atoms.

Warming the sample to room temperature regenerates the Gua absorption band and causes the disappearance of the Cl_2^- band.

Similar photochemical behavior was observed for Guo and GMP in terms of paramagnetic species produced. In both cases, the presence of Cl_2^- ions, trapped hydrogen atoms and electrons and base radicals was determined from their EPR spectra. On irradiation with visible light, the wide central singlet decreases in intensity (82% for Guo and 76% for GMP), while the Cl_2^- signal remains constant and the outside doublet due to trapped hydrogen atoms increases to about twice their original intensity. The EPR parameters for the species produced by UV irradiation and after irradiation with visible light are presented in Table 1.

However, optical studies showed different photochemical behavior for Guo. For this Gua nucleoside, when previously UV-photolyzed samples are irradiated with visible light an increase in absorbance in the 300 - 370 nm wavelength region is produced (Fig. 3). This increase is not due to an increase in the Cl_2^- concentration, as the corresponding EPR signal intensity does not change after bleaching, nor is it due to production of another paramagnetic intermediate since new peaks were not observed. Therefore, it is proposed that this increase in absorbance results from the absorption of an intermediate product resulting from either the reaction of a detrapped electron with a primary paramagnetic product to yield a diamagnetic species

$$e^- + Y\cdot \longrightarrow Y \qquad (1)$$

or from a reaction of a detrapped electron with a neutral Guo molecule to yield a radical anion which undergoes a reaction to produce a diamagnetic product absorbing in the UV region

$$e^- + Guo \longrightarrow Guo^{\cdot -} \longrightarrow D \qquad (2)$$

TABLE 1

Electron paramagnetic resonance spectral parameters for radical species produced in UV-irradiated 12 M LiCl glasses containing guanine, guanosine or guanosine monophosphate

Compound	*EPR spectral parameters*	
	After 60 s UV irradiation	*After 300 s bleaching*
Gua[a]	g = 2.0035 LW = 16 G	g = 2.0045 LW = 15 G
Guo[a]	g = 2.0035 LW = 17 G	g = 2.0049 LW = 15 G
GMP[a]	g = 2.0030 LW = 17 G	g = 2.0049 LW = 15 G
Gua[b]	g = 2.0038 LW = 15 G	g = 2.0041 LW = 16 G
Guo[b]	g = 2.0039 LW = 15 G	g = 2.0041 LW = 16 G
GMP[b]	g = 2.0042 LW = 16 G	g = 2.0040 LW = 16 G
Gua[c]	g = 2.0032 LW = 15 G	g = 2.0050 LW = 13 G
Guo[d]	g = 2.0031 LW = 11 G	g = 2.0054 LW = 15 G
GMP[d]	g = 2.0035 LW = 17 G	g = 2.0047 LW = 15 G

LW, linewidth.
[a]At a concentration of 2.5×10^{-4} M.
[b]At a concentration of 2.5×10^{-3} M with 0.025 M potassium ferricyanide as an electron scavenger.
[c]Saturated solution with 6.6×10^{-3} M potassium ferricyanide or potassium ferrocyanide.
[d]At a concentration of 2.5×10^{-3} M with 0.025 M potassium ferrocyanide as an electron donor.

The optical behavior of UV-irradiated and bleached samples of GMP in 12 M LiCl samples is similar to that of Gua.

Photolysis for long periods of time (5 h) of Gua, Guo and GMP showed the formation of a new photoproduct absorbing in the 224 nm region (Fig. 4). No trapped hydrogen atoms were observed on the EPR spectrum and the central part of it closely resembled the Cl_2^- spectrum, with a very small contribution from trapped electrons or base radical cations or anions. This is interpreted by assuming that, during the prolonged irradiation, the electrons can be photobleached and the hydrogen atoms can diffuse in the matrix and react to form diamagnetic species.

Attempts were made to identify the species generated by UV irradiation by generation of the Gua, Guo and GMP radical cations and anions by independent methods. To generate the radical cations of the guanines,

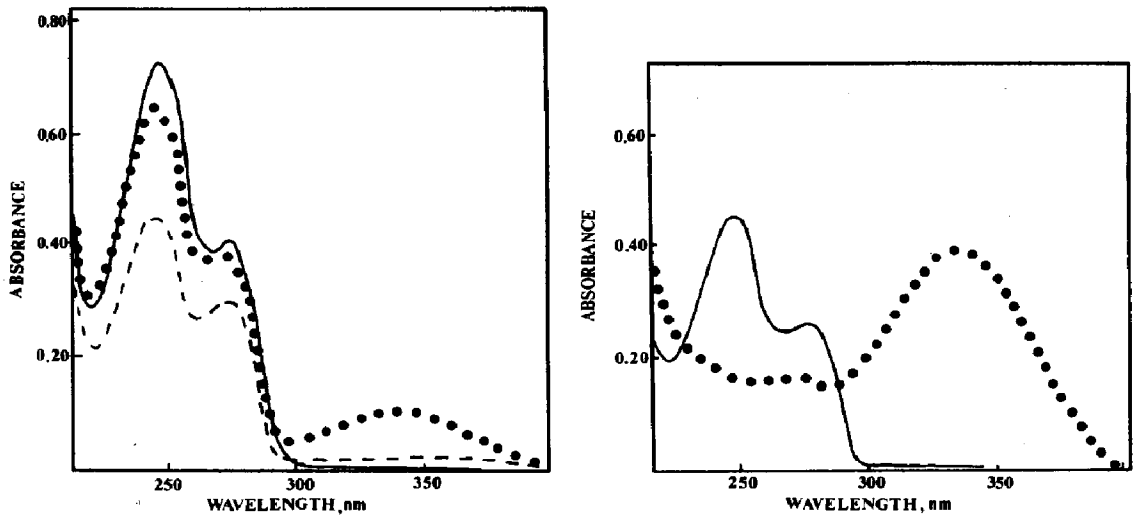

Fig. 3. Optical absorption spectra of Guo in a 12 M LiCl glass ([Guo] = 1.8×10^{-4} M; path length, 0.3 cm): ———, prior to irradiation; – – –, after 60 s UV irradiation; ● ● ●, after 60 s UV irradiation followed by 5 min bleaching.

Fig. 4. Optical absorption spectra of Guo in a 12 M LiCl glass ([Guo] = 1.5×10^{-4} M; path length, 0.3 cm): ———, prior to irradiation; ● ● ●, irradiated for 5 h.

potassium ferricyanide was used as an electron scavenger [15] with a ratio of concentration of scavenger to base of at least 10. Samples were UV irradiated for 120 s and bleached for 6 min with visible light. The EPR parameters for the species produced are included in Table 1. The changes in the *g* values after bleaching are due to the detrapping and consequent scavenging of the small number of trapped electrons present after UV irradiation. The linewidths and *g* values found for the Gua, Guo and GMP species produced in the presence of an electron scavenger are very similar to those found by Sevilla *et al.* [7] for the deoxyguanosine monophosphate (dGMP) cation in 8 M $NaClO_4$ glasses and by Sevilla and Mohan [16] for the Gua cations in a 12 M LiCl glass, confirming their designation as the corresponding radical cations. Figure 5, spectrum D, shows the EPR spectrum for the GMP radical cation.

Radical anions were generated using potassium ferrocyanide as an electron donor [17]. The ferrocyanide concentration was at least ten times that of the base. These results are also included in Table 1 and the EPR spectra for the generated anions are presented in Fig. 5, spectra A, B and C. The satellite lines, marked by arrows on Fig. 5, spectra A, B and C, have been assigned to the hydrogen addition radicals produced by radical anion protonation [18 - 20]. Therefore, the species produced by UV irradiation of Gua, Guo and GMP in the presence of potassium ferrocyanide are postulated to be the corresponding radical anions and small amounts of hydrogen addition radicals.

The *g* values for the paramagnetic intermediates observed in UV-photolyzed and bleached samples of Gua, Guo and GMP (Table 1) are intermediate

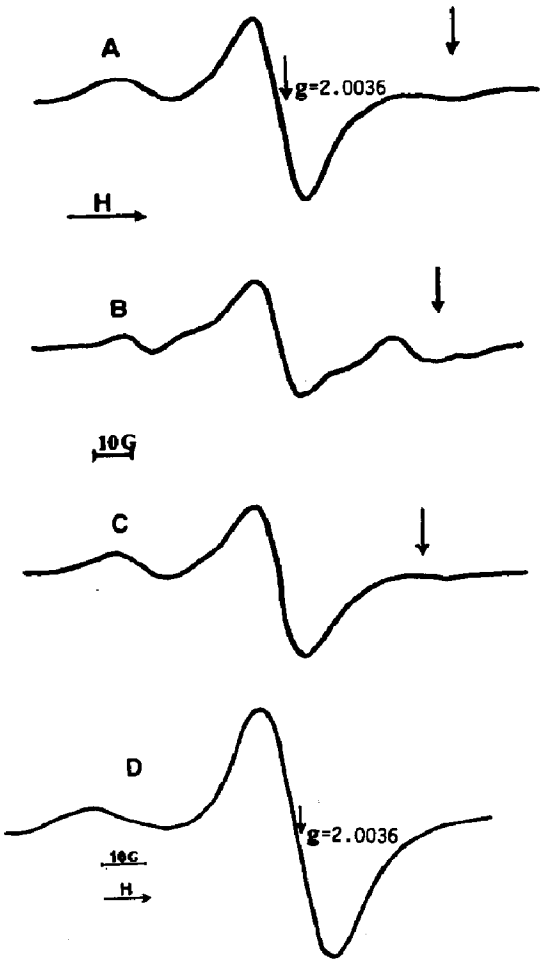

Fig. 5. EPR spectra of the species produced by UV irradiation and subsequent bleaching of Gua (spectrum A), Guo (spectrum B) and GMP (spectrum C) in the presence of potassium ferrocyanide (0.25 M) and GMP (spectrum D) (2.5×10^{-3} M) in the presence of potassium ferricyanide (0.025 M) in 12 M LiCl glass (saturated solution of Gua; [Guo] = [GMP] = 2.5×10^{-3} M; gain, 1.0×10^{5}; microwave power, 1 mW; modulation amplitude, 10 G). The satellite lines are due to hydrogen addition radicals produced on radical anion protonation.

between the values found for the corresponding radical cations and anions generated by the potassium ferricyanide and potassium ferrocyanide methods. High amplification of the EPR signals of the bleached samples shows the presence of the satellite lines characteristic of the hydrogen addition radicals for all the bases. Since the radical anions are postulated to be the precursors of these species, it can be said that radical anions are formed on bleaching of the UV-photolyzed base samples and that a fraction of these protonate to give the corresponding hydrogen addition radicals. We conclude that for Gua, Guo and GMP in 12 M LiCl glasses, UV photolysis and subsequent bleaching of the samples produces radical cations, trapped electrons, radical anions and hydrogen addition radicals as paramagnetic intermediates.

The possible reactions of the bases on UV irradiation and bleaching in 12 M LiCl glasses are summarized as follows:

$$G + h\nu \longrightarrow {}^{T}G \tag{3}$$

$${}^{T}G + h\nu' \longrightarrow G^{+\cdot} + e^{-} \tag{4}$$

$$e^{-} + G \longrightarrow G^{-\cdot} \tag{5}$$

$$G^{-} + H_2O \longrightarrow GH\cdot + OH^{-} \tag{6}$$

$$e^{-} + H_3O^{+} \longrightarrow H\cdot + H_2O \tag{7}$$

$$e^{-} + G^{+\cdot} \longrightarrow G\cdot \tag{8}$$

$$H\cdot + G \longrightarrow GH\cdot \tag{9}$$

where ${}^{T}G$ is the purine triplet, $G^{+\cdot}$ and $G^{-\cdot}$ are the radical cation and anion and GH· is a neutral radical. In addition, energy transfer to the solvent reactions also occurs. Studies of the dependence of the ionization rate on the incident light intensity show a quadratic dependence, indicative of a biphotonic mechanism for the photoionization of Gua and its derivatives in a 12 M LiCl glass.

Qualitative studies on the photochemistry of Gua in ethylene glycol–water mixtures and in 8 M $NaClO_4$ glasses were performed. No quantitative results were obtained because of the poor quality of the glasses formed in the optical cells (0.3 cm by 0.3 cm square) used in this work.

For ethylene glycol–water glasses (7:3, 6:4 and 1:1 by volume) irradiation with 254 nm light from a helical low pressure mercury lamp produces a faint blue coloration of the sample characteristic of trapped electrons [11], indicating photoionization of Gua in this glass. The EPR signal observed in irradiated samples consists of a triplet with a linewidth of 10.8 G and a splitting of 14 G (Fig. 6, spectrum 1). This spectrum is very similar to that of the glycol radical, $\cdot CH_2CH_2OH$, previously observed by Kaalhus and Mohan [21] on the γ radiolysis of ethylene glycol–water mixtures and by Steen [22] on UV irradiation of tryptophan solutions in ethylene glycol–water mixtures. The complex spectrum presented in Fig. 6, spectrum 1, thus consists of contributions from the glycol radical and the Gua radical cation. The glycol radical could result from a biphotonic energy transfer mechanism involving the Gua excited triplet state [22] or from hydrogen atom abstraction from the alcohol by an excited triplet state of Gua [14].

The EPR spectrum of an 8 M $NaClO_4$ Gua sample after UV irradiation is shown in Fig. 6, spectrum 2. Perchlorate ion can act as an electron scavenger [23] producing O^{-} radicals as in

$$ClO_4^{-} + e^{-} \longrightarrow ClO_3^{-} + O^{-} \tag{10}$$

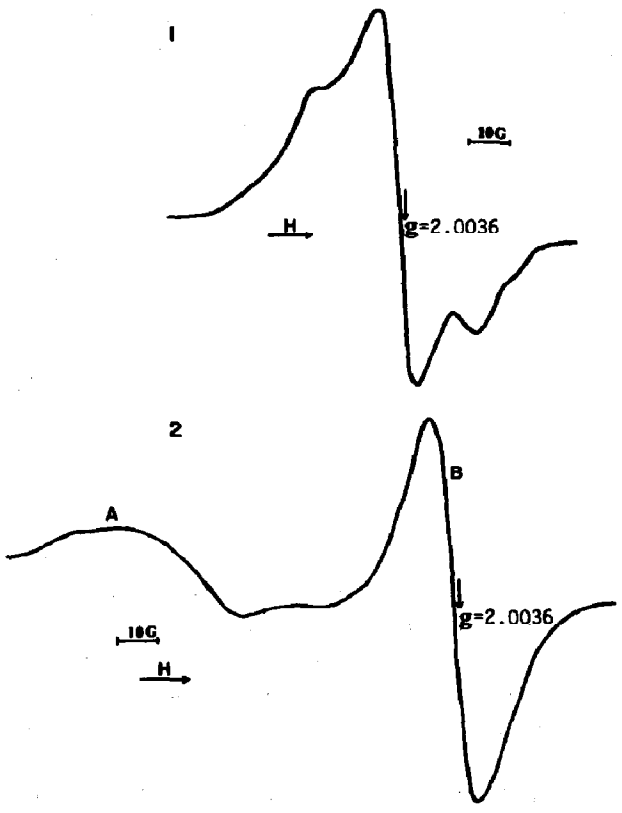

Fig. 6. EPR spectra of the species produced by UV irradiation and subsequent bleaching of a saturated Gua solution in an ethylene glycol–water (7:3) glass (spectrum 1) (30 s irradiation; gain, 8×10^5; microwave power, 1 mW; modulation amplitude, 10 G) and in an 8 M $NaClO_4$ glass (spectrum 2) (10 s irradiation; gain, 5×10^5; modulation amplitude, 10 G; microwave power, 1 mW).

The low field component of the spectrum in Fig. 6, spectrum 2, with g = 2.045 and designated with the letter A, closely resembles that obtained by Sevilla and D'Arcy [24] for the O^- radical in the photoionization of potassium ferrocyanide in 8 M $NaClO_4$ glass. Both the faint blue color of the photolyzed samples, which indicates the formation of trapped electrons, and the presence of the O^- radicals, indicate the photoionization of Gua. Therefore, the broad singlet in the EPR spectrum designated with the letter B has been assigned to the overlap of the EPR signals of trapped electrons, Gua cations and a small contribution from O^- radicals.

Optical examination of the irradiated Gua samples in either ethylene glycol–water or 8 M $NaClO_4$ glasses shows destruction of the base with the formation of a low intensity band in the 300 - 340 nm region which disappears on warming the photolyzed solution to room temperature. No quantitative studies were carried out because of the poor quality of the glasses formed.

3.2. Intermediates and photodestruction quantum yields

The quantum yield for the production of paramagnetic species for GMP in a 12 M LiCl glass (Table 2) is of the order of 10^{-3}, one order of magnitude higher than the value found by Graslund *et al.* [6] for the production of Gua cations in UV-irradiated samples of oriented DNA. The difference can be explained in terms of energy transfer from the excited triplet state of GMP to other bases in the DNA helix conformation. This energy transfer would leave the GMP molecule in its singlet ground state, therefore reducing its ionization probability. Although the GMP excited triplet state transfers energy to the solvent as one of its de-excitation mechanisms, this occurs to a lesser extent in 12 M LiCl. The order of reactivity towards the production of paramagnetic intermediates in neutral media for some purine bases is Gua > purine free base [9] > adenine (Ade) [10]. Photodestruction and trapped electron quantum yields are presented in Tables 3 and 4. In all cases, the photodestruction yields are higher than the trapped electron yields. This is due to a rapid electron reaction with H_3O^+ from the medium to produce trapped hydrogen atoms, to electron reaction with neutral bases to yield radical anions or to other photodestruction paths as well as photoionization. To estimate a photoionization yield, the trapped hydrogen concentration, calculated by comparison with a standard EPR radical concentration, was added to the concentration of base radical cation obtained from the total concentration of paramagnetic species with an equal contribution assumed for the total concentration of the paramagnetic species from the base radical cation and trapped electron and correction for any contributions from Cl_2^- radical ion or saturation effects. After these assumptions, a photoionization yield of 0.005 was determined. Addition of the hydrogen atom concentration to the trapped electron concentration determined by optical methods and using the Beer–Lambert law gave an ionization yield of 0.0016. The higher ionization yield obtained using EPR data alone (68% higher) indicates that, as well as trapped electrons and radical cations, other paramagnetic species are being produced from the UV irradiation of the bases as an alternative route to base destruction.

TABLE 2

Quantum yield for the total production of paramagnetic species for guanine and its derivatives in a 12 M LiCl glass[a]

Compound[b]	$[R] \times 10^5$ (M)	$I_{abs} \times 10^{-16}$ (photons cm^{-2} s^{-1})	ϕ[c]
Gua	8.7	2.92	0.009 ± 0.001
Guo	3.44	3.1	0.0033 ± 0.0003
GMP	3.19	2.88	0.0032 ± 0.0002

[a] Irradiation time, 60 s; volume irradiated, 0.3 cm^3.
[b] Initial base concentration, 2.5×10^{-4} M.
[c] Average value of four to five determinations; the uncertainty corresponds to the standard deviation.

TABLE 3

Photodestruction quantum yields for guanine and its derivatives in 12 M LiCl glass after 60 s UV irradiation[a]

Compound[b]	$I_{abs} \times 10^{-16}$ (photons cm^{-2} s^{-1})	$-[G] \times 10^5$ (M)	$\phi(-G)$[c]
Gua	2.91	5.47	0.0057 ± 0.0008
Guo	2.90	6.16	0.0062 ± 0.0008
GMP	3.45	6.58	0.0055 ± 0.0006

[a]Irradiated volume, 0.3 cm^3.
[b]Initial base concentration $[G]_0 = 2.5 \times 10^{-4}$ M.
[c]Average value of four to five determinations; the uncertainty corresponds to the standard deviation.

TABLE 4

Trapped electron quantum yields after 60 s UV irradiation of guanine and its derivatives in 12 M LiCl glass[a]

Compound[b]	$I_{abs} \times 10^{-16}$ (photons cm^{-2} s^{-1})	$[e_t^-] \times 10^5$ (M)	$\phi(e_t^-)$[c]
Gua	2.99	7.84	0.00079 ± 0.00002
Guo	3.30	10.02	0.00094 ± 0.00008
GMP	3.34	9.08	0.00093 ± 0.00008

[a]Irradiated volume, 0.3 cm^3.
[b]Initial base concentration, 2.5×10^{-4} M.
[c]Average value of four to five determinations; the uncertainty corresponds to the standard deviation.

The photodestruction yield for purine free base in an 8 M $NaClO_4$ glass has been found to be 0.011 [9]. For Ade and deoxyadenosine (dAdo) in 12 M LiCl glasses the values are 3.01×10^{-4} and 1.38×10^{-4} [10]. Thus, purine free base is almost twice as reactive towards destruction as Gua, while Gua and Guo are about ten times as reactive towards photodestruction as Ade or dAdo. The quantum yield for trapped electron formation for Ade in 12 M LiCl glasses is 0.000 86, which is about the same as for Gua. As there are large differences in the photodestruction yields with the Gua destruction yield being almost ten times that for Ade, this implies that, even if the photoionization process is as efficient for both bases, Gua has available additional destruction paths as well as ionization.

Photoconversion yields of 0.02 have been reported for Gua, Guo and GMP by Morgan and Callis [1]; this is four times higher than the photodestruction yield determined in our work. Morgan and Callis [1] postulated that the product formed on irradiation could be a diol addition product, which cannot be formed in our medium.

3.3. *Triplet states and intersystem crossing yields*

Phosphorescence of the Gua, Guo and GMP samples during and after UV irradiation was observed, and the corresponding triplet state EPR signals were recorded. The EPR characteristic data for these triplets are presented in Table 5. Both the decay time and the zero-field splitting parameters D^* identify the emitting triplet states to be of π,π^* character [27, 28]. No significant changes in D^* are observed when substitution of the hydrogen atom by the sugar or sugar phosphate groups takes place.

The EPR spectrum for the Gua triplet state showed a peculiar structure (Fig. 7). No EPR signal was observed for a 12 M LiCl glass without Gua and using Gua from different distributors gave identical EPR signals, so impurities from either the solvent or the solute were ruled out as the source of the EPR signal. Since the broad band excitation used to generate the triplet states included the two absorption maxima of Gua (246 nm and 272 nm), the

TABLE 5

Electron paramagnetic resonance characteristic data for the triplet states of guanine and its derivatives in 12 M LiCl glasses

Compound	*Linewidth* (G)	H_{min} (G)	τ (s)	ν (GHz)	D^* (cm^{-1})	ϕ_{phos}	ϕ_{ISC} [a, b]	ϕ_{ISC} [a, c]
Gua	25	1419	2.2	9.31	0.140	0.07[d]	0.15 ± 0.02	—[e]
Guo	35	1429	2.1	9.23	0.133	0.03[d]	0.31 ± 0.05	0.027 ± 0.002
GMP	38	1425	1.6	9.23	0.139	0.04[d] 0.42[f]	0.28 ± 0.01	0.015 ± 0.001

[a] ISC yields are average values of four to five determinations; the uncertainty corresponds to the standard deviation.
[b] Samples were irradiated with an Hg–Xe 1000 W lamp coupled to Corning 7-54 and $NiSO_4$ filters.
[c] Samples were irradiated with a Baush and Lomb grating monochromator at an excitation wavelength of 280 nm; volume irradiated, 0.129 cm^3.
[d] From ref. 25.
[e] Not detectable.
[f] From ref. 26.

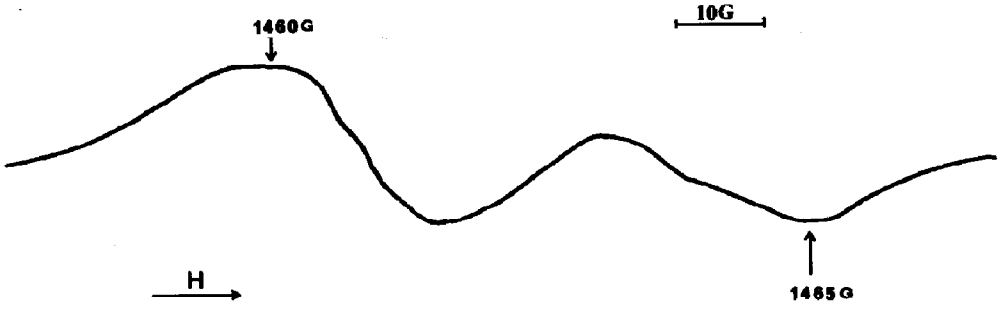

Fig. 7. EPR spectrum of Gua triplet states in a 12 M LiCl glass (saturated Gua solution; gain, 8 × 10^6; microwave power, 10 mW; modulation amplitude, 10 G).

possibility existed that each electronic transition gave rise to different triplet states with different paramagnetic properties and whose EPR spectra overlapped. However, irradiation using filters which had different transmittance values at each of the wavelengths of maximum absorption of Gua caused no change in the relative intensity of the bands as would have been the case if, in fact, these bands corresponded to two different triplet states. The decay times measured from plots of the logarithm of the triplet concentration *versus* time measured at 1400 and 1465 G, selected to ensure minimal overlap of the bands, were identical. All this evidence indicates that the observed EPR spectrum is due to only one type of emitting triplet state. Kottis and Lefebvre [29] have concluded, on the basis of theoretical calculations, that peaks or critical points for $\Delta m_s = 2$ transitions can also appear at magnetic field values corresponding to field orientations along the principal axes of the spin–spin dipolar tensor. Therefore, the shape of the observed EPR spectrum for the Gua triplet may be due to the observation of an additional $\Delta m_s = 2$ peak.

Values for the intersystem crossing (ISC) yields are presented in Table 5. From a comparison of the values for the phosphorescence yield with those of ISC, it can be seen that internal conversion reactions from the triplet state and/or energy transfer processes are very effective for Gua, Guo and GMP as the ISC yields are at least three times higher than the phosphorescence yields. The order of ISC yields is Guo > GMP > Gua, while phosphorescence yields [25] follow the order Gua > Guo > GMP. These results imply that internal conversion from the triplet state is a more efficient process for Guo and GMP than for Gua since, even when the ISC yields for Guo and GMP are almost twice the value for Gua, their phosphorescence yield is smaller.

Attempts were made to observe the triplet EPR signals using monochromatic light. Signals were observed for Guo and GMP concentrated solutions (3.5×10^{-2} M) on excitation with a wavelength of 280 nm through a high intensity Baush and Lomb monochromator with a narrow bandwidth. No triplet signal was observed for Gua under these conditions. The ISC yield values obtained under these conditions are presented in Table 5. A decrease of about an order of magnitude is found for yields obtained with 280 nm narrow band excitation compared with broad band excitation through a Corning 7-54 filter coupled with a 50% aqueous solution of $NiSO_4$. Morgan and Callis [2] found a wavelength dependence on the phosphorescence yields of GMP- and Gua-containing dinucleotides. The phosphorescence yield increased by a factor of 2 in going from 265 to 290 nm excitation. Since internal conversion processes should remain constant for excitation at different wavelengths, differences in phosphorescence yields must be due to differences in triplet state population or ISC. Thus, the different results found in this work for ISC yields at different excitation wavelengths can be accounted for by a strong wavelength dependence of the ISC process.

For purine free base, the ISC yield is 0.37 [30] while for Ade, dAdo and deoxyadenosine monophosphate (dAMP) the values are 0.17, 0.051 and

0.008 respectively in 12 M LiCl glasses. The smaller yield for Gua compared with purine indicates that the presence of the substituents in Gua decreases the efficiency of triplet state population, while emission from the singlet state is enhanced, as evidenced by the fluorescence yields of 0.02 for purine and 0.06 for Gua in neutral glasses [30]. Although Gua and Ade show almost identical values of ISC, Guo and GMP have yields at least ten times higher than dAdo or dAMP, indicating that for Gua and its derivatives the incorporation of a ribose or ribose phosphate increases the probability of ISC, while for Ade and its derivatives the effect is the reverse.

4. Conclusions

The intermediate species produced by UV irradiation of Gua, Guo and GMP in neutral media have been studied and characterized. The photoionization of the bases via an excited triplet state has been observed. Excited triplet states, radical cations and anions and neutral radicals have been identified as the reactive species produced. Photosensitization of the solvent was also observed.

The electronic structure of the triplet states does not change appreciably on substitution of the N(9) hydrogen by a sugar phosphate group, although the ISC yield changes, suggesting differences in the emission properties of the excited singlet states which are the precursors of the triplets. The ISC yields are higher than the ionization or destruction yields, which is in accordance with the results found indicating a biphotonic mechanism involving an excited triplet state for the ionization of the bases. The photodestruction yields are of the same order of magnitude for the three Gua derivatives while the ionization yields decrease in the order Guo $\approx$ GMP $>$ Gua. Other destruction paths are available as well as ionization. The destruction yields for Gua, Guo and GMP are higher than the corresponding yields for Ade, dAdo and dAMP in 12 M LiCl glass, confirming that, of the purine bases in DNA, Gua is the most photoreactive and may play the most important role in nucleic acid photochemistry.

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