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Monophotonic ionization of guanine components in alkaline aqueous solution by 248 nm laser light: identification of guanine-derived radical anions

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Abstract

The effect of 248 nm laser light on alkaline aqueous solutions of guanine (Gua), guanosine (Guo), 2′-deoxyguanosine 5′-monophosphoric acid (dGMP), and polyguanylic acid (5′)(poly[G]) was investigated. Under these conditions monophotonic ionization was suggested to occur as inferred from the linear dependences of the concentration of ejected electrons. The quantum yields of formation of hydrated electrons (Φ_{e^-}) are therefore independent of the laser pulse intensities (I_L), and were determined with KI solution used as a reference. On the basis of pH titration, it was confirmed that photoionization occurs on deprotonated Gua and its derivatives. As shown by comparison with our previous results obtained from the acetone-sensitized photolysis, the ionized Gua, Guo and dGMP anion, i.e. the neutral radicals, deprotonate in alkaline solution, yielding radical anions that are the same as those from acetone-sensitized excitation.

Keywords: Guanine; Alkaline aqueous solution; Photoionization; Monophotonic process; Radical anions

1. Introduction

One of the processes leading to light-induced chemical modification of DNA is ionization. Photoionization of bases in DNA by laser pulses, which is the so-called direct effect in radiation biology, thereby generating selective damage in nucleic acids and avoiding the indirect effect of ionizing irradiation, has been applied successfully [1]. The investigation of the formation of laser-induced strand breaks in DNA, involving radical cations, should lead to a better understanding of the chemical mechanisms resulting from in the direct effect. Monophotonic ionization of DNA and its constituents has been confirmed experimentally for 193 nm laser pulses in aqueous solution, and with either $\lambda_{irr} = 266$ or 248 nm radiation, photoionization occurs via a biphotonic process [2].

It is well known [1,3] that guanine (Gua) is the most easily oxidized nucleic acid base. As a result an electron-loss center created in DNA fragments containing the four nucleic acid bases will end up in Gua. However, the energy provided by one photon of about 250 nm is not sufficient to ionize Gua. The reported [4,5] ionizations of Gua and its derivatives on interaction with about 250 nm light are therefore due to biphotonic processes.

In the present work, we have demonstrated the occurrence of monophotonic ionization of Gua and its derivatives in alkaline solution. This results as the formation of radical anions, which may be also generated from acetone-sensitized excitation.

2. Experimental details

Gua, guanosine (Guo), 2′-deoxyguanosine 5′-monophosphoric acid (dGMP), and polyguanylic acid (5′) (poly[G]) from Sigma were used as received. NaOH and H_3PO_4 (analytic grade reagent) were used as received. The pH values of solutions were adjusted by NaOH and H_3PO_4 solutions, and kept at 11.0–11.5. The Gua and its derivative samples were freshly prepared in dilute solutions and the absorbance (A) in a 1 cm cuvette was adjusted typically to 1.5–2.5 at the wavelength of excitation, $\lambda_{exc} = 248$ nm. The absorption spectra were recorded on a Shimadzu 210A spectrophotometer. All samples were prepared in triply distilled water and studied in quartz cuvettes at 15 ± 2 °C. For the determination of Φ_{e^-} in aqueous solution, the same A_{248} (e.g. 1.5 in 1 cm) was taken for a series of guanines and KI, at several concen-

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trations ($A_{248}=0.1$–1), was used as reference ($\Phi_{e^-}=0.23$) [6].

Laser photolysis experiments were performed using a home-made excimer laser which provided 248 nm (KrF) laser pulse with a duration of 20 ns. The maximum laser energy was 100 mJ per pulse. The laser and analysing light beam passed perpendicularly through a quartz cell. The transmitted light entered a monochromator equipped with a R955 photomultiplier. The signals were collected using an HP54510B 300 MHz transient recorder and then processed with an PC-486 personal computer. Detailed descriptions of the equipment and experimental conditions have been given elsewhere [7].

The pulse radiolysis experiment was carried out using electron pulse with a duration of 8 ns delivered from a 10 MeV linear electron accelerator. The experimental methods and the specifications of equipment have been described in a previous paper [8].

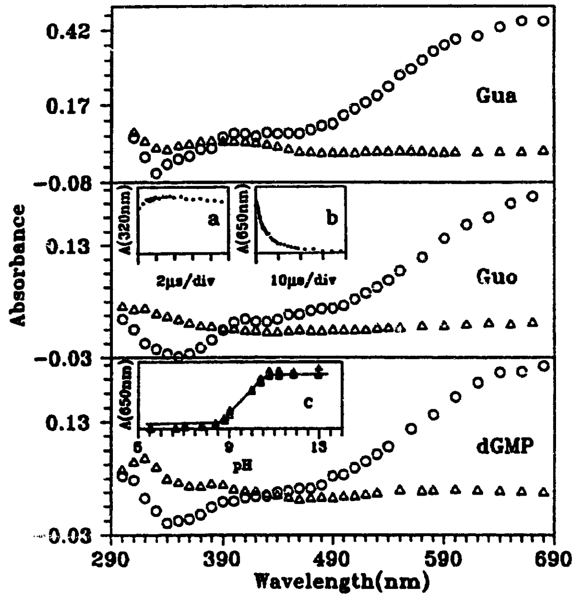


Fig. 1. Transient absorption spectra from photolysis of 0.3 mM G N_2-saturated aqueous solution at pH 11, Gua: 80 ns (○), 20 μs (△); Guo: 60 ns (○), 20 μs (△); dGMP: 60 ns (○), 20 μs (△), after the pulse. Insets: time dependence of the absorbance at (a) 320 nm and (b) 650 nm; (c) dependence on pH of 0.1 mM dGMP solution of the absorbance at 650 nm.

3. Results and discussion

3.1. Monophotonic ionization of Gua and its derivatives in alkaline aqueous solution

On 248 nm laser photolysis of deaerated alkaline solutions of Gua, Guo, dGMP and poly[G] relatively strong absorption changes were observed. The absorption spectra recorded at short time (about 100 ns) after the 20 ns pulse are characterized by three main features. These include a strong, broad band with a maximum at about 700 nm, a weaker absorption bands in the range 300–400 nm, and bleaching in the range 330–36 nm (see Figs. 1 and 2). The absorption at about 700 nm decays in deaerated solution within the 20 μs time range for Gua, Guo and dGMP (inset b of Fig. 1), and the 80 μs time range for poly[G]. The rate of the decay of about 700 nm is strongly increased by O_2 or N_2O. The transients absorbing at 300–400 nm decay in the millisecond time scale and are not affected by N_2O.

The absorption at about 700 nm is characteristic of the hydrated electron (e^-_{aq}). The appearance of e^-_{aq} is the direct evidence for ionization of guanine components.

For all of the guanine components studied, the amount of e^-_{aq} formed by ionization, expressed by absorbance at 650 nm measured 0.1 μs after the pulse, increases linearly with incident laser intensity (I_L) (insets a, b of Fig. 2). The ionization is therefore concluded to be a monophotonic process. The quantum yields of formation of hydrated electrons (Φ_{e^-}) have been determined, and are listed in Table 1.

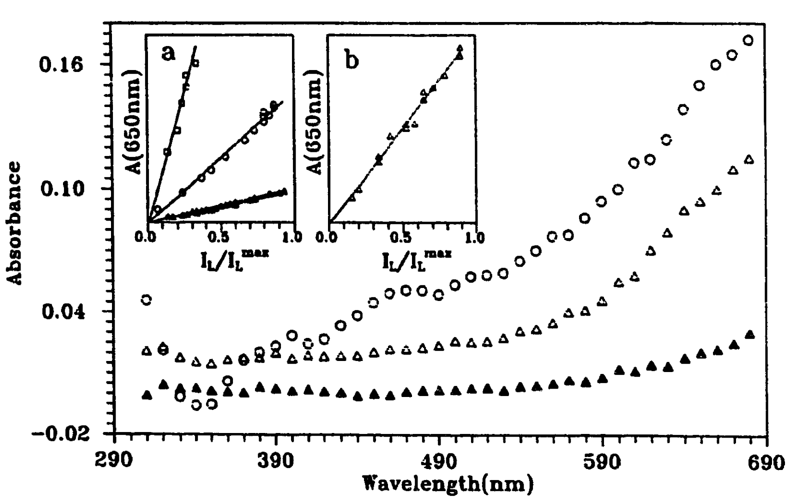


Fig. 2. Transient absorption spectra from photolysis of 0.5 mM poly[G] N_2-saturated aqueous solution at pH 11. 0.1 μs (○), 20 μs (△) and 75 μs (▲) after the pulse. Insets. (a) Dependence on laser intensity of the absorbance at 650 nm: KI (□, 6 mM), Gua (○, 0.3 mM), Guo (△, 0.3 Mm) and dGMP (▲, 0.3 mM). (b) Dependence on laser intensity of the absorbance at 650 nm: poly[G] (△, 0.3 mM).

Table 1
Quantum yield of e_{aq}^- and rate constants for reaction of e_{aq}^- with substrate

Compound	pK_a	Φ_{e^-} ($\times 10^{-4}$)		Laser photolysis [b]	Pulse radiolysis [c]
				$k(e_{aq^-})/(10^8\ M^{-1}\ s^{-1})$	
		248 nm [a]	193 nm		
Gua	9.6	610	570 [d]	14	9.1
Guo	9.2	160	730 [d]	6.3	6.4
dGMP	9.4	150	700 [d]	5.5	3.2
poly[G]		88	440 [e] 340 [f]	0.98 [g]	1.3 [g]

[a] Obtained from ΔA_{650} values 0.1 μs after the pulse in deaerated aqueous solution, at 15 ± 2 °C and pH 11.0–11.5.
[b] Rate constants for decay of e_{aq}^-, monitored by its absorption at 650 nm.
[c] Rate constants for reactions of e_{aq}^- with substrates, monitored by its absorption at 310 nm by pulse radiolysis.
[d,e,f] From Refs. [5,12,13], respectively.
[g] Rate constant per nucleotide unit.

In alkaline solution, Gua, Guo and dGMP (symbolized as G) deprotonate from N1 (Eq. (1)), and the pK_a values [9] are listed in Table 1. The pK_a values show that at pH 11 the predominant species of G is $G[-H^+]^-$.

The pH dependence on the amplitudes measured at 650 nm between pH 6 and 13 has a sigmoidal shape with an inflection point at pH = 9.5 (see inset c of Fig. 1), which is in accord with the pK_a value 9.4 of dGMP. Therefore, the photoionization occurs on the deprotonated Gua, Guo, dGMP and poly[G] ($G[-H^+]^-$). After G deprotonates from N1, the oxidation potential and ionization potential decrease [10,11], thus causing the monophotonic ionization of guanine components by 248 nm laser pulses. In addition, it may be predicted that monophotonic ionization of DNA will occur under 248 nm pulsed excitation in alkaline solution.

3.2. Identification of guanine-derived radical anions

The bleaching observed at 330–360 nm (Fig. 1) shows that the $G[-H^+]^-$, which absorbs in this wavelength range, is depleted upon photolysis owing to the formation of neutral radicals ($G[-H^+]^\bullet$) resulting from ionization of the guanine moiety (Eq. (2)). Since the pK values for protonation of $Guo[-2H]^{\bullet-}$ and $dGuo[-2H]^{\bullet-}$ were reported to be 10.7 and 10.8 respectively [14], the neutral radicals deprotonate rapidly in alkaline solution, and produce radical anions (Eq. (3)), which have absorption spectra with λ_{max} within the 300–400 nm range.

$$G \rightleftharpoons G[-H^+]^- + H^+ \tag{1}$$

$$G[-H^+]^- \xrightarrow{h\nu} G[-H^+]^\bullet + e_{aq}^- \tag{2}$$

$$G[-H^+]^\bullet \xrightarrow{+OH^-} G[-2H]^{\bullet-} + H_2O \tag{3}$$

The hydrated electrons formed by photoionization disappear in deaerated solutions with first-order kinetics, as shown by the exponential decay of absorbance at 650 nm. This is ascribed as the reactions of e_{aq}^- with untransformed guanine components predominantly, thus forming initially electron adducts, doubly negatively charged radicals, $G[-H^+]^{\bullet 2-}$ (Eq. (4)). The decay rate constants of e_{aq}^- have been listed in Table 1.

By use of pulse radiolysis with conductance detection, the radical anion of Guo has been found to be rapidly protonated by water at a heteroatom to give neutral radical $G[8CH]^\bullet$. It is therefore reasonable to assume that the dianionic radicals $G[-H^+]^{\bullet 2-}$ will undergo even faster protonation by water to give monoanionic radicals, $G^{\bullet-}$ [15] (Eq. (5)). These radical anions have absorption spectra with λ_{max} at 300–320 nm (results of pulse radiolysis) and their formation explains the second component of the growth trace observed at these wavelengths (see inset a of Fig. 1): the fast and concentration-independent component after the pulse is due to formation of the (deprotonated) neutral radicals [3] (Eqs. (2) and (3)).

$$G[-H^+]^- + e_{aq}^- \rightarrow G[-H^+]^{\bullet 2-} \tag{4}$$

$$G[-H^+]^{\bullet 2-} \xrightarrow{+H_2O} G^{\bullet -} + OH^- \tag{5}$$

The rate constants for the reactions of e_{aq}^- with the substrates which were obtained by pulse radiolysis are similar to those determined from laser photolysis (see Table 1). These rate constants are one order of magnitude lower than those in neutral solution [16]. It is reasonable to assume that electrostatic repulsion, owing to formation of anions resulting from the deprotonation of Gua moiety from N1 in alkaline solution, between the negative charged reactants, e_{aq}^- and anionic forms of the guanine components, retards the reaction of e_{aq}^- with substrate.

Recently, we have reported the formation of the radical anions of Gua, Guo and dGMP in the acetone-sensitized photolysis of the alkaline aqueous solutions. The radical anions are produced via an exciplex arising from interaction of triplet acetone ($^3A^*$) and $G[-H^+]^-$ [17]. Besides the electron transfer reaction, there is photoionization of $G[-H^+]^-$ upon the photolysis. However, the characteristic absorption spectrum of e_{aq}^- was not observed from the ace-

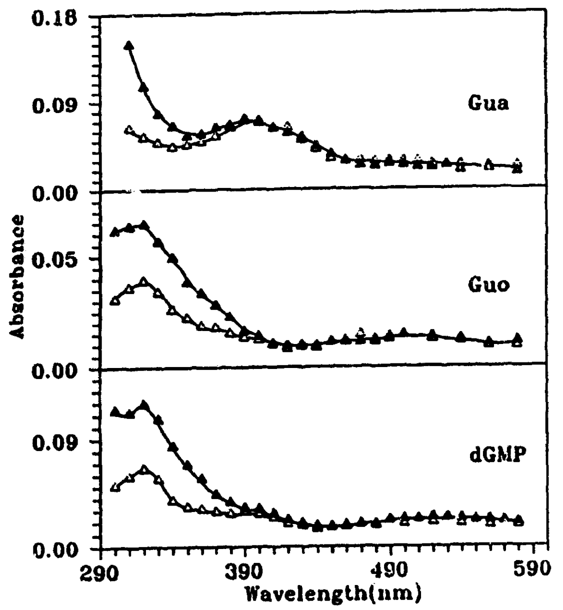


Fig. 3. Transient absorption spectra from photolysis of 0.3 mM G aqueous solution containing 20 mM tert-butyl alcohol N_2O-saturated at pH 11 (△, 1 μs) or 0.1 mM G aqueous solution containing 0.13 M acetone N_2-saturated at pH 11.3 (▲, 30 μs).

tone-sensitized photolysis [17]. The absence of the e_{aq}^- band can be explained in terms of a rapid reaction with acetone.

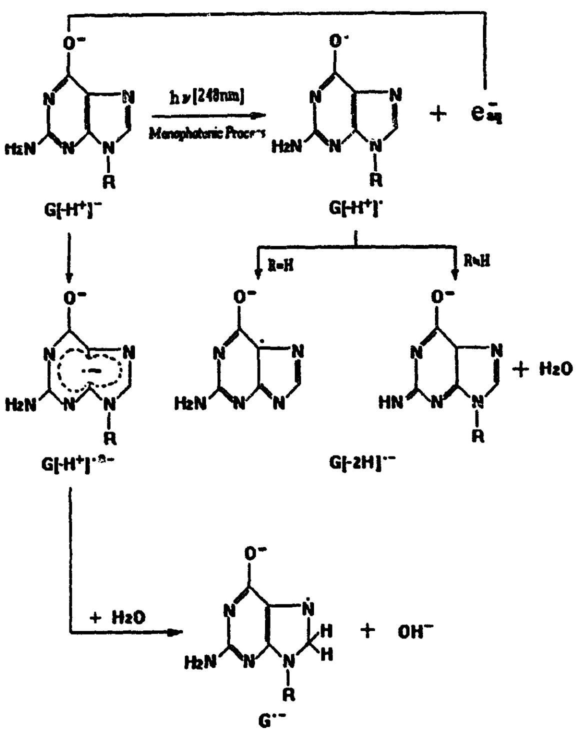
$$A + e_{aq}^- \rightarrow A^{\cdot -} \qquad (6)$$

Fig. 3 shows transient absorption spectra from the photolysis of the guanine components in the presence of tert-butyl alcohol saturated with N_2O and in the presence of acetone saturated with N_2, in alkaline solution, respectively. In the presence of an electron scavenger N_2O, the former absorption spectra result only from the radical anions, $G[-2H]^{\cdot -}$, because of the absence of reactions (4) and (5). The spectra in the region above 380 nm are identical with those of the photolysis of the solutions containing acetone, but in region 300–380 nm, the spectra obtained from the photoionization and from the acetone-sensitized excitation are not identical. This is ascribed as the absorption of isopropyl alcohol radical ($A[+H]^{\cdot}$) in the region for the solutions containing acetone [18].

Accordingly, it is concluded that the radical anions arising from neutral radicals formed by photoionization of the guanine components are the same as those from acetone-sensitized excitation in alkaline aqueous solution.

The transient absorption spectrum of $G[-2H]^{\cdot -}$ and that of $Guo[-2H]^{\cdot -}$ or $dGMP[-2H]^{\cdot -}$ are different as shown in Fig. 3. This may stem from different sites of deprotonation: on N9 for $Gua[-H^+]^-$ and on N2 for $Guo[-H^+]^-$ or $dGMP[-H^+]^-$ [17].

The results are summarized in Scheme 1.



G = Gua, Guo, dGMP or poly[G]

Scheme 1.

4. Summary and conclusions

It has been demonstrated that photoionization of the guanine components with 248 nm laser light occurs via a monophotonic process in alkaline solution, giving hydrated electrons and radical anions that results from the neutral radicals of the guanine components. The radical anions are the same as those from acetone-sensitized excitation. The quantum yield of photoionization decreases in the order Gua ≫ Guo > dGMP > poly[G]. The ionization occurs on the deprotonated Gua moiety.

For this reason, alkali is a sensitizer for the photochemical reaction of Gua and its derivatives. Monophotonic ionization of DNA may occur for 248 nm pulses in alkaline aqueous solution.

Acknowledgements

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