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Visible-light promoted photoprocesses on aqueous gallic acid in the presence of riboflavin. Kinetics and mechanism

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ABSTRACT

Within the frame of possible precursory photoreactions in the generation of humic substances, the visible-light promoted interaction between riboflavin (Rf), a native photosensitizer in aqueous systems, and gallic acid (GA), a polyphenol naturally formed after lignin degradation, was investigated. A systematic kinetic and mechanistic study was conducted under aerobic conditions in aqueous media, through visible-light continuous photolysis, polarographic detection of oxygen uptake, stationary and time resolved fluorescence spectroscopy, time resolved near-IR phosphorescence detection and laser flash photolysis techniques. GA is degraded relatively fast in pH 7 aqueous solutions, where singlet molecular oxygen ($O_2(^1\Delta_g)$), superoxide radical anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) – all three species photogenerated from triplet excited Rf – participate in the photoprocess. The general conclusion is that in natural waters GA can undergo spontaneous phototodegradation under environmental conditions. Radical species generated in the presence of Rf can participate in condensation or polymerization reactions promoting the natural synthesis of humic products.

1. Introduction

Humic acids (HA) play a dominant role in conditioning soil properties. They are species of relatively high molecular weight with several functional groups, predominantly carboxylic and phenolic ones [1]. Although the precise reaction sequence of natural HA synthesis is uncertain, apparently the oxidative polymerization of polyphenols in waters and soils is thought to be among the major processes of formation of natural humic substances [2]. Recent studies relating polyphenol content in olive-mill composting and lignin content suggest that polyphenols contribute to the synthesis of humic substances [3]. In fact, very recently was synthesized a water soluble humic-acid-like polycondensate, which mimics fundamental physicochemical and spectroscopic properties of natural humic acids [4]. The polymer was produced under oxygenated ambient by oxidative co-polymerization of gallic acid (GA) and protocatechuic acid. On these grounds, the knowledge of different natural oxidation channels and the generation of potentially reactive radicals that could operate in the early steps of carboxylic polyphenols oxidation may be of importance in order to understand the HA generation, under environmental conditions.

Many papers have been published on polyhydroxybenzenes and particularly on GA oxidation, through photochemical, enzymatic and thermal reactions [5–11]. GA abounds in nature since it is among the low molecular weight phenolic acids formed after lignin degradation [12]. In natural waters, the colorless GA could be oxidized under solar irradiation if some compounds, named photosensitizers, able to absorb visible light and to generate reactive excited or reactive oxygenated species (ROS), are present in the same aqueous environment. Several dissolved organic compounds usually present in natural aqueous media can act as solar photosensitizers [13]. Riboflavin (Rf, vitamin B_2), a well-known natural pigment present as traces in waters of rivers, lakes and seas [14,15] has been repeatedly postulated as a possible sensitizer for the photooxidative degradation of natural substrates and contaminants [16] and as a photoinitiator of polymerization in aqueous media [17]. The mechanism of sensitization with Rf is rather complex, in many cases with the concurrent involvement of the ROS $O_2(^1\Delta_g)$ and $O_2^{\bullet-}$, both generated with quantum yields of 0.49 and 0.009, respectively [18].

The aim of the present work was to carry out a kinetic and mechanistic study on the photoprocesses that operate on the polyhydroxylaromatic compound GA, under conditions fre-

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quently found in nature: an aqueous environment, in the presence of the native photosensitizer Rf, and under visible-light illumination. This knowledge could contribute to the understanding of possible mechanistic pathways for the initiation of condensations or polymerization reactions involving GA and other polyphenols in the medium, promoting the generation of humic substances.

2. Materials and methods

2.1. Materials

Riboflavin (Rf), deuterium oxide 99.9% (D_2O), superoxide dismutase (SOD) from boverythrocytes and gallic acid (GA), pyrogallol (PG), sodium azide (NaN_3) and catalase from bovine liver (CAT) were purchased from Sigma Chem. Co. Rose Bengal (RB), perinaphtenone (PN) and furfuryl alcohol (FFA) were from Aldrich. Hydrogen peroxide (H_2O_2), 100 vol. was from Parsol (Argentine). D_2O was employed in the time resolved determinations of $O_2(^1\Delta_g)$ in order to enlarge the lifetime of this species [16]. Water was triply distilled. Methanol (MeOH), HPLC quality, was provided by Sintorgan (Argentine).

The pH of the final aqueous solutions (Rf + GA or Rf + PG, in water) for all photochemical experiments was in the range 7 ± 0.2, employing buffered aqueous solutions prepared, with KH_2PO_4 0.025 M/Na_2HPO_4 0.025 M [19]. The presence of the salts in the mentioned concentrations did not affect neither the lifetimes nor the profiles of the optical spectra of Rf electronically excited states, as compared to those obtained in pure water.

2.2. Absorption and fluorescence measurements

Ground state absorption spectra were registered employing a Hewlett-Packard 8452A diode array spectrophotometer. Fluorescence lifetimes were determined with a time-correlated single photon counting technique (SPC) on an Edinburgh FL-9000CD instrument. Excitation and emission wavelengths for Rf were 445 and 515 nm, respectively. A classical Stern–Volmer treatment of the data was applied through Eq. (1), where $^1\tau$ and $^1\tau_0$ are the respective fluorescence lifetimes of Rf in the presence and in the absence of GA, and ^1k_q is the rate constant of the quenching of excited singlet Rf (^{1}Rf*) by GA:

$$\frac{^1\tau_0}{^1\tau} = 1 + {}^1k_q{}^1\tau_0[\mathrm{GA}] \quad (1)$$

2.3. Laser flash photolysis experiments

Argon-saturated 0.04 mM Rf aqueous solutions were photolysed using a flash photolysis apparatus. A ns Nd:Yag laser system (Spectron) at 355 nm was the excitation source, employing a 150 W Xenon lamp as analyzing light. The detection system comprised a PTI monochromator and a red-extended photomultiplier (Hamamatsu R666). The signal, acquired and averaged by a digital oscilloscope (Hewlett-Packard 54504A), was transferred to a PC via a Hewlett-Packard Interface Bus (HPIB), where it was analyzed and stored.

Triplet Rf (^{3}Rf*) was generated by a 355 nm laser pulse, and its disappearance was monitored from the first order decay of the absorbance at 670 nm, a zone where the interference from other possible species is negligible. The triplet decay was measured at low Rf concentration (typically 0.05 mM) and at low enough laser energy, to avoid self-quenching and triplet–triplet annihilation. The rate constant for the interaction ^{3}Rf*–GA (^3k_q, reaction (7) in Scheme 1) was determined from a Stern–Volmer treatment (Eq. (2)):

$$\frac{^3\tau_0}{^3\tau} = 1 + {}^3k_q{}^3\tau_0[\mathrm{GA}] \quad (2)$$

where $^3\tau$ and $^3\tau_0$ are the experimentally determined lifetimes of ^{3}Rf* in the presence and in the absence of a GA, respectively.

2.4. Time resolved $O_2(^1\Delta_g)$ phosphorescence detection (TRPD)

The total quenching rate constant (k_t, see Scheme 1) for $O_2(^1\Delta_g)$ deactivation by GA, was graphically determined by near-IR time resolved phosphorescence, employing Eq. (2), being τ and τ_0 the respective $O_2(^1\Delta_g)$ lifetimes in the presence and in the absence of GA.

$$\frac{\tau_0}{\tau} = 1 + k_t\tau_0\,[\mathrm{GA}] \quad (3)$$

The third harmonic (λ = 355 nm) from a Nd:Yag laser (Spectron) was used as the excitation source. The emitted ($O_2(^1\Delta_g)$) phosphorescence at 1270 nm was detected at right angles using a Edinburgh EI-P Germanium detector, after having passed through 1270 nm-interference and two wratten filters. The output of the detector was coupled to a 400 MHz digital oscilloscope (HP 54504A) and to a personal computer to carry out the signal processing. Usually, 10 shots were needed for averaging so as to achieve a good signal to noise ratio, from which the decay curve was obtained. Air equilibrated solutions were employed in all cases.

2.5. Stationary photolysis and oxygen uptake experiments

Stationary aerobic photolysis of aqueous solutions containing typically 0.2–0.5 mM GA or PG and 0.04 mM Rf was carried out in a PTI unit, provided with a high pass monochromator and 150 W Xe lamp, irradiating with 445 ± 10 nm, or in a home-made photolyser for non-monochromatic irradiation (150 W quartz-halogen lamp). In this case cut-off filters (360 nm) ensured that the light was only absorbed by the sensitizer.

The Rf-sensitized photooxygenation rates of 0.5 mM GA and PG and 0.02 mM Rf were determined by evaluation of the initial slopes of oxygen consumption *vs.* irradiation time, employing a specific oxygen electrode (Orion 97-08).

Oxygen uptake in water was monitored with a 97-08 Orion electrode. Assuming that the reaction of RB-generated $O_2(^1\Delta_g)$ with the quencher is the only way of oxygen consumption, the ratio of the first order slopes of oxygen uptake by GA, each at the same concentration, yields k_r/k_{rR}. The reference was FFA, with a reported pH-independent k_r value of 1.2×10^8 M^{-1} s^{-1} [20].

3. Results

3.1. The sensitized photooxidation process

The main photoinduced processes that take place when a solution containing GA and Rf is irradiated with visible light in the presence of oxygen, are shown in Scheme 1. These processes include photoinduced reactions, both in the presence and in the absence of a substrate, the prevalence of which usually depends on the experimental conditions and on the involved compounds GA and Rf. This self-explanatory Scheme has been previously discussed in regard with similar processes applied to other substrates [16]. P(n) represent different photoproducts. The reported pK values for GA are 4.21 and 8.54 and, respectively correspond to the acidic ionization of carboxylic group and the ionization of the first phenolic group [21]. Hence, in the following the symbol GA will represent the monoanionic form of gallic acid, the species present

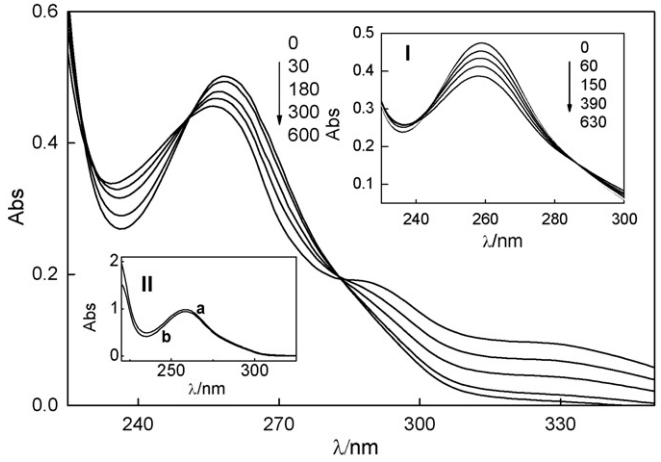

Fig. 1. Changes in the UV–vis absorption spectrum of a pH 7 aqueous solution of 0.04 mM riboflavin (Rf) plus 0.06 mM gallic acid (GA), taken *vs.* solvent, upon irradiation ($\lambda_{irr} > 360$ nm) under air-saturated conditions; inset I: changes in the UV–vis absorption spectrum of a pH 7 aqueous solution of Rose Bengal (RB) ($A(549) = 0.41$) plus 0.06 mM GA, taken *vs.* RB ($A(549) = 0.41$), upon irradiation under the same conditions of the main figure. Inset II: UV–vis absorption spectra of pH 7 aqueous solutions of GA 0.12 mM *vs.* solvent (a) and the same taken *vs.* solvent plus H_2O_2 0.05 mM in the presence of H_2O_2 0.05 mM.

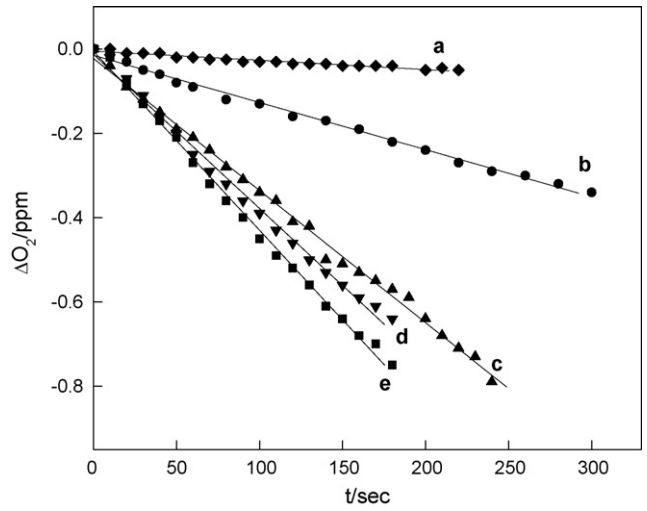

Fig. 2. Oxygen uptake as a function of photoirradiation time of pH 7 aqueous solutions containing 0.02 mM Rf (a) and 0.02 mM Rf plus: 5 mM GA and 2 mM NaN_3 (b); 0.5 mM GA plus 1 μg/ml CAT (c); 0.5 mM GA plus 1 μg/ml SOD (d) and 0.5 mM GA (e).

at pH 7.

$$Rf + h\nu \rightarrow {}^1Rf* \rightarrow {}^3Rf* \quad (4)$$

$${}^1Rf* + GA \rightarrow Rf + GA \text{ or } P(5), \quad \text{rate constant } {}^1k_q \quad (5)$$

$${}^3Rf* + O_2({}^3\Sigma_g^-) \rightarrow Rf^{\bullet+} + O_2^{\bullet-} \quad (6)$$

$${}^3Rf* + GA \rightarrow Rf^{\bullet-} + GA^{\bullet+}, \quad \text{rate constant } {}^3k_q \quad (7)$$

$$Rf^{\bullet-} + H^+ \leftrightarrow RfH^{\bullet} \quad (8)$$

$$2RfH^{\bullet} \rightarrow Rf + RfH_2 \quad (9)$$

$$RfH_2 + O_2({}^3\Sigma_g^-) \rightarrow RfH_2^{\bullet+} + O_2^{\bullet-} \quad (10)$$

$$RfH_2^{\bullet+} + O_2^{\bullet-} \rightarrow Rf + H_2O_2 \quad (11)$$

$$O_2^{\bullet-} + GA \rightarrow P(12) \quad (12)$$

$$H_2O_2 + GA \rightarrow P(13) \quad (13)$$

$${}^3Rf* + O_2({}^3\Sigma_g^-) \rightarrow Rf + O_2({}^1\Delta_g), \quad \text{rate constant } k_{ET} \quad (14)$$

$$O_2({}^1\Delta_g) \rightarrow O_2({}^3\Sigma_g^-) \quad (15)$$

$$O_2({}^1\Delta_g) + GA \rightarrow O_2({}^3\Sigma_g^-) + GA, \quad \text{rate constant } k_q \quad (16)$$

$$O_2({}^1\Delta_g) + GA \rightarrow P(17), \quad \text{rate constant } k_r \quad (17)$$

Being $k_t = k_r + k_q$.

Scheme 1

3.2. Stationary irradiation of solutions of GA in the presence of sensitizers

The visible-light irradiation of 0.06 mM GA, in pH 7 water and in the presence of 0.04 mM Rf, produces spectral modifications that indicate, as shown in Fig. 1 (main), possible transformations of both GA and Rf.

In parallel experiments, oxygen consumption was detected upon irradiation of solutions containing 0.04 mM Rf and 0.4 mM GA (Fig. 2).

The results herein shown clearly indicate that either Rf electronic excited states or ROS produced through these states, or even both processes operating simultaneously, are responsible for the photodegradation of GA. It is known that Rf is highly reactive under visible-light irradiation of its solutions, due to the generation of ROS [15,16]. On this basis, we have carried out a systematic kinetic study in order to evaluate and characterize the nature, mechanism and extent of the possible processes involved in the Rf-sensitized degradation of GA.

The rates of oxygen consumption by GA were determined using Rf, PN or RB as sensitizers. In the case of Rf oxygen was slightly consumed in the absence GA, as shown in Fig. 2. This rate of oxygen uptake must be considered negligible as compared to that observed in the presence of GA. Oxygen consumption by a GA solution in the absence of light was practically null, within the same time scale.

In the Rf-sensitized experiments, the individual presence of SOD (1 μg/ml), NaN_3 (2 mM) or CAT (1 μg/ml) caused a delay in the oxygen uptake (Fig. 2). Similar experiments have been formerly employed to confirm/discard the participation of $O_2({}^1\Delta_g)$, $O_2^{\bullet-}$ and H_2O_2 in a given oxidative event, respectively [22–24]. The enzyme SOD dismutates the species $O_2^{\bullet-}$ (reaction (18)), whereas CAT decomposes H_2O_2 (reaction (19)), and NaN_3 efficiently quenches $O_2({}^1\Delta_g)$ by a physical fashion (reaction (16), with NaN_3 instead of GA).

$$2O_2^{\bullet-} + 2H^+ + SOD \rightarrow O_2({}^3\Sigma_g^-) + H_2O_2 \quad (18)$$

$$2H_2O_2 + CAT \rightarrow 2H_2O + O_2({}^3\Sigma_g^-) \quad (19)$$

The viability of the reaction between GA and H_2O_2 (reaction (13)), was independently checked. Fig. 1, inset II, shows the absorption spectra of 0.12 mM GA aqueous solution after and before addition of 0.05 mM of H_2O_2. The spectrum was taken immediately after the addition of H_2O_2. It was controlled in the next five minutes, and remains unmodified. Neat spectral changes indicate the oxidation of GA.

The rates of oxygen consumption for PG, upon Rf and PN sensitization, were determined for comparative purposes. Results are shown Fig. 3A and B. All runs were performed under identical experimental conditions. It can be seen that the rate of oxygen uptake by PG upon PN-sensitization is higher than the corresponding one for GA with the same sensitizer, whereas an opposite behaviour is observed for the case of Rf-sensitization.

PG is the primary product identified by thermal degradation of GA, indicating that decarboxylation is an effective degradation pathway [25]. PG is a moderate quencher of $O_2({}^1\Delta_g)$ with reported rate constant values $k_t = 5 \times 10^7\ M^{-1}\ s^{-1}$ and $k_r = 1.9 \times 10^7\ M^{-1}\ s^{-1}$ determined in aqueous solutions [5].

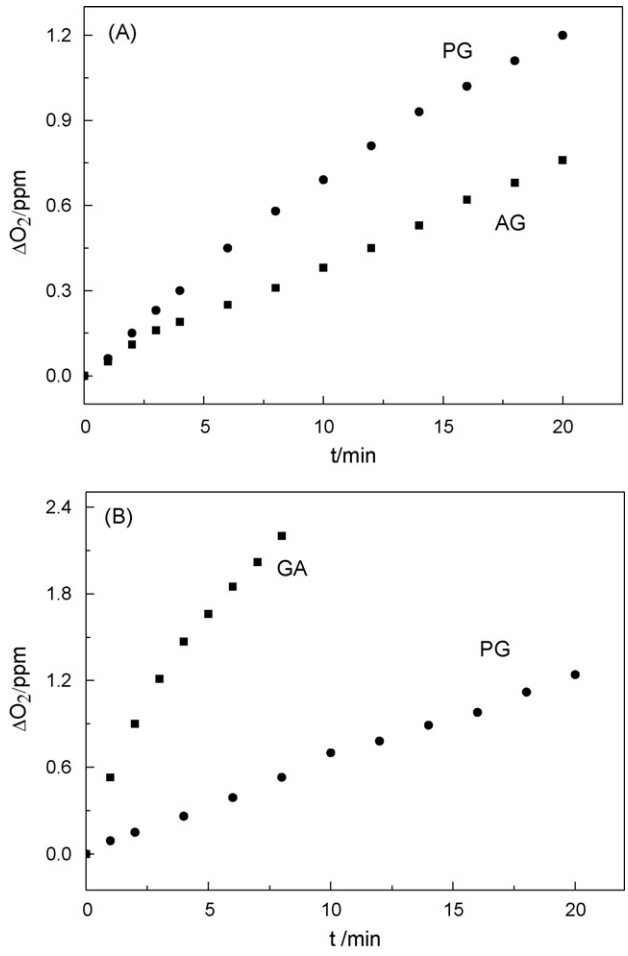

Fig. 3. Oxygen uptake as a function of photoirradiation time of pH 7 aqueous solutions containing: (A) perinaphtenone $A(370) = 0.3$ plus 0.5 mM PG (●); perinaphtenone $A(370) = 0.3$ plus 0.5 mM GA (■) and (B) 0.04 mM Riboflavin plus 0.5 mM GA (■); 0.04 mM riboflavin plus 0.5 mM PG (●).

3.3. Interaction $^1Rf^*$–GA

Rf presents in water an intense fluorescence emission band centered at 515 nm, with a reported [26] fluorescence quantum yield value of 0.25. The presence of GA, the quenching of $^1Rf^*$ (Scheme 1, process (5)) produces a decrease in the stationary emission intensity, but the shape of the emission spectrum does not change. The fluorescence decay of Rf in the absence and in the presence of a GA, as determined by the SPC technique, was monoexponential. Fig. 4 shows the Stern–Volmer plot obtained, from which a rate constant $^1k_q = 1.2 \pm 0.1 \times 10^{10}\ M^{-1}\ s^{-1}$ (process (5)) was evaluated.

3.4. The quenching of $^3Rf^*$ by GA

Addition of GA to a pH 7 aqueous solution of Rf 0.01 mM shortened the $^3Rf^*$ lifetime. The rate constant ^3k_q graphically determined for process (7) was $2.7 \pm 0.1 \times 10^7\ M^{-1}\ s^{-1}$ (Fig. 5).

The transient absorption spectrum of the solution, taken at 5 μs after the laser pulse (Fig. 5, trace (a)), is similar to that reported for $^3Rf^*$ in H_2O [27]. The trace (b), recorded at the same time after the laser pulse, under identical experimental conditions but in the presence of GA 3.6 mM, has a similar shape to that produced by the species ($RfH^\bullet$) [28,29]. This result could indicate that the quenching of $^3Rf^*$ by GA is due to an electron transfer process from the GA aromatic ring, with the concomitant production of $Rf^{\bullet -}$ (reaction (7), Scheme 1), followed by a rapid protonation

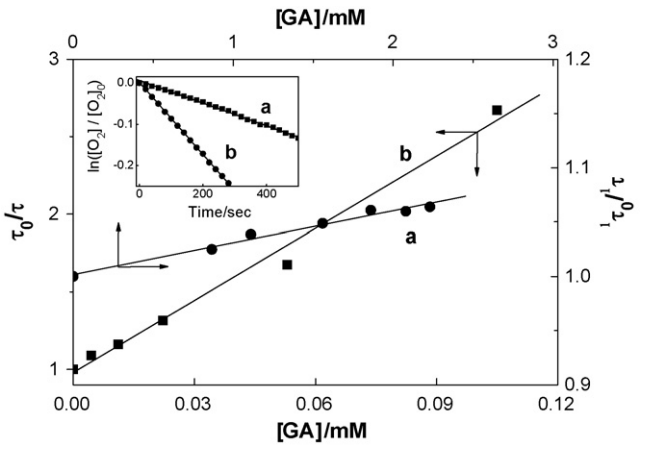

Fig. 4. Stern–Volmer plots for the quenching of (a) time resolved riboflavin fluorescence by GA; (b) time resolved phosphorescence emission of $O_2(^1\Delta_g)$. Inset: first order plots for oxygen uptake by 0.5 mM GA (a) and furfuryl alcohol 0.5 mM (b).

(step (8)). A pK value of 8.3 has been reported for the species $RfH^\bullet$ [30].

3.5. Interaction between $O_2(^1\Delta_g)$ and GA

When solutions of GA were irradiated with visible light in the presence of RB or PN ($Abs_{530} = 0.5$), two well-known $O_2(^1\Delta_g)$ generators [31,32], spectral changes shown in Fig. 1, inset were observed, strongly suggesting some degree of interaction of $O_2(^1\Delta g)$ with the substrates. The decay kinetics of $O_2(^1\Delta_g)$ phosphorescence in pD = 7 D_2O solutions was first order, and the lifetime agreed well with literature data [33]. The addition of a GA as a quencher led to a decrease of the $O_2(^1\Delta_g)$ lifetime, unambiguously confirming the interaction $O_2(^1\Delta_g)$–GA. The k_t value graphically obtained through expression (3), was $2.6 \times 10^8\ M^{-1}\ s^{-1}$ (Fig. 4). The rate constant for the reactive interaction, $k_r = 3.8 \times 10^7\ M^{-1}\ s^{-1}$ process (17) was obtained by the already mentioned actinometric method, measuring oxygen consumption (Fig. 4, inset).

The $O_2(^1\Delta_g)$-mediated photooxidation quantum efficiency [34] is not easy to evaluate, particularly in natural environments, because the determination includes the knowledge of the concentration of the photooxidizable substrate. A simpler and useful

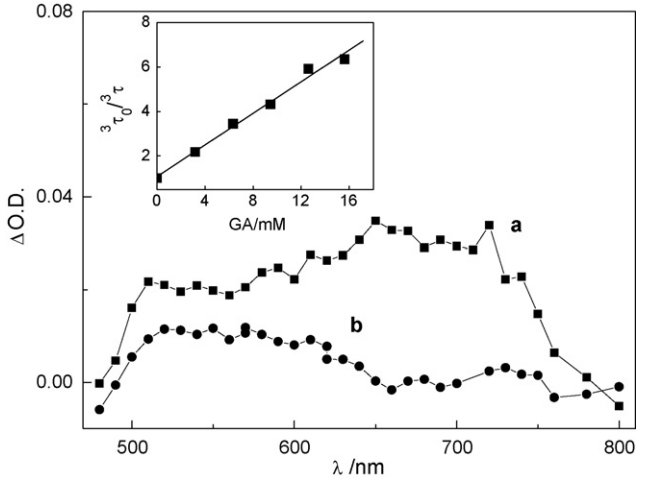

Fig. 5. Transient absorption spectra of riboflavin 0.02 mM in argon-saturated pH 7 aqueous solution, taken 10 μs after the laser pulse in the absence (a) and in the presence of 3.6 mM GA. Inset: Stern–Volmer plot for the quenching of riboflavin triplet state by GA in argon-saturated solutions.

approach is the evaluation of the k_r/k_t ratio, which indicates the fraction of overall quenching of $O_2(^1\Delta_g)$ by the substrate that effectively leads to a chemical transformation. In the present case $k_r/k_t = 0.14$ for GA, at pH/pD 7.

4. Discussion

GA interacts physically and chemically with $O_2(^1\Delta_g)$. In the cases of phenol and polyhydroxybenzenes [34], the accepted mechanism is the initial formation of an encounter complex $O_2(^1\Delta_g)$–substrate with partial charge-transfer character, from which an irreversible electron transfer process would yield the photooxidation products. The formation of this complex is governed by the electron-donor ability of the substrate. In the case of phenols the most photooxidizable species are the OH-ionized ones, due to their enhanced electron-donor capacities. As said, GA is in a monoanionic form at pH 7. The OH group remains in the molecular form and for this reason the reactivity of GA towards $O_2(^1\Delta_g)$ is only moderate. Nevertheless, although the value of 0.15 for the ratio k_r/k_t indicates a considerable degree of physical component in the quenching of $O_2(^1\Delta_g)$, oxygen consumption experiments and the evolution of GA absorption spectrum upon Rf-sensitized photoirradiation, demonstrate that the tri-hydroxy derivative is effectively photooxidated by this mechanism. The parent compound PG is more reactive towards the oxidative species. In this case, the quotient $k_r/k_t = 0.4$, according to published data [5]. This increase in photooxidation efficiency is due to the presence of a significant fraction of ionized OH group in PG, given that the pK for the ionization of the first phenol group is 7.26 [5].

In the visible-light irradiation of systems Rf–GA in the presence of 2 mM NaN_3, the $O_2(^1\Delta_g)$ lifetime should be reduced by a factor of ca. 15. This value was calculated through a simple Stern–Volmer treatment employing $k_t = 4.5 \times 10^8\ M^{-1}\ s^{-1}$ [35] and an $O_2(^1\Delta_g)$ lifetime $\tau_0 = 4\ \mu s$ [33]. However, the rate of oxygen uptake by GA under these conditions was only reduced by a factor of ca. 3.8 (Fig. 2). This fact strongly supports the existence of additional channels of oxygen consumption different from the $O_2(^1\Delta_g)$-driven process, possibly represented by $O_2^{\bullet-}$ and H_2O_2 mediated mechanisms. A decrease in the oxidation rate in the presence of SOD (Fig. 2) has already been reported for other hydroxyaromatic compounds [36]. In our case, a decrease in the rate of oxygen uptake was observed, due to the dismutation of $O_2^{\bullet-}$ (reaction (18)) demonstrating the involvement of this species in the overall oxidative event.

The decrease in the rate of oxygen uptake in the presence of CAT (Fig. 2, reaction (19)) confirms the participation of H_2O_2 as a reactive species. Besides, independent results demonstrate the viability of the reaction between GA and H_2O_2 (Fig. 1, inset B).

As said, the quantum yield for the straightforward generation of $O_2^{\bullet-}$ by ^{3}Rf* (reaction (6)) is extremely low: 0.009 [18] and should be ignored as a possible $O_2^{\bullet-}$ source for the ROS in the present case. Besides, the possibility of $O_2^{\bullet-}$ generation from ^{1}Rf* must be neglected on the basis of the sub-mM values of GA concentration employed in the stationary photolysis experiments, unable to effectively quench ^{1}Rf*, according to the observed ^1k_q value. The thermodynamic feasibility of the electron transfer, process (7), for the case of Rf–GA can be evaluated by means of the Gibbs free energy for electron transfer, $\Delta_{ET}G_0 = E_{0(GA/GA+)} - E_{0(Rf/Rf-)} - E_{Rf^*} + C$, where $E_{0(GA/GA+)} = 0.799$ V [37] is the standard electrode potential of the donor cation radical, $E_{0(Rf/Rf-)}$ is the standard electrode potential of the acceptor Rf (−0.80 V), E_{Rf^*} is the ^{3}Rf* energy (2.17 eV), and C is the coulombic energy term (−0.06 V) [38]. The so-calculated $\Delta_{ET}G_0$ value (−0.63 V) indicates that process (7) may be operative and, consequently, that the species $O_2^{\bullet-}$ could be formed by electron transfer from Rf (process (10)), if process (7) is kinetically competitive with the $O_2(^1\Delta_g)$ generation (reaction (14)). In principle, the dominant mechanism will depend on the predominance of either $O_2(^3\Sigma_g^-)$ or GA in the ^{3}Rf* quenching. It is known that reaction (14) occurs with a rate constant k_{ET} in water of $7 \times 10^8\ M^{-1}\ s^{-1}$, equivalent to 1/9 of the diffusional value [39,40]. Taking into account the experimental ^3k_q value found, and considering for comparative purposes equal concentrations of dissolved oxygen and GA, it can be deduced that the product k_{ET} $[O_2(^3\Sigma_g^-)]$ (reaction (14)) is ca. 25 times higher than the corresponding product ^3k_q [GA] (reaction (7))). This kinetic balance indicates that, under work conditions, the rate of generation of $Rf^{\bullet-}$ – the H_2O_2 precursor species – could compete, with the rate of $O_2(^1\Delta_g)$ production. In other words, the generation of H_2O_2 will be dependent on the actual concentration of GA. Oxygen concentration is constant, approximately 0.4 mM under air-saturated conditions.

The comparison of the rates of oxygen uptake at pH 7 for GA and PG, employing Rf or PN as sensitizers (Fig. 3) clearly indicates that the efficiencies for the respective reaction mechanisms, or the photogenerated ROS involved, are different each other. In the case of PN-sensitization the only oxidative species is $O_2(^1\Delta_g)$, provided that the quantum yield for the generation of this ROS by this dye is practically 1 [32], and the efficiency of oxygen uptake in much higher for PG. To the contrary, in the Rf-sensitized runs, the rate of oxygen uptake by GA is neatly higher than the corresponding one for PG. This result points out the importance of the $O_2^{\bullet-}$ and H_2O_2 mediated mechanisms in the case of GA.

Finally, we can say that although Rf also participates as a reactant in the proposed mechanism, the sensitizer is almost not degraded. Rf is reduced by processes (7), with $^3k_q = 2.7 \times 10^7$ and also reacts with $O_2(^1\Delta_g)$, by process (16), replacing Rf by GA, with a rate constant $k_{rRf} = 6 \times 10^7\ M^{-1}\ s^{-1}$. Nevertheless, processes (10) + (11) constitute a source for Rf regeneration by back electron transfer to ground state oxygen, a naturally-occurring reaction in biological environments that protects the dye against photodestruction. Furthermore, under work conditions, is $10 \times [Rf] \sim [GA]$ and being the respective rate constants for $O_2(^1\Delta_g)$ quenching by the vitamin and by GA quite similar each other, the sensitizer is neither decomposed in a considerable extent by this mechanism.

In conclusion, the experimental evidence and thermodynamic evaluation demonstrate that the simultaneous presence of dissolved Rf and GA upon visible-light irradiation, in aqueous-aerobic medium, induce photoprocesses that mainly degrade GA. The overall oxidative mechanism involves the participation of the ROS $O_2(^1\Delta_g)$, $O_2^{\bullet-}$ and H_2O_2 and includes as intermediary radical species of GA and Rf. In natural waters, these species could contribute to the initiation of polymerization reactions on GA and other polyphenols in the medium, promoting the generation of humic substances.

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