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Characterization of reactive intermediates in laser photolysis of guanine and its derivatives using acetone as photosensitizer: the pH dependence

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Abstract

The pH effects on photochemical reactions of guanine (Gua), guanosine (Guo) and 2′-deoxyguanosine-5′-monophosphoric acid (dGMP) using acetone as a photosensitizer have been investigated by 248 nm laser flash photolysis. Laser-induced transient species have been characterized according to kinetic analysis and quenching experiments by Mn^{2+} and O_2. In acid aqueous solutions, the intermediates recorded are assigned to the excited triplet states and the dehydrogenated radicals of Gua, Guo and dGMP. The excited triplet states produced via triplet–triplet excitation transfer and the dehydrogenated radicals arising from electron transfer reactions have been identified for the first time. In alkaline aqueous solutions, transient absorption spectra of dehydrogenated radical anions have been observed, but no triplet states of Gua, Guo and dGMP could be detected. The kinetics of the formation and decay of the exciplex arising from interaction of $^3A^*$ and $Gua[-H^+]^-$ and its derivatives have been offered for the first time. However, in neutral aqueous solution the intermediates recorded are assigned to the excited triplet state and dehydrogenated radical of dGMP arising from energy transfer and an electron transfer reaction. The sites of dehydrogenation have been suggested. Furthermore, four sets of kinetic parameters of the triplet decay of Gua and its derivatives have also been determined.

Keywords: Guanine; Guanosine; dGMP; Triplet states; Laser flash photolysis; Dehydrogenated radicals

1. Introduction

Guanine (Gua) has been widely accepted as the most easily oxidized among DNA components. In addition, many observations have demonstrated that purine components, particularly guanine and its derivatives, are more readily photooxidized by a variety of sensitizers [1–4]. Although extensive studies on the triplet states of pyrimidines and their nucleosides and nucleotides have been performed, triplet–triplet (T–T) absorption spectra and direct measurement of triplet state kinetics of purine bases and their nucleosides and nucleotides have not yet been observed owing to their low intersystem crossing quantum yields and very small extinction coefficient at room temperature [1,2].

Arce and Rivera [5] characterized the reactive intermediates resulting from laser photolysis of aqueous guanine system at different pH values. The fast decay of a higher intensity band in the visible region with λ_{max} around 700 nm was attributed to a hydrated electron. The UV band was assigned to the radical cation, radical anion and/or radicals resulting from neutralization of these charged radicals.

Candeias and Steenken [6] observed absorption spectra arising from 193 nm laser photolysis of aqueous guanosine (Guo). On the basis of a comparison with a pulse radiolysis study, it was concluded that photoionization of purine derivatives in deaerated neutral solution resulted in two different neutral radicals: a deprotonated radical cation and a protonated radical anion.

Kasama et al. [7] observed the transient absorption spectra of guanine derivatives in the presence of acetone excited by 248 nm laser and in the absence of acetone excited by 193 nm laser. It was suggested that the radical cation of guanosine was produced, but triplet guanosine was hardly observed.

Recently, a breakthrough in detection of absorption spectra and direct triplet states kinetics of DNA components with a KrF laser under acetone-sensitized excitation has been achieved at our laboratory [8–10]. Jian et al. [8] investigated the photochemical reactions of aqueous solution of guanosine using acetone as photosensitizer. On the basis of detailed kinetic analysis and the estimation of the free energy change of the electron transfer reaction between Guo and triplet acetone, it was suggested that the dehydrogenated radical of Guo and triplet Guo were produced via electron transfer and T–T excitation transfer respectively.

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In this work, characterization of the excited triplet state and radical intermediates of the guanine system from KrF laser photolysis of aqueous solutions at several pH values using acetone as sensitizer has been performed.

2. Experimental details

Gua, Guo and 2′-deoxyguanosine-5′-monophosphoric acid (dGMP) obtained from Sigma were used as received. Acetone (analytic grade reagent) was distilled before use. $MnCl_2$ and $Na_2S_2O_8$ were recrystallized from triply distilled water. NaOH and phosphate (analytic grade reagent) were used as received. The pH values of solutions were adjusted with NaOH and H_3PO_4 solutions. All samples were prepared in triply distilled water and deaerated by high purity nitrogen (99.99%) bubbling for 20 min. All experiments were carried out at room temperature.

Laser photolysis experiments were performed using a home-made excimer laser which provided a 248 nm (KrF) light pulse with a duration of 20 ns. The maximum laser energy was 100 mJ per pulse. The signals were collected using an HP54510B 300 MHz transient recorder and then processed with a PC-486 personal computer. Detailed descriptions of the equipment and experimental conditions were given elsewhere [9].

3. Results and discussion

3.1. Identification of transient species

3.1.1. Acid aqueous solution

As shown in Fig. 1 transient absorption spectra were observed from laser photolysis of 0.1 mM Gua aqueous solution containing 0.13 M acetone at pH 1.0. At the end of the laser pulse, an absorption band appears in the wavelength region 300–380 nm which is similar to that from photolysis of neat acetone aqueous solution and thus should be assigned to the absorption of triplet acetone ($^3A^*$). Another absorption spectrum different from $^3A^*$ rises to a maximum 2 μs after the pulse. The absorption maximum at 360 nm disappears completely 25 μs after the pulse and a slow-decaying spectrum with absorption maximum at 310 nm and another weak absorption peak at 390 nm appears subsequently.

The band with absorption maximum at 360 nm decays with first-order kinetics and can be quenched by triplet quenchers Mn^{2+} and O_2. Thus, the novel transient species should be assigned to the excited triplet state of protonated Gua ($^3[GuaH^+]^*$) because the pK value of protonation of Gua was reported to be 3.00 [11]. So, at pH 1.0 the concentration of protonated Gua is 2 orders of magnitude more than that of the parent, Gua. This is equivalent to saying that $^3[GuaH^+]^*$ is prevailing, whereas the excited triplet state of the parent is undetectable owing to the very low concentration of the parent.

The slowly decaying absorption spectrum, which is very similar to that from one-electron oxidation of Gua by $SO_4^{\bullet -}$

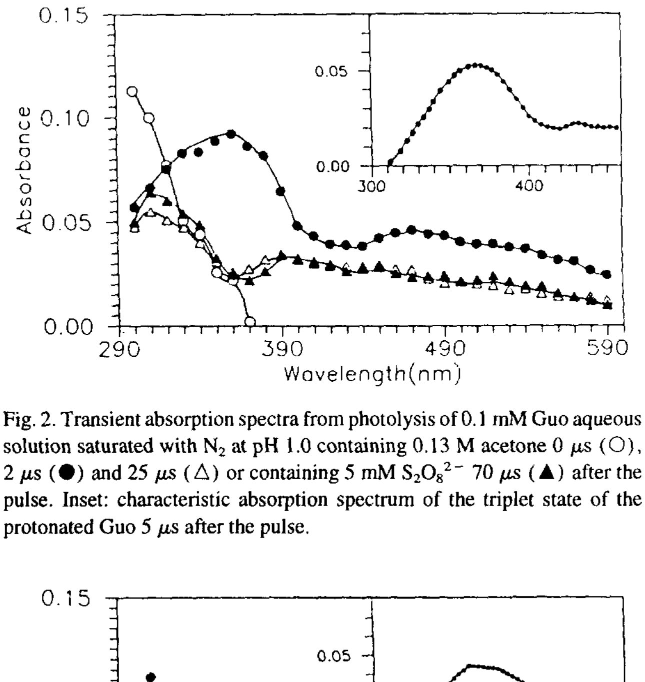


Fig. 2. Transient absorption spectra from photolysis of 0.1 mM Guo aqueous solution saturated with N_2 at pH 1.0 containing 0.13 M acetone 0 μs (○), 2 μs (●) and 25 μs (△) or containing 5 mM $S_2O_8^{2-}$ 70 μs (▲) after the pulse. Inset: characteristic absorption spectrum of the triplet state of the protonated Guo 5 μs after the pulse.

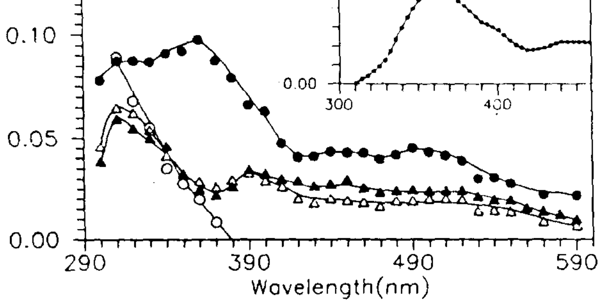


Fig. 1. Transient absorption spectra from photolysis of 0.1 mM Gua aqueous solution saturated with N_2 at pH 1.0 containing 0.13 M acetone 0 μs (○), 2 μs (●) and 25 μs (△) or containing 5 mM $S_2O_8^{2-}$ 70 μs (▲) after the pulse. Inset: characteristic absorption spectrum of the triplet state of the protonated Gua 5 μs after the pulse.

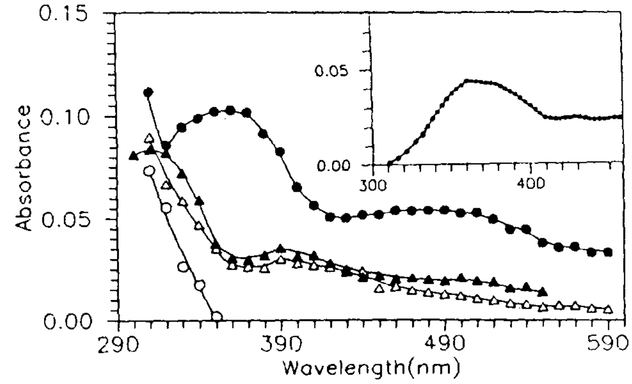
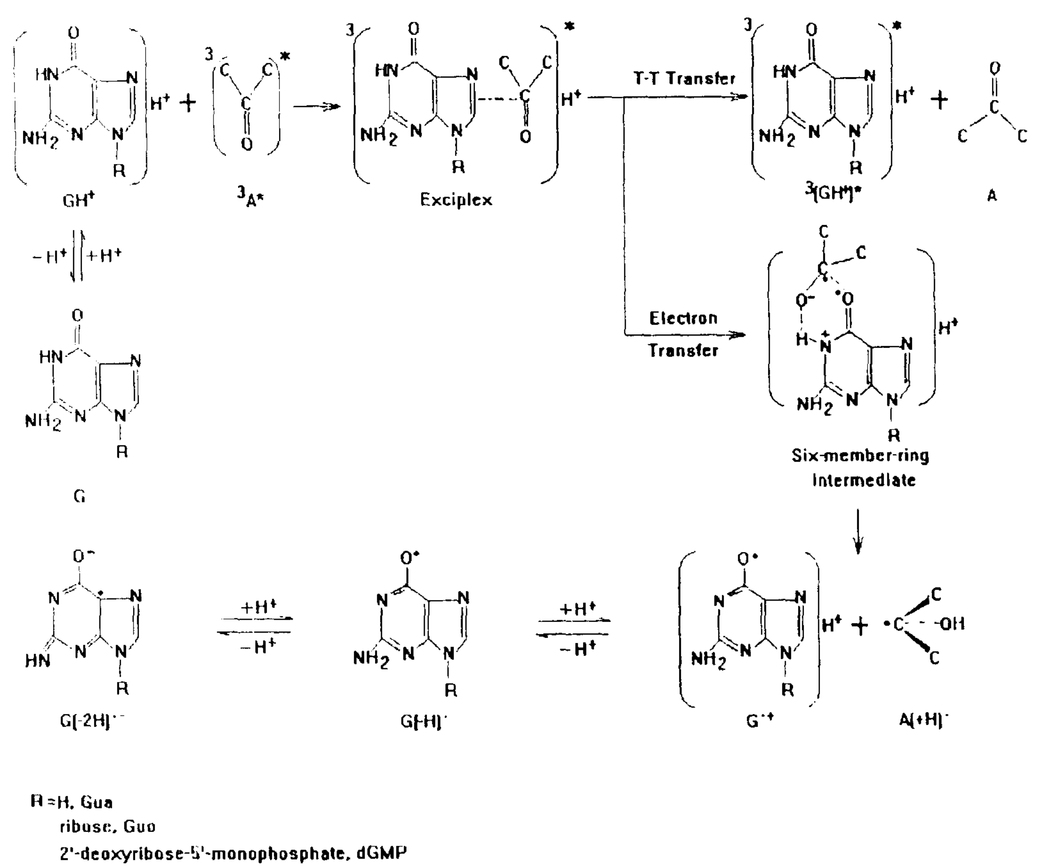


Fig. 3. Transient absorption spectra from photolysis of 0.2 mM dGMP aqueous solution saturated with N_2 at pH 1.0 containing 0.13 M acetone 0 μs (○), 2 μs (●) and 25 μs (△) or containing 5 mM $S_2O_8^{2-}$ 70 μs (▲) after the pulse. Inset: characteristic absorption spectrum of the triplet state of the protonated dGMP 5 μs after the pulse.



Scheme 1.

(see Fig. 1), should be ascribed to Gua[−H]H$^{•+}$, because in acid solution Guo[−H]$^{•}$ is protonated to give a radical cation (Guo[−H]H$^{•+}$) with a pK value of 3.9 [12].

As shown in Fig. 2 and Fig. 3, similar transient absorption spectra have been obtained from photolysis of 0.1 mM Guo and 0.2 mM dGMP aqueous solutions containing 0.13 M acetone at pH 1.0. According to the quenching experiments with Mn^{2+} and O_2 and kinetic analysis of the decay traces in the wavelength region 300–600 nm, the absorption maxima at 360 nm have been assigned to triplet states of protonated Guo (^{3}[GuoH$^+$]*) and dGMP (^{3}[dGMPH$^+$]*) respectively. For the same reason described above, the absorptions with λ_{max} at 310 nm and a weak absorption peak at 390 nm should be assigned to the dehydrogenated radicals of the protonated Guo (Guo[−H]H$^{•+}$) and dGMP (dGMP[−H]H$^{•+}$) respectively.

On the basis of the above systematic identification of the laser-induced transient species of Gua, Guo and dGMP (symbolized as G), it is reasonable to propose that both the excited triplet states and the dehydrogenated radicals of the protonated G have been induced by ^{3}A*, namely the triplet states have been produced via T–T excitation transfer from ^{3}A* to G, and the dehydrogenated radicals formed via electron transfer from G to ^{3}A*.

As shown in Figs. 1–3, the characteristic absorption spectra of the dehydrogenated radicals of the protonated Gua, Guo and dGMP, with absorption maxima at 310 nm and another weak absorption peak at 390 nm, are very similar to those from laser-induced oxidation of Gua, Guo and dGMP by $SO_4^{•-}$ in our experiments or from pulse radiolytically induced oxidation of dGuo by $SO_4^{•-}$ [12]. Furthermore, both Candeias and Steenken [12,13] and O'Neill and Davies [14] assumed that "O6-centred" dehydrogenated radicals of (d)Guo were produced from deprotonation on N1 of the radical cations. On the basis of the similarity of transient spectra from acetone-mediated dehydrogenated radicals of G to that from oxidation by $SO_4^{•-}$, we propose that the site of dehydrogenation is also on N1 of the guanine moiety, which interacts with triplet acetone to form a six-membered ring intermediate followed by fast deprotonation within the electron–donor–acceptor intermediate.

The proposed mechanisms are summarized in Scheme 1.

3.1.2. Neutral aqueous solution

Fig. 4 shows transient absorption spectra of the photolysis of 0.2 mM dGMP neutral aqueous solution containing 0.13 M acetone. On the basis of quenching experiments with Mn^{2+} and O_2 and kinetic analysis of the decay traces in the 340–600 nm region, the absorption with λ_{max} at 370 nm has been assigned to the triplet state of dGMP. The absorption with λ_{max} at 320 nm should be assigned to the dehydrogenated radical of dGMP (dGMP[−H]$^{•}$), since it is quite similar to our previous finding for Guo[−H]$^{•}$ arising from electron transfer reaction between Guo and ^{3}A* [8]. So, we suggest

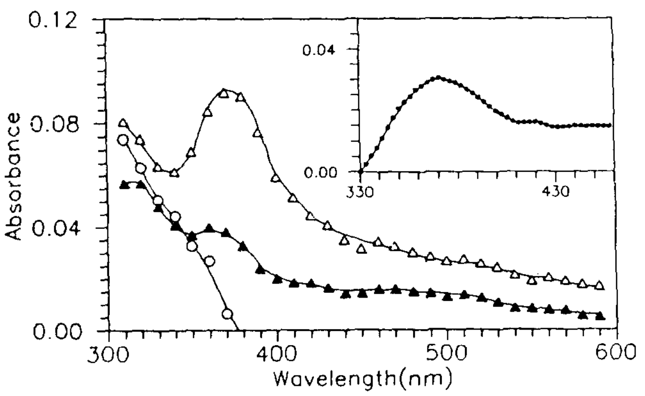


Fig. 4. Transient absorption spectra from photolysis of 0.2 mM dGMP neutral aqueous solution containing 0.13 mM acetone saturated with N_2 0 μs (○), 2 μs (△) and 25 μs (▲) after the pulse. Inset: characteristic absorption spectrum of the triplet state of dGMP 5 μs after the pulse.

that dGMP[− H]$^\bullet$ may result from electron transfer within an exciplex arising from the interaction of dGMP and ${}^3A^*$ followed by fast deprotonation of the dGMP$^{\bullet+}$–A$^{\bullet-}$ geminate ion pair; the site of deprotonation is also on N1 of the guanine moiety.

However, the transient absorption spectrum from the photolysis of Gua with 0.13 M acetone at pH 7.0 is very different from that in Fig. 4. The marked change is that beyond the absorption of ${}^3A^*$ in the wavelength region 300–380 nm no signal from Gua could be detected, because the solubility of Gua in neutral aqueous solution is very low.

3.1.3. Alkaline aqueous solution

As shown in Fig. 5, transient absorption spectra were obtained from photolysis of 0.1 mM Gua aqueous solution containing 0.13 M acetone at pH 11.3. The absorption rises to its maximum around 2 μs after the pulse. It is composed of a strong absorption around 310 nm and another λ_{max} at 400 nm. This slow-decaying absorption band, which cannot be quenched by O_2, decays according to the second-order rate law. Thus, this long-lived species can be assigned to a dehydrogenated radical anion (Gua[− 2H]$^{\bullet-}$) produced via electron transfer reaction between Gua anion (Gua[− H$^+$]$^-$) and ${}^3A^*$.

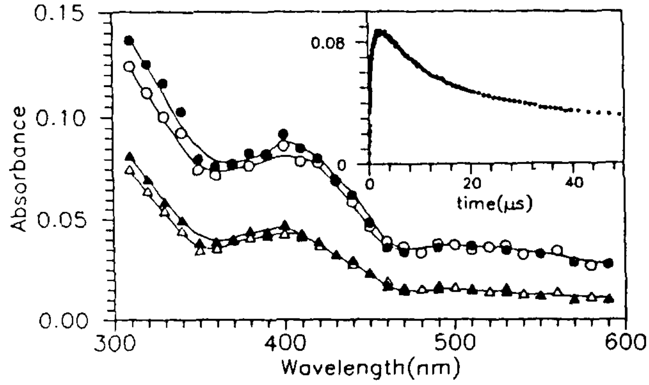


Fig. 5. Transient absorption spectra from photolysis of 0.1 mM Gua aqueous solution containing 0.13 M acetone at pH 11.3 saturated with N_2 2 μs (○) and 25 μs (△) or saturated with air 2 μs (●) and 25 μs (▲) after the pulse. Inset: growth–decay trace at 400 nm.

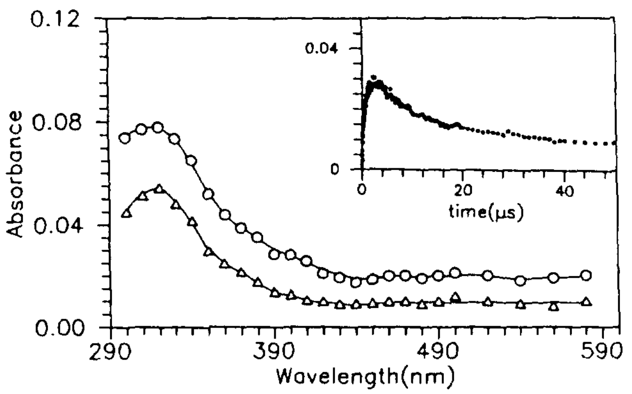


Fig. 6. Transient absorption spectra from photolysis of 0.1 mM Guo aqueous solution containing 0.13 M acetone saturated with N_2 at pH 11.3 at 2 μs (○) and 25 μs (△) after the pulse. Inset: growth–decay trace at 400 nm.

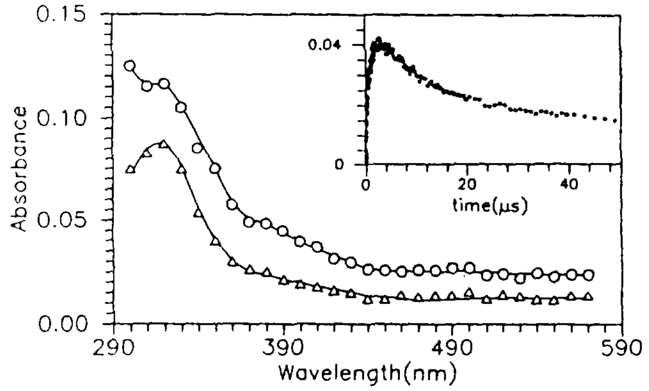


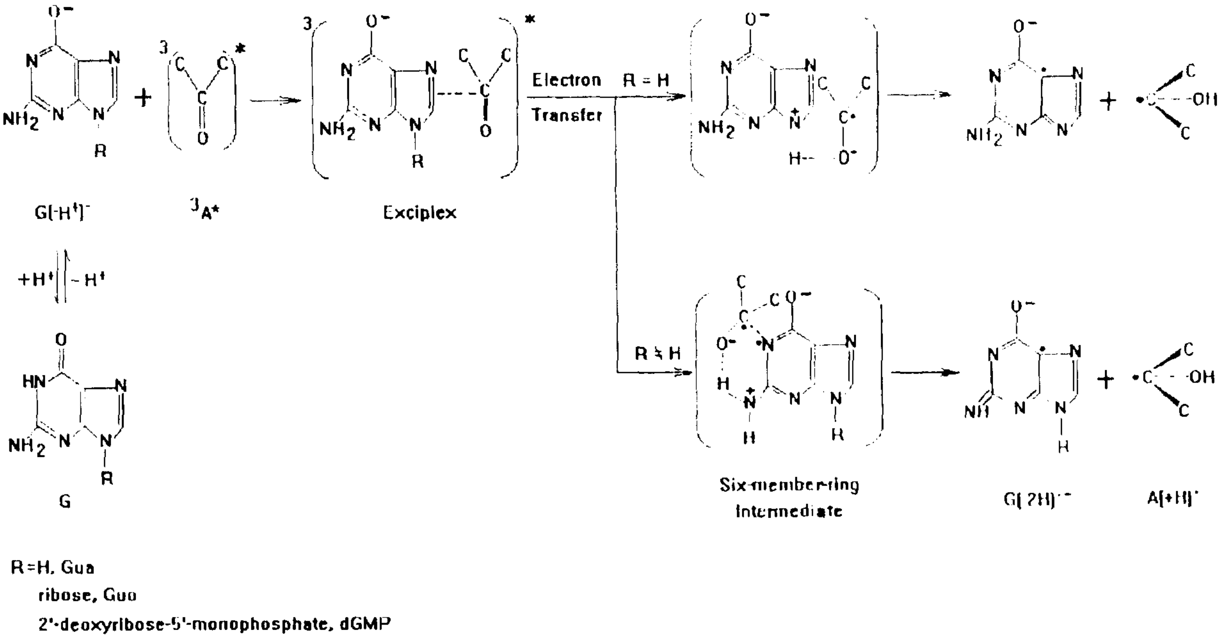
Fig. 7. Transient absorption spectra from photolysis of 0.1 mM dGMP aqueous solution containing 0.13 M acetone saturated with N_2 at pH 11.3 at 2 μs (○) and 25 μs (△) after the pulse. Inset: growth–decay trace at 400 nm.

Fig. 6 and Fig. 7 show the transient absorption spectra obtained from photolysis of 0.1 mM Guo and 0.2 mM dGMP aqueous solutions containing 0.13 M acetone at pH 11.3. The slow-decaying absorptions with only one λ_{max} at 320 nm, which cannot be quenched by O_2 and decay according to the second-order rate law, should be assigned to the dehydrogenated radical anions of Guo and dGMP respectively.

Since the pK values for protonation of Guo[− 2H]$^{\bullet-}$ and dGuo[− 2H]$^{\bullet-}$ were reported to be 10.7 and 10.8 respectively [12], the radical anion of the guanine system, G[− 2H]$^{\bullet-}$, is the prevailing species at pH 11.3.

On the basis of a comparison of the transient absorption spectra of Gua[− 2H]$^{\bullet-}$ and those of Guo[− 2H]$^{\bullet-}$ or dGMP[− 2H]$^{\bullet-}$, we suggest that the difference in shape of the dehydrogenated radical anions may stem from different sites of deprotonation.

In basic aqueous solutions of Gua and Guo, N1 is the preferred site of deprotonation with pK_a of 9.6 and 9.2 [8,15]. So, it is reasonable to suppose that in the case of deprotonated Gua (Gua[− H$^+$]$^-$), there are two possible sites of dehydrogenation: N2 position and N9 position. Owing to the high



Scheme 2.

electron density on the imidazole moiety, the electron on C8 can be abstracted by triplet acetone, followed by deprotonation on N9 forming a radical anion. However, in the case of Guo$[-H^+]^-$ and dGMP$[-H^+]^-$, the hydrogen atoms on N9 are substituted by ribose and deoxyribose monophosphate; therefore, only the N2 position is a possible site of dehydrogenation forming imino-substituted radical anions. The radical anions produced via electron transfer may be within a six-membered ring exciplex arising from the interaction of Guo$[-H^+]^-$, dGMP$[-H^+]^-$ and $^3A^*$.

As shown in the insets of Fig. 5, Fig. 6 and Fig. 7, the growth–decay absorption traces offer the kinetics of formation and decay of the exciplex. Firstly, the traces rise according to a pseudo-first-order rate law characterizing the formation kinetics of exciplex, because the concentration of $^3A^*$ is much less than that of G$[-H^+]^-$. The rate constants of formation of exciplex have been deduced to be 2.8×10^6 s^{-1}, 1.2×10^6 s^{-1} and 1.1×10^6 s^{-1} for Gua$[-H^+]^-$, Guo$[-H^+]^-$ and dGMP$[-H^+]^-$ respectively. Then the traces of exciplex decay rapidly with a first-order rate law indicating the production of G$[-2H]^{\bullet-}$ and A$[+H]^\bullet$. Afterwards, the traces decay according to the second-order rate law revealing the decay off G$[-2H]^{\bullet-}$.

The results are summarized in Scheme 2.

3.2. Characteristic absorption spectra of the transient species

As shown in Fig. 1, Fig. 2 and Fig. 3, the slowly decaying species characterized by an absorption maximum at 310 nm and another weak absorption peak at 390 nm 25 μs after the pulse are assigned to the protonated O^6-centred dehydrogenated radicals of Gua, Guo and dGMP respectively. The absorptions at 310 nm 5 μs after the pulse, which decay with a second-order rate law, should be attributed to the protonated dehydrogenated radicals, but the absorptions in the wavelength region longer than 310 nm are composed of the absorption of the excited triplet states of the protonated Gua, Guo and dGMP and their protonated dehydrogenated radicals, while 25 μs after the pulse the radicals exist alone. The absorbance at wavelength λ (nm) and t (μs) after the pulse is symbolized by A_t^λ. According to the subtraction method suggested by Jian et al. [8], we have derived the characteristic absorption spectra of the excited triplet states of the protonated Gua, Guo and dGMP at 5 μs by subtracting $A_{25}^\lambda A_5^{310}/A_{25}^{310}$ from A_5^λ. The characteristic spectra are shown in the insets of Figs. 1, 2 and 3 respectively.

Using the same subtraction method, the characteristic absorption spectrum of the excited triplet state of dGMP in neutral aqueous solution at 5 μs was obtained as shown in the inset of Fig. 4.

3.3. Determination of the kinetic parameters of the excited triplet states

The triplet states of Gua and its derivatives ($^3G^*$) decay according to pseudo-first-order kinetics. Their apparent decay rate constants can be expressed as

$$k_{obs}=k_0+k_{sq}[G]+k_q[Q]$$

The decay trace of $^3[GuaH^+]^*$ at $\lambda=360$ nm has been obtained by subtracting the absorbance A_t^{310} at 310 nm multiplied by $A_{25}^{360}/A_{25}^{310}$ from A_t^{360} at 360 nm using the same subtraction method as above. The decay trace of $^3[GuaH^+]^*$ (Fig. 8, curve c) follows well first-order kinetics as shown in Fig. 9. The value of the slope is the apparent decay rate constant k_{obs} of $^3[GuaH^+]^*$. The plot of k_{obs} against the concentration of Gua is a straight line as shown in Fig. 10.

Table 1
Kinetic parameters of triplet states of Gua, Guo and dGMP

	pH	k_0 ($\times 10^5$ s^{-1})	k_{sq} ($\times 10^8$ dm^3 mol^{-1} s^{-1})	k_q ($\times 10^8$ dm^3 mol^{-1} s^{-1})	
				Mn^{2+}	O_2
Gua	1.0	1.4	3.9	1.8	17
Guo	1.0	1.2	1.5	1.4	14
	7.0	1.3	1.7	1.5	8.5
dGMP	1.0	1.0	1.2	2.3	12
	7.0	1.1	1.3	2.5	11

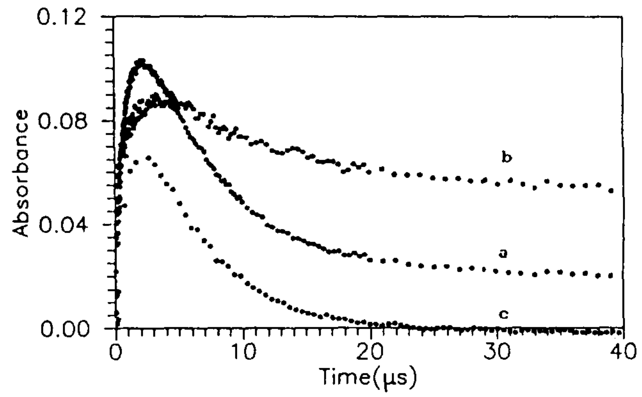


Fig. 8. Transient absorption decay traces obtained by photolysis of 0.1 mM Gua aqueous solution containing 0.13 M acetone saturated with N_2 at pH 1.0: trace a, decay at 360 nm; trace b, decay at 310 nm; trace c, decay obtained by subtracting trace b multiplied by $A_{25}^{360}/A_{25}^{310}$ from trace a.

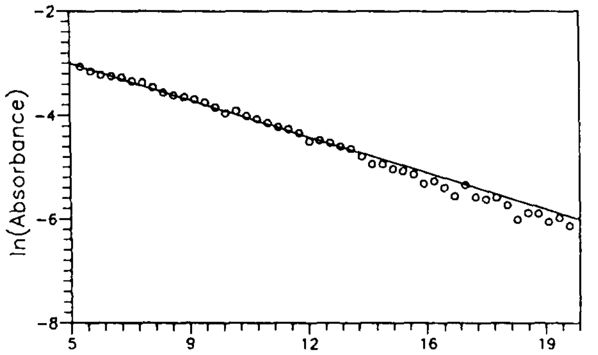


Fig. 9. Plot of the logarithm of the absorbance of ^{3}[GuaH$^+$]* at 360 nm vs. time, from trace c of Fig. 8.

From the value of the slope, the rate constant k_{sq} of ^{3}[GuaH$^+$]* quenched by its ground state has been deduced to be 3.9×10^8 dm^3 mol^{-1} s^{-1}. The value of k_0, representing the rate constant of the unimolecular decay, has been obtained from the intercept of Fig. 10 to be 1.4×10^5 s^{-1}. Through the same data processing as for Fig. 8, in the solutions containing 0.1 mM, 0.3 mM, 0.5 mM Mn^{2+} and 0.25×10^{-3} M O_2, the pseudo-first-order decay rate constants $k_{obs} = k'_{obs} + k_q[Q]$ of ^{3}[GuaH$^+$]* have been obtained, and then the quenching rate constant k_q of Mn^{2+} and O_2 on ^{3}[GuaH$^+$]* have been deduced to be 1.8×10^8 dm^3 mol^{-1} s^{-1} and 1.7×10^9 dm^3 mol^{-1} s^{-1} respectively.

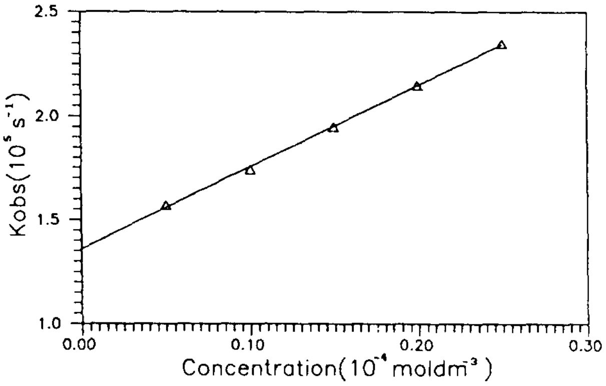


Fig. 10. Plot of the apparent decay rate constant of ^{3}[GuaH$^+$]* vs. the concentration of Gua.

The kinetic parameters of triplet Guo and dGMP at two pH values have been obtained through the same data processing method and listed in Table 1.

From Table 1, it is obvious that the kinetic parameters of triplet decay of Guo and dGMP at pH 1.0 are approximately equal to those at pH 7.0. Therefore, we can predict that the kinetic parameters of Gua at pH 7.0 may approximate those at pH 1.0.

4. Summary and conclusions

The total picture of T–T absorption spectra and direct triplet state kinetics of the guanine system under acetone-sensitized excitation have been offered for the first time. This novel finding extends our recent breakthrough in the direct detection of triplet excited states of DNA components. On the basis of the similarity of the absorption spectra of dehydrogenated radicals of guanine system to those from oxidation by SO_4^-, it is suggested that the site of dehydrogenation may be on N1 of the guanine moiety in acid or neutral solution. However, in alkaline solution the site of dehydrogenation may be on N9 of Gua, but on N2 of Guo or dGMP. Thus, it may be concluded that the sites of dehydrogenation depend on both the pH of solution and the structure of the guanine components. Furthermore, the kinetics of the formation and decay of the exciplex arising from interaction of ^{3}A* and G[$-H^+$]$^-$ has been offered for the first time.

Acknowledgement

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