Research article

Visible-light-promoted degradation of the antioxidants propyl gallate and *t*-butylhydroquinone: Mechanistic aspects

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The kinetic and mechanistic aspects of the visible-light-mediated photodegradation of the phenolic antioxidants (PA), propyl gallate (PG), and *t*-butylhydroquinone (TBHQ), employing riboflavin (Rf) as photosensitizer, have been studied by time-resolved and stationary techniques. The photosensitizer Rose Bengal (RB) was used for auxiliary experiments. Results show the occurrence of chemical transformations on PA with the participation of electronically excited states of Rf and different reactive oxygen species (ROS) generated from these states. With 0.02 mM Rf and 1.0 mM PA, the electronically excited triplet state of Rf is quenched by PA, in a competitive manner with the dissolved oxygen. As a consequence, a cascade of photoprocesses produces singlet oxygen ($O_2(^1\Delta_g)$) and H_2O_2 in the case of PG and, $O_2(^1\Delta_g)$, H_2O_2 and $HO^\bullet$ in the case of TBHQ. The participation of these species is supported by experiments of oxygen consumption carried out in the presence of specific ROS scavengers. TBHQ has a relatively high capacity for $O_2(^1\Delta_g)$ physical deactivation and a low photodegradation efficiency by the oxidative species. Comparatively, it can be asserted that TBHQ has a higher antioxidant capacity than PG.

Keywords: Antioxidants, Propyl gallate, *t*-buthylhydroquinone, Photooxidation, Riboflavin, Reactive Oxygen Species

Introduction

Lipid oxidation in food is one of the main reasons for quality degradation during processing and storage. Deterioration in flavor, color, texture, nutritive value, and the production of toxic compounds are some of the negative consequences of lipid oxidation.[1–3] There have been numerous methods developed to control the rate and extend of lipid oxidation in foods, with one the most effective being the addition of antioxidants.[2,3] Butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate (PG), and *t*-butylhydroquinone (TBHQ) are the most widely used antioxidants in the food industry, especially for oils and fatty foods.[4,5] These synthetic antioxidants are exposed to daylight during the manufacture and storage of foods. Nevertheless, the antioxidants are transparent to visible light and their photodegradation depends on the presence in the protected medium of an adequate daylight-absorbing substance, named photosensitizer. Most of the commercial compounds are colored and/or possess different dyes and pigments that can act as photosensitizers and start an unpredictable series of photoreactions, many of them driven through the action of the so-called reactive oxygen species (ROS).[4] These photoreactions can involve antioxidant preservatives and affect their specific capacity, being this aspect of enormous importance in the commercial, health, and safety fields. The most frequent ROS generated by photosensitized processes are singlet oxygen ($O_2(^1\Delta_g)$), superoxide radical anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^\bullet$), and different free radicals, generally produced as primary reaction products.[6]

We have recently studied kinetic and mechanistic aspects of the photosensitized reactions involving butylated hydroxyanisole and butylated hydroxytoluene. Both $O_2^{\bullet-}$ and $O_2(^1\Delta_g)$ produce the photodegradation of butylated hydroxytoluene. Butylated hydroxyanisole mainly interacts with $O_2(^1\Delta_g)$ and exhibits a desirable property as antioxidant: a relatively high capacity for $O_2(^1\Delta_g)$ physical deactivation and a low photodegradation efficiency by oxidative species.[7]

The main aim of this work is to investigate kinetic and mechanistic aspects of the sensitized photoreactions involving the phenolic antioxidants (PA), PG,

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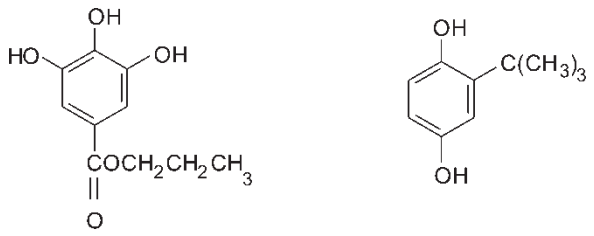


propyl gallate (PG) | *t*-butylhydroquinone (TBHQ)

Scheme 1 Chemical structures of propyl gallate and *t*-butylhydroquinone.

and TBHQ (generically named PA in the following). The structural formulae of both are shown in Scheme 1. For this purpose riboflavin (Rf; vitamin B2) was used as dye-photosensitizer. It can act as a prooxidant for food components under visible light. Rf-photosensitization causes production of free radicals and ROS such as $O_2(^1\Delta_g)$, $O_2^{\bullet-}$, H_2O_2, and $HO^{\bullet}$. These species could produce not only the destruction of lipids, proteins, carbohydrates, and vitamins, but also the formation of off-flavor and loss of nutrients in foods.[8–11]

As PA and Rf can occupy common locations in foods, kinetic, and mechanistic information about the visible-light-promoted interactions between the antioxidants and the vitamins can contribute to the understanding of important aspects connecting the photoprotection activity and the degradability of these antioxidants.

Materials and methods

Materials

PG, TBHQ, Rf, Rose Bengal (RB), sodium azide (NaN_3), catalase (CAT) from bovine liver, and superoxide dismutase (SOD) from bovine erythrocytes were purchased from Sigma Chem. Co. (St Louis, USA). Benzoic acid sodium salt (NaBz), 9,10-dimethylanthracene (DMA), D-mannitol and deuterated methanol (MeOD) was provided by Aldrich (St Louis, USA); whereas methanol (MeOH), HPLC quality, by Sintorgan and H_2O_2, by Tensol (Argentine).

Methods

Stationary photolysis

Stationary aerobic photolysis of MeOH solutions containing 0.04–1.0 mM PA and 0.02 mM Rf was carried out in a home-made photolyser. This is provided with quartz-halogen lamp (OSRAM XENOPHOT® HLX 64640, 150 W–24 V–G6.35–6000lm, OSRAM Augsburg, Germany) which generates a continuous spectrum of light that ranges from the central ultraviolet through the visible and into the infrared wavelength regions (*ca.* 300–2000 nm). A cut-off filter of $\lambda > 350$ nm was used in order to ensure that the light was only absorbed by the photosensitizer. The light was passed through a water filter and focused on the reaction vessel (either a hermetically sealed reaction cell, with an oxygen electrode or a 1×1-cm spectrophotometric cuvette) containing the continuously stirred solutions.

The experiments of oxygen uptake by 1.0 mM PA were determined with the specific oxygen electrode Orion 97-08.

The Rf-photosensitized rates of oxygen consumption were determined by evaluation of the initial slopes of oxygen uptake vs. irradiation time. In order to investigate the eventual involvement of ROS, experiments of oxygen uptake in the absence and in the presence of different additives with ROS-scavenging capacity were carried out. The following compounds were employed as ROS scavengers: 10.0 mM NaN_3 for $O_2(^1\Delta_g)$; 1 mg/100 ml SOD for $O_2^{\bullet-}$; 1 mg/100 ml CAT for H_2O_2 and 10.0 mM NaBz for $HO^{\bullet}$ (or 5.0 mM D-mannitol).

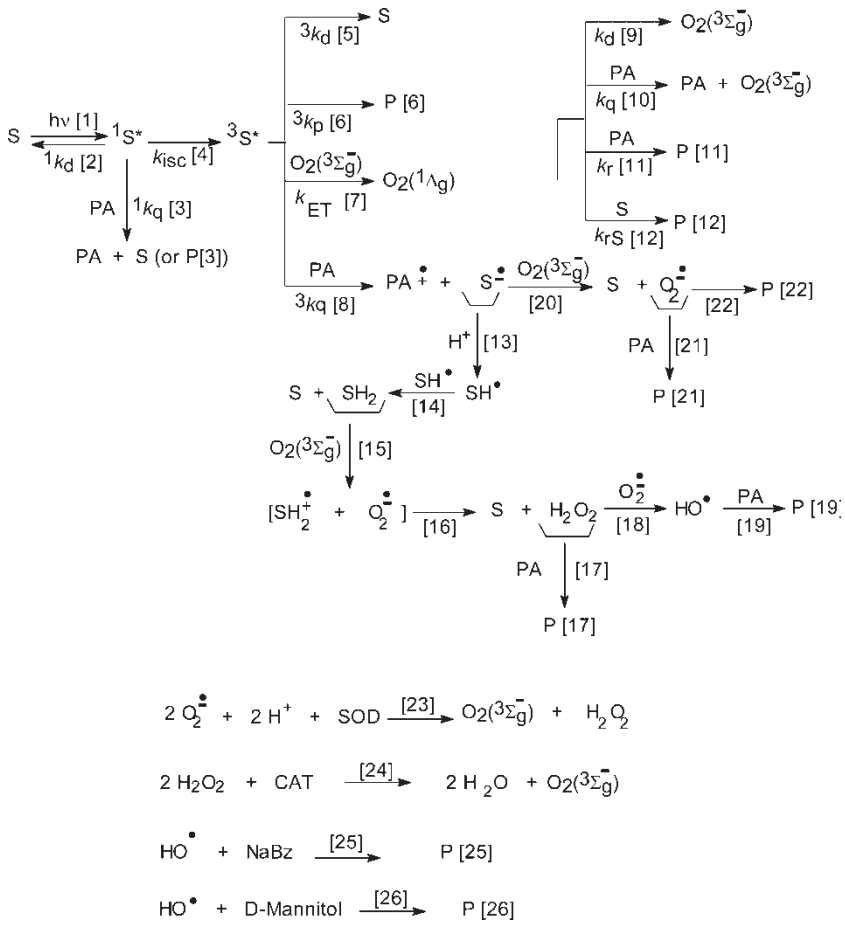
The rate constant of the chemical reaction of PA with $O_2(^1\Delta_g)$ (reaction [11], Scheme 2) was determined in MeOH by means of the method introduced by Foote and Ching.[12] The synthetic dye-photosensitizer RB was used as $O_2(^1\Delta_g)$-generator.[13] Assuming that the reaction of $O_2(^1\Delta_g)$ with the substrate is the only way of oxygen consumption, the ratio of the slope of the first-order plot for substrate consumption, measured by the initial decrease in the respective absorption spectra of the PA and a reference compound (with a k_{rRef} value known) upon photosensitization, both at identical concentrations, is equal to the ratio k_r/k_{rRef}.

Ground state absorption spectra were registered in a Hewlett Packard 8452A diode array spectrophotometer. In all cases, quartz cells of 1.0-cm pathlength were used.

All rate constant values were determined by triplicate. The reported values in Table 1 are the mean value and the error bars correspond to the extreme values obtained.

Time-resolved $O_2(^1\Delta_g)$ phosphorescence detection

The laser-kinetic spectrophotometer for time resolved $O_2(^1\Delta_g)$ phosphorescence detection (TRPD) has been previously described.[14] In brief, it consisted of a Nd:YAG laser (Spectron Laser System Ltd, Rugby, UK) as the excitation source. The output at 532 nm was used to excite the phtosensitiser RB. The emitted radiation (mainly 1270 nm) was detected at right angles using an amplified Judson J16/8Sp germanium detector after passing through appropriate filters. The output of the detector was coupled to a digital oscilloscope and to a personal computer for processing the signal. Sixteen shots were usually averaged in order to get a good signal-to-noise ratio, from



Scheme 2 Possible reaction pathways in the sensitized photoirradiation of propyl gallate and *t*-butylhydroquinone, and reactions of ROS with specific auxiliary quenchers. S represents the photosensitizer and PA the phenolic antioxidants.

which the decay times were determined. Solution of RB with absorbance at the laser wavelength of *ca.* 0.2 was used. The decay kinetics was first order in all cases. The experiments were made in MeOD instead of MeOH, due to the enlargement of the $O_2(^1\Delta_g)$ lifetime in this solvent, as already discussed elsewhere.[15] $O_2(^1\Delta_g)$ lifetime was evaluated in the absence (τ_0) and in the presence (τ) of PA and the ratio τ_0/τ was plotted as a function of the PA concentration, according to a simple Stern-Volmer treatment, using equation (1):

$$\tau_0/\tau = 1 + k_t\tau_0\,[\mathrm{PA}] \qquad (1)$$

where k_t (addition $k_q + k_r$, processes [10] and [11], respectively, in Scheme 2) is the overall rate constant for $O_2(^1\Delta_g)$ deactivation.

Table 1 Relative rates of oxygen photoconsumption by propyl gallate and *t*-butylhydroquinone sensitized by riboflavin (V_{-ox}(rel); rate constants for the overall (k_t) and reactive (k_r) quenching of singlet oxygen; k_r/k_t ratios; rate constants for the quenching of electronically excited singlet state of riboflavin (^1k_q); rate constants for the quenching of electronically excited triplet state of riboflavin (^3k_q); slope of oxygen uptake sensitized by riboflavin in the absence (slope$_0$) and in the presence (slope$_x$) of different scavengers ($X = NaN_3$, SOD, CAT, or NaBz). Solvent: methanol.

	Propyl gallate	*t*-butylhydroquinone
$V_{-ox\ (rel)}$	1.0 ± 0.1	0.40 ± 0.04
$k_t \times 10^{-8}$ (1/M second)	0.14 ± 0.01	0.55 ± 0.05
$k_r \times 10^{-8}$ (1/M second)	0.020 ± 0.002	0.030 ± 0.003
k_r/k_t	0.14	0.05
$^1k_q \times 10^{-8}$ (1/M second)	41 ± 4	57 ± 6
$^3k_q \times 10^{-8}$ (1/M second)	42 ± 4	30 ± 3
slope$_{NaN3}$/slope$_0$	0.7	0.4
slope$_{SOD}$/slope$_0$	1	1
slope$_{CAT}$/slope$_0$	0.7	0.4
slope$_{NaBz}$/slope$_0$	1	0.6

Stationary and time-resolved fluorescence experiments

For the stationary Rf fluorescence experiments, a Spex Fluoromax spectrofluorometer was used.

Fluorescence lifetimes were measured using a time-correlated single photon counting technique (SPC) on an Edinburgh FL-9000CD instrument (Edinburgh Instruments Ltd, Edinburgh, UK).

The excitation and emission wavelengths were 445 and 518 nm, respectively. All the measures were made at 25 ± 1°C in air equilibrated solutions of MeOH. Quartz cells of 1.0-cm path-length were used.

In order to determine the values of ^1k_q (process [3] in Scheme 2), as before, a classical Stern–Volmer treatment of the data was applied through equation (2) where, $^1\tau_0$ and $^1\tau$ are the fluorescence lifetimes in the absence and in the presence of different concentrations

of PA, respectively.

$$^{1}\tau_0/^{1}\tau = 1 + {}^{1}k_q^{1}\tau_0\,[\mathrm{PA}] \qquad (2)$$

Laser flash photolysis experiments

Nitrogen-saturated Rf MeOH solutions were irradiated using a laser flash photolysis apparatus with the frequency-doubled output of a Nd:YAG laser (Spectron) at 355 nm as excitation wavelength, using a 150-W xenon lamp as analyzing light.

The detection system comprised a PTI monochromator and a red-extended photomultiplier (Hamamatsu R666, Hamamatsu Photonics, Hamamatsu, Japan). The signal, acquired and averaged by a digital oscilloscope (Hewlett-Packard 54504A, Agilent Technologies, Santa Clara, USA), was transferred to a PC via a Hewlett-Packard Interface Bus (HPIB) parallel interface, where it was analyzed and stored.

Solutions of Rf with absorbance *ca.* 0.2 at excitation wavelength were used.

A Stern–Volmer treatment was applied (equation (3)) in order to determine the values of $^{3}k_q$ (process [8], in Scheme 2).

$$^{3}\tau_0/^{3}\tau = 1 + {}^{3}k_q^{3}\tau_0\,[\mathrm{PA}] \qquad (3)$$

where $^{3}\tau_0$ and $^{3}\tau$ are the respective lifetimes of electronically excited triplet state of Rf in the absence and in the presence of PA.

Test of H_2O_2-mediated oxidation

The thermal reaction between PA and 20.0 mM H_2O_2 was evaluated monitoring the respective fluorescence spectra of the antioxidants in MeOH, after and before addition of the peroxide. The respective excitation and emission wavelengths were 280 and 370 nm for 0.05 mM PG, and 290 nm and 325 nm for 0.03 TBHQ.

Results

Photodegradation kinetics

The photoirradiation of the system 0.05–0.2 mM PA/0.02 mM Rf in air saturated MeOH solutions, produces changes in the PA absorption spectra and also in the absorption bands of the photosensitizer (Fig. 1, main and inset A). In parallel, oxygen consumption by PA was observed, but not in the dark or in the absence of PA within of the irradiation times used in these experiments.

It is known that the anaerobic photodegradation of Rf under visible light irradiation predominantly proceeds through the electronically excited triplet state,[8,16] and the rate of the process can be estimated by absorption spectroscopy. Comparative irradiations of N_2-saturated MeOH solutions of 0.02 mM Rf in the absence and in the presence of PA demonstrated

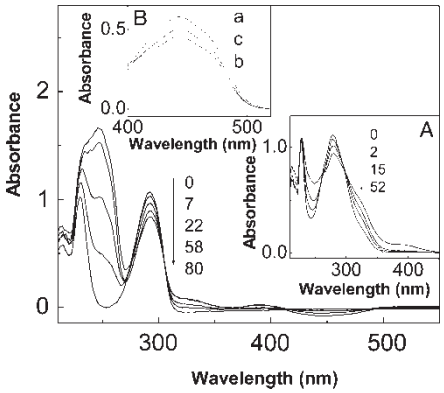


Figure 1 Spectral changes of *ca.* 0.2 mM *t*-butylhydroquinone + 0.02 mM riboflavin vs. 0.02 mM riboflavin upon visible-light photoirradiation. *Inset A*: Spectral changes of 0.1 mM propyl gallate + 0.02 mM riboflavin vs. 0.02 mM riboflavin upon visible-light photoirradiation. Numbers on the spectra represent irradiation time, in minutes. *Inset B*: Spectral changes in a N_2-saturated solution of 0.02 mM riboflavin upon visible-light photoirradiation; a, without additives and non-irradiated; b, without additives, 25 minutes irradiation; c, in the presence of 0.15 mM *t*-butylhydroquinone, 25 minutes irradiation. Solvent: MeOH.

that this rate decreases in the presence of PA (*ca.* 0.15 mM) (Fig. 1, inset B).

Results strongly suggest that under visible light irradiation the interaction of Rf–PA could include the participation of electronically excited states of the dye and/or ROS formed from these states. In order to elucidate kinetic and mechanistic aspects of the sensitized-photooxidation of PA a systematic study was developed.

Participation of ROS in the sensitized-photodegradation of PA

Singlet oxygen

The rate of oxygen consumption of the individual PA/Rf-photomediated systems diminished in the presence of 10.0 mM NaN_3 (Table 1). This salt is an efficient quencher of $O_2(^1\Delta_g)$ with reported[17] k_t values in water in the range $(2–5)\times10^8$/M second. NaN_3 competes with PA for the quenching of $O_2(^1\Delta_g)$, diminishing the rate of oxygen consumed by the reaction of the excited oxygen species with PA. Nevertheless, it is known that the ion quenches the $^{3}Rf^{*}$[18] (no data for the quenching rate constants is available) and that its oxygen-consumption inhibitory effect must be carefully considered. The NaN_3 test only suggests the possible presence of $O_2(^1\Delta_g)$ as an oxidative species.

In order to evaluate the eventual participation of the species $O_2(^1\Delta_g)$ and to achieve as much information as possible about pure potential interactions of $O_2(^1\Delta_g)$–PA, the photosensitizer RB, a profusely used $O_2(^1\Delta_g)$-generator,[13] was used. The overall and reactive interactions between $O_2(^1\Delta_g)$ and PA were

quantified through the rate constants k_t and k_r, respectively.

The k_t values (addition $k_q + k_r$, processes [10] and [11], Scheme 2) were determined in MeOD, employing TRPD technique (see Experimental section). They do not depend either on the type of photosensitizer or on potential interactions of the substrate with electronically excited states of the photosensitizer involved in $O_2(^1\Delta_g)$-generation. The $O_2(^1\Delta_g)$ lifetimes were evaluated in the absence and in the presence of PA, and the data plotted as a function of substrate concentration, according to equation (1) (Fig. 2, Table 1).

The k_r values (process [11]) were determined, in MeOH, from the initial decrease in absorption band at 275 nm for PG, 292 nm for TBHQ, and 355 nm for DMA, upon RB-sensitized photoirradiation. In all cases, conversions lower than 10% were used to avoid possible interference from photoproducts absorption. A first order kinetic treatment of the data of substrate consumption for PG, TBHQ, and DMA was employed to determine the k_r values (Fig. 2, inset; Table 1). The hydrocarbon was used as reference compound with $k_{rRef} = 2.4\ 10^7/M$ second in MeOH.[17]

Superoxide radical anion, hydrogen peroxide, and hydroxyl radical

Experiments of oxygen uptake upon Rf-photosensitized irradiation in the absence and in the presence of different additives with ROS-scavenging capacity SOD, CAT, and NaBz were carried out. These scavengers have been already used in similar concentrations to confirm/discard the participation of $O_2^{\bullet-}$, H_2O_2, and $HO^{\bullet}$, respectively, in a given oxidative process.[9,19–26]

The rate of oxygen consumption of the individual PA/Rf system was practically the same in the presence

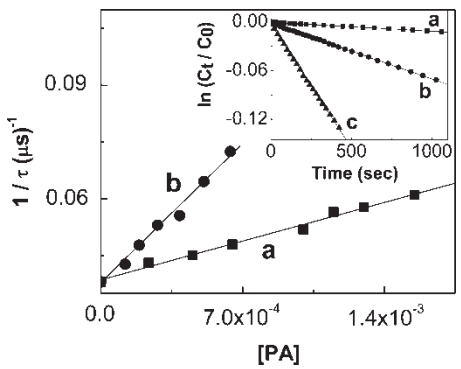


Figure 2 Stern–Volmer plot for the quenching of $O_2(^1\Delta_g)$-phosphorescence emission by PA (a) propyl gallate and (b) *t*-butylhydroquinone, in MeOD. *Inset*: first order plots for PA consumption in MeOH of (a) propyl gallate; (b) *t*-butylhydroquinone; (c) 9,10-dimethylanthracene (reference compound).

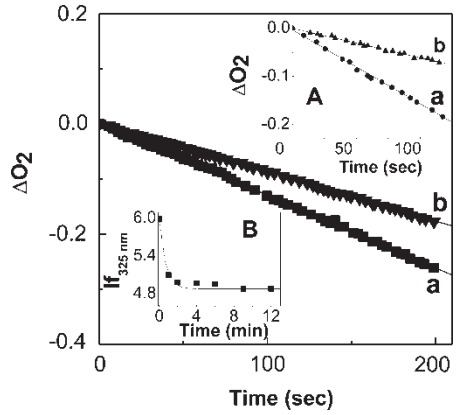


Figure 3 Profiles of oxygen consumption, in MeOH solutions, upon riboflavin-sensitized photoirradiation (a) 0.02 mM Riboflavin + 1.0 mM propyl gallate; (b) 0.02 mM riboflavin + 1.0 mM propyl gallate + 1 mg/100 ml catalase. *Inset A*: Profiles of oxygen consumption, in MeOH, upon riboflavin-sensitized photoirradiation (a) 0.02 mM riboflavin + 1.0 mM *t*-butylhydroquinone; (b) 0.02 mM Riboflavin + 1.0 mM *t*-butylhydroquinone + 1 mg/100 ml catalase. *Inset B*: Profile of 0.03 mM *t*-butylhydroquinone consumption, monitored through the decay of stationary fluorescence intensity at 325 nm in the presence of 20.0 mM H_2O_2 as a function of time, in MeOH.

of SOD (1 mg/100 ml). The enzyme SOD dismutates the species $O_2^{\bullet-}$ (reaction [23], Scheme 2).

The participation of H_2O_2 species in the Rf-sensitized photooxidation of PA is supported by the oxygen-uptake inhibition observed in the presence of CAT (Fig. 3 main and inset A). This enzyme decomposes[9] H_2O_2 as shown in reaction [24], Scheme 2.

We independently tested the thermal reaction between 0.05 mM PG, 0.03 mM TBHQ, and 20.0 mM H_2O_2, in MeOH solution. A decrease in the PA fluorescence intensity upon addition of the peroxide indicates the occurrence of chemical transformations in the antioxidants (Fig. 3, inset B).

The behavior of the profile for oxygen uptake by PG and TBHQ upon Rf-sensitized in the presence of NaBz is somewhat different. In the case of PG, the addition of 10.0 mM salt did not produce any change in the rate of oxygen consumption. Nevertheless, for TBHQ the rate of oxygen uptake in the presence of the salt was decreased. A similar concentration of NaBz as used in these experiments has been used by other authors[22] for the specific quenching of the species $HO^{\bullet}$. The results with NaBz were confirmed using D-mannitol as additional scavenger of $HO^{\bullet}$. D-Mannitol reacts with the species $HO^{\bullet}$ with a second order rate constant of $1.9\ 10^9/M$ second in water[20] (reaction [26]). Similar concentrations of D-mannitol have been previously used as an efficient trappers in $HO^{\bullet}$-mediated photooxidations.[22,24,27]

As additional information, and in order to eliminate possible interference of the quenchers in the oxygen uptake experiments, SOD and NaBz were checked to

control that they not produced a noticeable oxygen uptake when exposed to light in the presence of 0.02 mM Rf. Besides, both scavengers did not interact with the electronically excited states of the vitamin.[27] The last asseveration is also valid for CAT, whereas the enzyme, in the described conditions, produced an oxygen consumption of *ca.* 10% as compared with the corresponding values for PA.

The values of the relative rates of oxygen uptake in the presence and in the absence of specific quenchers of ROS are shown in Table 1. These rates constitute the mean values of three independent runs which do not differ over 3% from each other.

Participation of electronically excited states of Rf in the photodegradation of PA

The former experimental evidence clearly indicates that at least a ROS-mediated mechanism operates in the aerobic sensitized photodegradation of PA. Consequently, the possibility of an additional photodegradation mechanism based on the interaction of PA with electronically excited states of the Rf was investigated.

Quenching of electronically excited singlet state of Rf

The fluorescence properties of Rf are well known.[8] In air-equilibrated MeOH solution, Rf shows a fluorescence emission band centered at 518 nm. In the presence of ≥5.0 mM PA, the intensity of the stationary emission of ^{1}Rf* (^{1}S*, in Scheme 2) decreased, but the shape of the emission spectrum did not change. In parallel, the fluorescence decay of Rf was evaluated in the absence and in the presence of PA by means of time-resolved methods (SPC technique). From the classical Stern–Volmer treatment (Eq. (2)) the rate constant ^1k_q (process [3], Scheme 2) was graphically determined (Fig. 4, Table 1). In all cases, the fluorescence decay of Rf in MeOH was monoexponencial with a value $^1\tau_o$ of 5.4 ns, in excellent agreement with previous published data.[28]

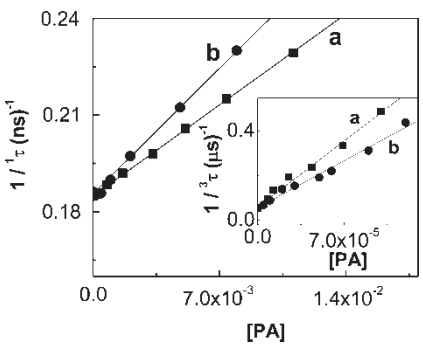


Figure 4 Stern–Volmer plots for the time-resolved quenching of electronically singlet excited riboflavin (main figure) and electronically triplet excited riboflavin (inset) in MeOH, by (a) propyl gallate; (b) *t*-butylhydroquinone.

Quenching of electronically excited triplet state of Rf

The decay of ^{3}Rf* (^{3}S*, in Scheme 2) in N_2-saturated MeOH solution was determined at low Rf concentration and at laser energy low enough to avoid undesired effects such as self-quenching and triplet–triplet annihilation. The disappearance of ^{3}Rf*, generated by a 355-nm laser pulse was monitored from the first-order decay of the absorbance at 670 nm, at wavelength where the interference from other possible species is negligible. Under these conditions, ^{3}Rf* lifetime appreciably decreases in the presence of PA.

As before, a Stern–Volmer treatment of the triplet quenching (equation (3)) yielded the bimolecular rate constants ^3k_q, (process [8]) (Fig. 4, inset; Table 1).

Discussion

Scheme 2 shows the set of reactions employed for interpretation and discussion of the results. It depicts a generic photosensitized process, in which the absorption of visible light promotes the dye-photosensitizer (S) to the electronically excited singlet (^{1}S*) and triplet (^{3}S*) states (processes [1] and [4]). ^{3}S* can transfer energy to ground state oxygen ($O_2(^3\Sigma_g^-)$) in the aerated solution, generating $O_2(^1\Delta_g)$ (process [7]). These species can decay by collision with solvent molecules (process [9]), and can interact physically (rate constant k_q) and/or chemically (rate constant k_r) with the substrate PA (processes [10] and [11], respectively), with overall rate constant $k_t = k_q + k_r$. Typical electron donors, as phenols,[29] can also transfer an electron to ^{3}S*, giving rise to the respective semireduced ($S^{\bullet-}$) and semioxidised ($PA^{\bullet+}$) forms (process [8]).

The interaction of $S^{\bullet-}$ with $O_2(^3\Sigma_g^-)$ could generate the reactive species $O_2^{\bullet-}$ through process [20]. In the case of Rf, the neutral radical ($SH^\bullet$) would be formed after the protonation of the species $S^{\bullet-}$ (process [13]). The bimolecular decay of $SH^\bullet$ is known[30,31] to proceed through disproportion reaction to yield equimolar S and fully reduced S (SH_2) (process [14]), which in the presence of $O_2(^3\Sigma_g^-)$ is reoxidised to give initially $SH_2^{\bullet+}$ and $O_2^{\bullet-}$ (process [15]) and finally S and H_2O_2 (process [16]). Under these reaction conditions, H_2O_2 together with $O_2^{\bullet-}$ could give rise to $HO^\bullet$ (process [18]). The processes [16] and [20] regenerate ground state S. These processes represent crucial steps in living organisms, for which it is well established that ROS are a key intermediate in the oxygen redox chemistry.[32]

According to the results herein shown, PA interact with electronically excited singlet and triplet states of Rf, as well as with ROS generated from these excited states.

Although the interaction of PA with electronically excited singlet state of Rf (process [3], Scheme 2) occurs with a rate constant close to the diffusion limit, relatively high concentrations of PA, much

higher than those employed in the kinetic experiments, are necessary to partially hinder electronically excited triplet state generation. As a consequence, it can be assumed that the population of ^{3}Rf* is practically not affected by any quenching effect on ^{1}Rf* under our experimental conditions (*ca.* 1.0 mM PA).

It is currently accepted[33] that, in general, the quenching of ^{3}S* by $O_2(^3\Sigma_g^-)$ to produce $O_2(^1\Delta_g)$ occurs with a rate constant k_{ET} of 1/9 of the diffusional value. A kinetic analysis of laser flash photolysis data for Rf, considering a rate constant k_{ET} *ca.* 1.2×10^9/M second in MeOH[34] and a mean value for ^3k_q of 3.6×10^9/M/second, indicates that the quenching of ^{3}Rf* by PA (process [8]) could compete with the generation of $O_2(^1\Delta_g)$ (process [7]) at concentrations typically employed in this work (*ca.* 1.0 mM PA and 2.0 mM dissolved $O_2(^3\Sigma_g^-)$ in MeOH).[35] In other words, both $O_2(^1\Delta_g)$ and $Rf^{\bullet-}$ are formed from ^{3}Rf*. The last species is generated by an electron transfer process from PA toward ^{3}Rf*, with the concomitant production of $Rf^{\bullet-}$ and $PA^{\bullet+}$ (process [8]). From $Rf^{\bullet-}$, the species $RfH^{\bullet}$ may be formed in the presence of proton-donating substrates[29,36,37] (process [13]) and triggers a cascade of photoprocesses that may produce the oxidative species $O_2^{\bullet-}$, H_2O_2, and/or $HO^{\bullet}$ that in a further step can react with PA or with Rf (Scheme 2).

According to Scheme 2, both $RfH^{\bullet}$ and $O_2^{\bullet-}$ are straightforwardly generated from $Rf^{\bullet-}$ (processes [13] and [20], respectively) and could act as precursors of the species H_2O_2 and $HO^{\bullet}$. Results show that different ROS participate in the Rf-sensitized photooxidation of PG and TBHQ.

In the case of PG, the observed decrease in the rate of oxygen consumption in the presence of CAT strongly suggests that H_2O_2 species is formed during the oxidative process and may eventually react with PG. The result obtained from the thermal reaction between PG and H_2O_2 demonstrates the viability of a H_2O_2-mediated oxidation of PG. In parallel, the lack of any effects on the oxygen consumption in the presence of SOD and NaBz leads us to discard the participation of $O_2^{\bullet-}$ and $HO^{\bullet}$ as oxidative agents under work conditions. However, the species $O_2^{\bullet-}$ should be generated because the formation of H_2O_2 occurs via $O_2^{\bullet-}$ (processes [15] and [16]). In this way, the case of TBHQ is somewhat different. The decrease in the rate of oxygen consumption in the presence of CAT or NaBz, accounting for the respective presence of H_2O_2 and $HO^{\bullet}$, suggests that both oxidative species may be formed and could react with TBHQ. The presence in the photoreaction of $HO^{\bullet}$ is supported by additional experiments of oxygen consumption carried out in the presence of D-mannitol. Although other radicals such as alkyl-peroxy could also react with $HO^{\bullet}$, their presence in our system is not evident. Hence, on the basis of the available experimental evidence, the interaction of TBHQ with $HO^{\bullet}$ remains a possibility. The generation of $HO^{\bullet}$ depends on the previous H_2O_2 and $O_2^{\bullet-}$ generation (processes [15] and [16], respectively). The scavenging of either of the two species reduces the rate of oxygen uptake in a $HO^{\bullet}$-mediated TBHQ oxidation. Following this line, $HO^{\bullet}$ is a final product, so that it is the only species that may be independently and selectively detected by a specific inhibitor like NaBz or D-mannitol. The CAT test effectively recognizes the presence of H_2O_2 as an initial oxidative species, but does not demonstrate the direct participation of this ROS in the oxidative process. Already described experiments support the occurrence of thermal reaction between TBHQ and H_2O_2, as shown in Fig. 3, inset B.

In contrast, the observed inhibition of the oxygen uptake runs in the presence of NaN_3 and the results mentioned above for the flash photolysis experiments, suggest the participation of $O_2(^1\Delta_g)$ in the Rf-sensitized photoreaction of both PA. The quenching of $O_2(^1\Delta_g)$ can occur through collisions with surrounding solvent molecules (process [9]), by interaction with Rf (process [12]) for which a rate constant k_{rRf} of 6×10^7/M second has been reported in MeOH[38] or by interaction with PA (processes [10] and [11]). The value for overall rate constant ($k_t = k_q + k_r$) obtained by time-resolved methods and the reactive rate constant (k_r) suggest that the photooxidation of PA by $O_2(^1\Delta_g)$ is possible. According to these rate constant values, we can said that the quenching ability of both PA towards $O_2(^1\Delta_g)$ is low-to-moderate, in accordance with values reported for different alkyl-substituted phenols.[39]

The distinction between physical and chemical contributions (k_q, process [10] and k_r, process [11], respectively) is highly significant because the physical contribution usually is interpreted, in practical terms, as a form of self-protection against $O_2(^1\Delta_g)$-mediated photooxidations. Nevertheless, no relevant information about these photoreactions can be obtained from the straightforward analysis of isolated k_t, k_q, and k_r values. A simple and useful approach is the evaluation of the k_r/k_t ratio, which can be envisaged as the fraction of the overall interaction $O_2(^1\Delta_g)$-substrate that leads to effective chemical transformation. Results show that TBHQ has a high component of O_2 $(^1\Delta_g)$-physical deactivation and low photodegradation efficiency by the oxidative species. In comparative terms, TBHQ has a higher antioxidant capacity than PG.

With regard to the photooxidation products $O_2(^1\Delta_g)$ mediated for phenols and polyhydroxybenzenes,[39,40] the accepted mechanism of the quenching 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 products. This primary encounter complex can also give, through a (2 + 4) cycloaddition of $O_2(^1\Delta_g)$, an unstable endoperoxide and, later, a quinonic compound.[39,41,42]

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