Laser Flash Photolysis Studies on Gallic Acid

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The transient species of gallic acid (GA) have been studied by 266 nm nanosecond laser flash photolysis in aqueous solution and acetonitrile. The intermediate with absorption at 320 nm was identified as excited triplet state (3GA*), the decay rates of which were obtained in aqueous solution and acetonitrile respectively. Energy transfer from 3GA* to β-carotene was observed and the energy transfer rate constant k_{ent} was determined to be 2.2 \times 10^9 \text{ mol}^{-1}\text{L}\cdot\text{s}^{-1}. GA underwent photoionization during photolysis and the quantum yield of photoionization was determined to be 0.12 at room temperature with KI as a reference.

**Keywords** gallic acid, photolysis, triplet state, photoionization

**Introduction**

It has been widely accepted that oxidative free radicals are involved in damage to biomolecules. There are enzymic and non-enzymic antioxidant defense systems in human body, but these systems are not perfect. When the balance between free radical production and antioxidant defense system is damaged, oxidative stress occurs which related to aging and various diseases.1-5

Antioxidants can scavenge reactive oxygen species (ROS), inhibit the production of ROS or repair the oxidized substrate so as to delay or inhibit the oxidation of lipids, nucleic acids and proteins. Growing evidences show that sufficient supplement of antioxidants has beneficial effect on disease prevention. Results have shown that maximum life span is positively related to antioxidant defenses.2 Hydroxycinnamic acid, flavonoid and carotenoid are among the most widely researched antioxidants, the antioxidant properties of which are studied using pulse radiolysis, trolox equivalent antioxidant capacity (TEAC) assay and other methods.6-10

Phenolics behave as antioxidants by electron donors or hydrogen donors to free radical oxidants and metal chelation. The beneficial health effects of “Mediterranean Diet” are partly ascribed to their containing high concentration of phenolics. Considerable results have evidenced that consumption of foods rich in phenolics can reduce incidence of heart disease, cancer and cardiovascular disease.11,12

Gallic acid (GA, 3,4,5-trihydroxybenzoic acid, Figure 1) is a good antioxidant found in a variety of natural products such as tea, grapes and various medicinal plants. It is one of the phenolic antioxidants used as an additive of food and pharmaceutical products. GA showed cytotoxicity against cancer cells but no cytotoxicity against normal lymphocytes.13 GA and its derivatives are known to inhibit human low density lipoprotein oxidation.14 It has also been reported to scavenge free radicals.15 Richards and Adams16 have shown that GA is able to repair the tryptophan radical to produce phenoxyl radical. Recently, it was reported that although GA is a free radical scavenger, it may exhibit prooxidant properties under certain conditions.17-19

![Figure 1](image)

The structure of gallic acid.

**Experimental**

Materials and methods

GA was purchased from Sinopharm Chemical Reagent Company (Shanghai, China) and used as received. Acetonitrile and β-carotene were obtained from Sigma. KI (analytical grade reagent) was recrystallized in pure water. The other chemicals used in this work were of the purest available grade and used without further purification.
All solutions were prepared freshly with pure water provided by Millipore purification system. UV-Visible absorption spectra were recorded with a Zeiss Specord S10 spectrophotometer. Laser flash photolysis experiments were carried out using a Nd:YAG laser which provides 266 nm pulses with a duration of 5 ns and a maximum energy of 80 mJ per pulse. The laser and analyzing light beam passed perpendicularly through a 1 cm quartz cell. The transmitted light entered a monochromator equipped with an R955 photomultiplier (Hamamatsu). The signals were collected using an HP54510B digital oscillograph and then transferred to computer to be treated.

Results and discussion

Photolysis of GA in aqueous solution

Figure 2 shows that a transient absorption spectrum at about 310 nm is formed together with a weak band longer than 500 nm after the 266 nm laser irradiation of 0.05 mmol/L GA in N₂-saturated aqueous solution. In order to identify the 500—700 nm absorbing species, the experiment was carried out using O₂ and N₂O as electron scavenger (inset of Figure 2 and Figure 3). It can be seen that the absorption band at 500—700 nm disappeared under O₂ or N₂O saturation, which means the absorption in the 500—700 nm region is assigned to hydrated electrons (e_q^-). The information of hydrated electrons shows that GA undergoes photoionization. The formed cation radical GA⁺ (pK’a = 5.0) turned into neutral radical by deprotonation under our experimental condition. According to the report in literature, the species with absorption at about λmax 310 nm was assigned to the neutral radical arising from deprotonation of cation radical.

Photolysis of GA in acetonitrile

In view of that excited triplet state is formed more easily in acetonitrile, photolysis of 0.05 mmol/L GA was performed in N₂-saturated acetonitrile solution (Figure 4). A comparison of the spectral data from aqueous solution and acetonitrile solution indicated that in the latter case a broader band was observed. When 0.5 mmol/L fumaronitrile was added to the solution as a triplet scavenger, the species with absorption at λmax 320 nm was affected distinctly. However, no effect on the decay of the species with absorption at λmax 300 nm was detected even in the presence of 50 mmol/L fumaronitrile. So, the intermediate with absorption at 320 nm was identified as excited triplet state of GA (³GA*) and the intermediate with absorption at 300 nm as the neutral radical of GA.

β-Carotene (Car) is a good triplet energy acceptor due to its low triplet energy (ET = 88 kJ/mol). The triplet nature of the species (320 nm) was further confirmed in the present work by quenching with β-carotene. Figure 5 shows transient absorption spectra observed by the 266 nm laser light irradiation of GA in the presence of β-carotene in acetonitrile. It is evident that a new absorption band appeared at 510 nm at 3 μs after laser irradiation. This novel transient species can be assigned to the triplet-excited state of β-carotene (³Car*). Inset of Figure 5 displays the growth and decay for the transients. The appearance of ³Car* with the decay of ³GA* supports the following energy transfer
Transient absorption spectra observed by 266 nm laser flash photolysis of 0.05 mmol/L GA in the presence of 0.5 mmol/L β-carotene in N₂-saturated acetonitrile solution at 0.1 µs (○) and 3 µs (■). Inset: absorption-time profiles at 320 and 510 nm.

The kinetic analysis showed that 3Car* grows in first order process. From a dependence of the generation rate of 3Car* on the concentration of Car, an energy transfer rate constant $k_{\text{ent}}$ can be estimated to be $2.2 \times 10^9$ mol$^{-1}$L$^{-1}$s$^{-1}$.

Based on these results, a general scheme of GA photolysis can be drawn.

$\text{GA*} + \text{Car} \rightarrow k_{\text{ent}} \rightarrow \text{GA} + 3\text{Car*}$

Decay rate constants and lifetime of $^3\text{GA*}$

$^3\text{GA*}$ decays via a first-order process in both aqueous solution and acetonitrile. Triplet decay rate was increased with the increasing of GA concentration, which may be attributed to the triplet quenching by ground-state GA. The absorbance was measured at 320 nm as a function of the concentration of GA. From a plot of the pseudo-first-order rate constant of the triplet decay versus the ground state concentration, bimolecular rate constants of $1.4 \times 10^9$ and $6.8 \times 10^9$ mol$^{-1}$L$^{-1}$s$^{-1}$ were calculated for the self-quenching. Lifetimes of 0.3 µs and 1.2 µs were measured for the decay of $^3\text{GA*}$ in N₂-saturated aqueous solution and in N₂-saturated acetonitrile solution respectively (0.05 mmol/L GA).

Quantum yield of photoionization

In order to determine whether the photoionization of GA by 266 nm photons is caused by a monophoton process, the yield of $e_{\text{aq}}^-$ at 650 nm formed by ionization was measured from the optical density (OD) value

$\text{GA} \xrightarrow{\text{hv}} ^1\text{GA*} \xrightarrow{\text{intersystem crossing}} ^3\text{GA*}$

$\text{GA} \xrightarrow{\text{hv}} \text{GA}^+ + e_{\text{aq}}^-$

To determine the quantum yield of photoionization in aqueous solution, a series of potassium iodide KI solution was used as a reference (the quantum yield of $e_{\text{aq}}^-$, $\Phi_e = 1$). Concentrations of GA were varied from 0.02 to 0.12 mmol/L to have the same absorbance at 266 nm with KI solutions. By comparing the slope at 650 nm, the quantum yield of photoionization $\Phi_{\text{PI}}$ was determined to be 0.12 relative to that of KI at room temperature (Figure 7). This result shows that the ionization of GA in water is an efficient process.

Conclusion

GA can undergo photoionization by 266 nm laser flash photolysis. The quantum yield of photoionization was determined to be 0.12 at room temperature. The intermediate with the absorption at 320 nm is $^3\text{GA*}$ whose lifetime is about 0.3 µs in aqueous solution and 1.2 µs in acetonitrile. $^3\text{GA*}$ can be quenched by β-carotene and the quenching rate constant was obtained. Elucidating the mechanisms of the actions of antioxidants is of great significance in fields including nutrition.
medicine, and radiation chemistry, etc. Since the effect of antioxidants is often executed in complex biological mixtures where various interactions may take place, much work remains to be done in vivo.

References