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A kinetic study on the inhibitory action of sympathomimetic drugs towards photogenerated oxygen active species. The case of phenylephrine

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Abstract

Kinetics and mechanism of the aerobic Riboflavin (Rf, vitamin B2) sensitized photodegradation of Phenylephrine (Phen), a phenolamine belonging to the sympathomimetic drugs family, has been studied in water, employing continuous photolysis, polarographic detection of oxygen uptake, steady-state and time-resolved fluorescence spectroscopy, time-resolved IR-phosphorescence and laser flash photolysis. Results indicate the formation of a weak dark complex Rf–Phen, with an apparent association constant of $5.5 \pm 0.5\ M^{-1}$, only detectable at Phen concentrations much higher than those employed in the photochemical experiments. Under irradiation, an intricate mechanism of competitive reactions operates. Phen quenches excited singlet and triplet states of Rf, with rate constants of 3.33 ± 0.08 and $1.60 \pm 0.03 \times 10^9\ M^{-1}\ s^{-1}$, respectively. With the sympathomimetic drug in a concentration similar to that of dissolved molecular oxygen in water, Phen and oxygen competitively quench triplet excited Rf, generating superoxide radical anion and singlet molecular oxygen ($O_2(^1\Delta_g)$) by processes initiated by electron- and energy-transfer mechanisms respectively. As a global result, the photodegradation of the vitamin, a known process taking place from its excited triplet state, is retarded, whereas the phenolamine, practically unreactive towards these oxidative species, behaves as a highly efficient physical deactivator of $O_2(^1\Delta_g)$. The phenolamine structure in Phen appears as an excellent scavenger of activated oxygen species, comparatively superior, in kinetic terms, to some commercial phenolic antioxidants.

Keywords: Phenylephrine; Photo-oxidation; Riboflavin; Singlet molecular oxygen; Superoxide radical anion

1. Introduction

Light-promoted-degradation is being increasingly investigated in substrates of relevance in biology and medicine [1–5]. Sympathomimetic drugs (SD), belong to this class of biologically active and commercially valuable substrates. SD consist of a series of compounds with properties resembling the neurotransmitters epinephrine, norepinephrine, and dopamine [6].

Since SD are transparent to daylight, their decomposition due to direct environmental irradiation can be disregarded. Nevertheless, during elaboration, storage, or in vivo, after medicinal administration of the drugs, in the presence of photosensitising substances they might be able to absorb visible light and generate potentially aggressive species. These species can be transient entities consisting of the electronically excited states of a photosensitiser or some of the so called oxygen active species (OAS), generated from these excited states. A particularly interesting daylight-absorber sensitiser is Vitamin B2 (Riboflavin, Rf, see Scheme 1) which is a natural compound present in most living organisms [7]. The

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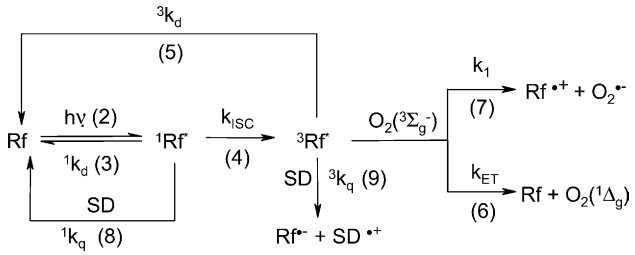
usual mechanism of action of this sensitiser is rather complex, in many cases with the concurrent involvement of the oxidative species singlet molecular oxygen ($O_2(^1\Delta_g)$) and superoxide radical anion ($O_2^{\bullet-}$) which are produced with quantum yields of 0.49 and 0.009, respectively [8]. In the presence of interacting species such as SD, the mentioned mechanism could be summarised as follows:

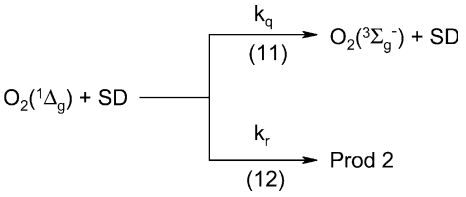
$$Rf + SD \overset{K_{ass}}{\rightleftharpoons} [Rf \cdots SD] \quad (1)$$



Process (1) represents the association Rf–SD, a kind of dark interaction which has been repeatedly reported for couples of Rf with different types of substrates [2,9–11]. Upon absorption of a photon, Rf excited singlet state (^{1}Rf*) is generated (2). It can decay to ground state (3) or, through an intersystem crossing Process (4), it can produce excited triplet Rf (^{3}Rf*). The last species can also decay to ground state Rf (5) or can be quenched by ground state oxygen dissolved in the solution ($O_2(^3\Sigma_g^-)$), generating OAS. That is, singlet molecular oxygen ($O_2(^1\Delta_g)$) (6) and superoxide radical anion ($O_2^{\bullet-}$) are produced by an energy transfer process and by electron transfer to oxygen, respectively (7). Besides, the transient species (^{1}Rf*) and (^{3}Rf*) can also interact with SD (Reactions (8) and (9)).

The interaction of OAS with pharmaceutical products is particularly important since, as a result of the photo-damage, the drug can degrade, either decreasing its original therapeutic activity or even worse, modifying its specific effects and/or eventually generating toxic products (Reactions (10)–(12)).

$$O_2^{\bullet-} + SD \underset{(10)}{\overset{k_{10}}{\rightarrow}} \text{Prod 1}$$



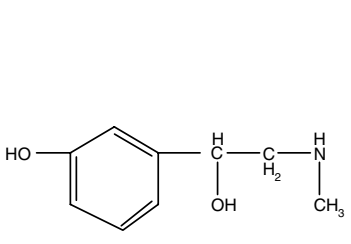
Nevertheless, a positive effect that can arise from the interaction of the drug with some photogenerated oxidative species is the eventual scavenging of the oxygenated species (Reactions (10)–(12)). In this context, the prevalence of the physical Process (11) is a desirable possibility since the final result is the elimination of the oxidative species without considerable loss of the scavenger.

In this paper we present a kinetic and mechanistic study on the behaviour of the SD derivative Phenylephrine (Phen, see Scheme 1) in the presence of molecular or radical species produced through daylight-promoted Rf–sensitised processes. Phen is a phenolamine and it is well known that several compounds with phenol-like structures are susceptible to degradation by reacting with environmentally-photogenerated oxidative species [12,13].

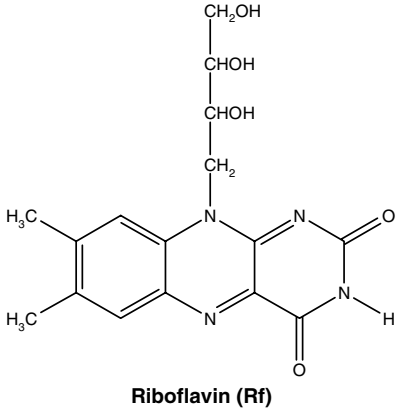
2. Materials and methods

2.1. Materials

Riboflavin (Rf), deuterium oxide 99.9% (D_2O), L-Tryptophan (Trp), Trolox and L-Phenylephrine Hydrochloride (Phen) were purchased from Sigma Chem. Co. Rose Bengal (RB), and furfuryl alcohol (FFA) were from Aldrich. All these chemicals were used



Phenylephrine (Phen)



Riboflavin (Rf)

Scheme 1. Chemical structures of Phenylephrine (Phen) and Riboflavin (Rf).

as received. Zinc tetraphenylporphyrine (ZnTPP) was prepared as previously published [14]. Water was triply distilled and benzene, HPLC quality, was from Sintorgan. All the measurements were carried out at room temperature and with freshly prepared solutions.

2.2. Methods

Ground state absorption spectra were registered employing a Hewlett Packard 8452A diode array spectrophotometer.

The apparent association constant (K_{ass}, Process (1), with SD = Phen) was evaluated through the Benesi–Hildebrand method [15], using (2.1).

$$1/\mathrm{OD} = (1/bK_{ass}\varepsilon_C[\mathrm{Rf}]_0)1/[\mathrm{Phen}]_0 + 1/b\varepsilon_C[\mathrm{Rf}]_0, \quad (2.1)$$

where OD is the absorbance of the complex at the monitoring wavelength (489 nm), b is the path-length (1 cm), K_{ass} is the apparent association constant, ε_C is the molar absorption coefficient of the complex, and $[\mathrm{Rf}]_0$ and $[\mathrm{Phen}]_0$ are the initial concentrations of the Rf and Phen, respectively. From a plot of 1/OD versus $1/[\mathrm{Phen}]_0$, the respective values of K_{ass} and ε_C can be obtained.

The total quenching rate constant of deactivation of the $O_2(^1\Delta_g)$ by Phen (k_t, see Reactions (11) and (12), being $k_t = k_r + k_q$ and SD = Phen), was determined using a system previously reported [16]. Briefly, 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 Judson J16/8Sp Germanium detector, after having passed through 1270 nm-interference and two wratten filters. The output of the detector was coupled to a digital oscilloscope 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.

The concentration of the sensitizer (Rf) was 0.02 mM. D_2O was employed in the dynamic determinations, instead of H_2O as solvent, in order to enlarge the lifetime of $O_2(^1\Delta_g)$ [17]. The $O_2(^1\Delta_g)$ lifetimes were evaluated in the presence (τ) and in the absence (τ_0) of the quencher; the data were plotted as a function of Phen concentration, according to a simple Stern–Volmer treatment ((2.2) see Reactions (11) and (12)).

$$1/\tau = 1/\tau_0 + k_t[\mathrm{Phen}]. \quad (2.2)$$

The reactive rate constant k_r for the reaction of the Phen with $O_2(^1\Delta_g)$ (Reaction (12), with SD = Phen), was determined using the method described by Scully and Hoigné [18] ((2.3)), for which the knowledge of the reactive rate constant for the photo-oxidation of a reference compound R is required

$$\mathrm{slope}/\mathrm{slope}_R = k_r[\mathrm{Phen}]/k_{rR}[\mathrm{R}], \quad (2.3)$$

where slope and slope_R are the respective slopes of their first-order plots of oxygen consumption by Phen and by a reference compound. Assuming that the reaction of $O_2(^1\Delta_g)$ with the quencher is the only possible pathway of oxygen consumption, the ratio of the first order slopes of oxygen uptake by the substrate and the reference compound, each at the same concentration (slope substrate/slope reference) yields k_r/k_{rR}. The reference was FFA, with a reported pH-independent k_{rR} value of $1.2 \times 10^8\ \mathrm{M^{-1}\,s^{-1}}$ [19]. Rose Bengal was used as dye-sensitizer in the determination of k_r.

The Rf-sensitised photooxygenation rates of Phen were determined by evaluation of the initial slopes of oxygen consumption as a function of the irradiation time, employing a specific oxygen electrode (Orion 97-08). These experiments were performed in water. Similarly, the Rose Bengal-sensitised photooxidation rates of Trp were determined by evaluation of the initial slopes of Trp consumption as a function of irradiation time, by monitoring the decrease in the 280 nm-absorption band of Trp.

Steady-state fluorescence was measured with a Spex Fluoromax spectrofluorometer at 25 ± 1 °C in air-equilibrated solutions. Fluorescence lifetimes were determined with a time-correlated single photon counting technique (SPC) on an Edinburgh FL-9000CD instrument, equipped with a nF900 nanosecond flashlamp. Excitation and emission wavelengths for Rf were 445 and 515 nm, respectively.

For the determination of the interactions of ^{1}Rf* with Phen (Reaction (8)) a classical Stern–Volmer treatment of the data was applied through Eqs. (2.4) and (2.5), where I, I_0, $^1\tau$ and $^1\tau_0$ are the respective intensities and lifetimes of Rf fluorescence in the presence and in the absence of Phen, being $K_{SV} = {}^1k_q\,{}^1\tau_0$

$$I_0/I = 1 + K_{SV}[\mathrm{Phen}], \quad (2.4)$$

$${}^1\tau_0/{}^1\tau = 1 + {}^1k_q\,{}^1\tau_0[\mathrm{Phen}]. \quad (2.5)$$

Continuous aerobic photolysis of aqueous solutions of Rf or Rose Bengal as sensitisers, and different substrates was carried out in a home made photolyser with a 150 W quartz-halogen lamp. In all cases continuous irradiation was performed at $\lambda > 350$ nm (cut-off filter). The aerobic and anaerobic photodegradation rates of Rf were evaluated from the decrease in the 445 nm absorption band as a function of the irradiation time.

Transient absorption spectra were determined in Argon-saturated 0.04 mM Rf aqueous solutions using a flash photolysis apparatus. The above described Nd:YAG laser was employed to generate ^{3}Rf* and a 150 W Xenon lamp as a source for the analysing light. The detection system comprised a PTI monochromator and a red-extended photomultiplier (Hamamatsu R666). The signal, acquired and averaged by a digital oscillo-

scope (Hewlett–Packard 54504A), was transferred to a PC via an HPIB parallel interface, where it was analysed and stored. ^{3}Rf* 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. To avoid self-quenching and triplet–triplet annihilation, the triplet decay was measured at low Rf concentration (typical 0.05 mM) and at low enough laser energy.

For the determination of the rate constant for interaction of ^{3}Rf*–Phen (Reaction (9)), the Stern–Volmer expression ((2.6)) was employed

$$1/{}^3\tau = (1/{}^3\tau_0) + {}^3k_q[\text{Phen}], \tag{2.6}$$

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

The efficiency of the electron transfer (2.7) processes (Reaction (9)) was calculated according to

$$\xi = \frac{A_0({}^3\text{Rf}^*)}{A_\infty(\text{RfH}^\bullet)} \frac{\varepsilon_{\text{RfH}^\bullet}}{\varepsilon_{{}^3\text{Rf}^*}}, \tag{2.7}$$

where $A_0(^3$Rf*) is the absorbance at 670 nm of the ^{3}Rf* species observed immediately after the flash, and the A_∞ (RfH$^\bullet$) is the absorbance of the RfH$^\bullet$ (see Reaction (13)), determined after completion of its grown-in at the maximum of the absorption spectrum (570 nm). The following molar absorption coefficients were used: riboflavin triplet, $\varepsilon_{3\text{Rf}^*} = 4.4 \times 10^3$ M^{-1} cm^{-1} [20]; riboflavin neutral radical, $\varepsilon_{\text{RfH}^\bullet} = 5.1 \times 10^3$ M^{-1} cm^{-1} [21]. Electron transfer quantum yield was determined relative to the triplet yield of zinc tetraphenylporphyrin (Φ_T) in benzene, employing (2.8) [22].

$$\Phi_{\text{Rf}^{\bullet-}} = \Phi_T \text{OD}_{T\varepsilon_T} / \text{OD}_{\text{RfH}^\bullet} \varepsilon_{\text{RfH}^\bullet}, \tag{2.8}$$

where OD_T is the triplet absorbance at 470 nm immediately after the laser pulse, ε_T is the molar absorption coefficient of ZnTPP triplet, and $OD_{RfH.}$ and $\varepsilon_{RfH.}$ are the absorbance and the molar absorption coefficients of the riboflavin radical anion at 570 nm. Values of 7.3×10^4 M^{-1} cm^{-1} and 0.83 were used for ε_T and Φ_T, respectively [23].

3. Results

3.1. Dark complexation Rf–Phen

The absorption spectrum of Rf (42 μM) in water suffers a number of perturbations in the presence of Phen (examined up to 85 mM Phen), that do not obey to the simple addition of the individual spectra of both components of the mixture. The spectral changes, which are imperceptible in the sub-mM range of Phen concentrations, can be better visualised by taking the difference spectra (Rf + Phen vs. Rf), as shown in Fig. 1. Considering that no absorption from Phen is expected over 300 nm, the species absorbing in this region should be ascribed to the complex Rf–Phen (Process (1)). From the slope and ordinate of Fig. 1 inset, $K_{ass} = 5.5 \pm 0.5$ M^{-1} and $\varepsilon_C = 5.3 \pm 0.5 \times 10^3$ M^{-1} cm^{-1} were determined (2.1).

3.2. Continuous photolysis experiments and the time-resolved determination of k_t

As shown in Fig. 2, the visible-light-photoirradiation of the mixture Rf (0.024 mM)–Phen (0.51 mM) in water produces spectral changes that can be mainly attributed to transformations in Rf and, to a lesser extent, in Phen. For comparative purposes, Fig. 2 inset, also shows the difference spectra Rf + Phen vs. Rf before and after photoirradiation. Phenylephrine practically does not absorb light at wavelengths higher than 290 nm. The shoulder observed in Fig. 2, between 290 and 375 nm approximately, correspond to absorption by the photoproducts. Since our kinetic determinations were performed at low conversions (the degree of conversion reached in Fig. 2, inset, was only on demonstrative grounds), the products absorption can be ignored, especially in the presence of Riboflavin.

In parallel, we observed that both the anaerobic and the aerobic photodegradation rates of Rf, processes that are well known to occur from electronically excited states of the vitamin (anaerobically) [7] and/or from autosensitisation via $O_2(^1\Delta_g)$ (aerobically) [20], suffer a decrease in the presence of Phen, in a concentration in the order of 0.1 mM. This fact, illustrated in Fig. 3, suggests an interaction between Phen and ^{1}Rf* and/or ^{3}Rf*.

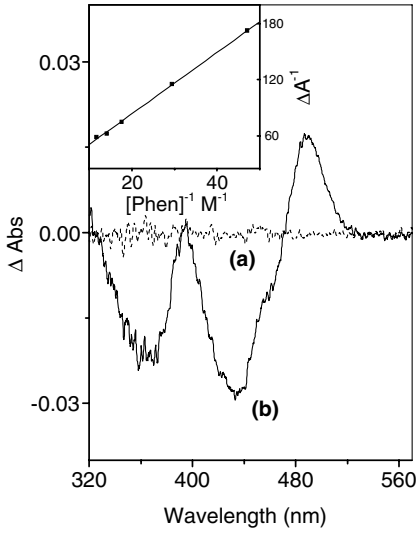


Fig. 1. Difference spectra of Rf + Phen vs. Rf in water. Rf 0.06 mM: (a) without Phen; (b) Phen 85 mM. Inset : Benesi–Hildebrand plot (see text) for the association Rf–Phen in water.

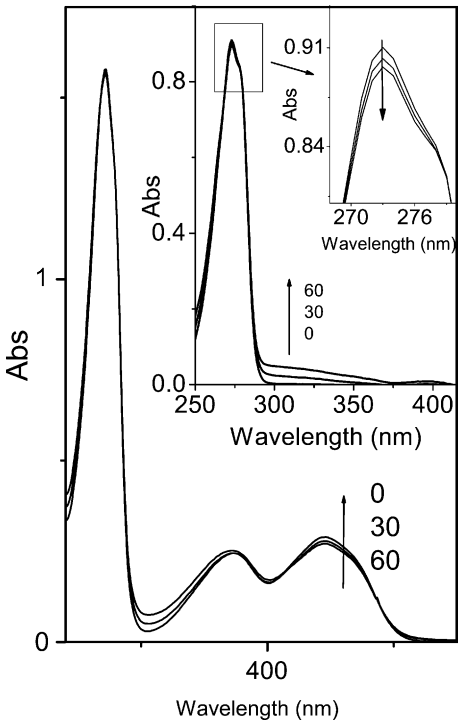


Fig. 2. Changes in the UV–Vis absorption spectra of Rf (0.024 mM) + Phen (0.51 mM) vs. water, upon irradiation at 445 nm under air-saturated conditions. Inset: changes in the UV–Vis absorption spectra of Rf (0.024 mM) + Phen (0.51 mM) vs. Rf (0.024 mM), upon irradiation at 445 nm under air-saturated conditions Numbers on the spectra represent the irradiation time in minutes. Cut-off 400 nm.

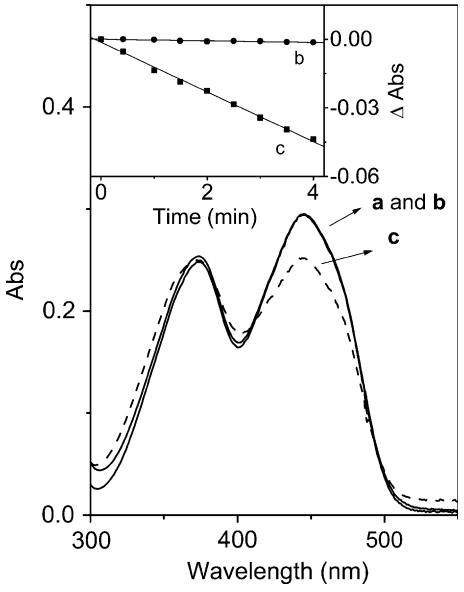


Fig. 3. Changes in the absorption spectra of N_2-saturated solutions of Rf (0.024 mM) in water (a) non-photolised; (b) photolised 5 min in the presence of Phen (0.7 mM); (c) photolised 5 min. Inset: Δ absorbance at 445 nm vs. irradiation time (min) of aqueous N_2-saturated solutions of Rf (0.024 mM) in the presence (b) and in the absence (c) of Phen (0.7 mM). Cut-off 400 nm.

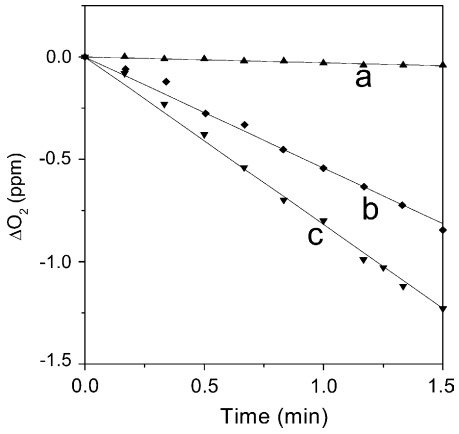


Fig. 4. Rates of oxygen uptake upon visible-light irradiation (cut-off 400 nm) of aqueous solutions containing: (a) Rf (0.04 mM)) + Phen (0.2 mM); (b) Rf (0.04 mM)) + FFA (0.2 mM); (c) Rf (0.04 mM)) + Trolox (0.2 mM).

In order to establish the possible involvement of OAS in the evolution of the photoirradiated mixtures, experiments of oxygen uptake upon Rf-photosensitised irradiation of Phen, Trolox and FFA are shown in Fig. 4. Oxygen consumption was scarcely detected for the system containing the sensitiser plus Phen 0.2 mM, whereas substantially higher rates of oxygen uptake could be observed when Phen was replaced by Trolox or FFA in said concentration. Trolox is a known phenolic antioxidant considered the water-soluble analog of α-tocopherol with regard to its properties as OAS scavenger [24]. FFA reacts with is $O_2(^1\Delta_g)$ and is currently used as a reference compound for kinetic determinations in photodynamic action [18].

The experimental evidence here shown, strongly suggests that the incidence of visible light on solutions containing the system Rf–Phen starts a series of processes that could include reactions of Rf electronically excited states and OAS with ground state of both Phen and the very Rf. Hence, we decided to systematically investigate these possibilities.

3.3. Quenching of $O_2(^1\Delta_g)$ by Phen

Due to the phenolic structure of Phen, and to the known properties of this family of compounds as $O_2(^1\Delta_g)$ quenchers, the rate constants k_t and k_r (being $k_t = k_q + k_r$ (Reactions (11) and (12)), were determined in water. $O_2(^1\Delta_g)$ phosphorescence was quenched by Phen in the sub mM concentration range, as shown in Fig. 5, inset. Through a simple Stern–Volmer treatment, an overall quenching rate constant value, $k_t = 2.2 \pm 0.2 \times 10^8\ M^{-1}\ s^{-1}$, was determined employing Rf as a sensitizer (inset Fig. 5). This experiment unambiguously demonstrates an interaction between $O_2(^1\Delta_g)$ and Phen, which may be merely physical in nature (Pro-

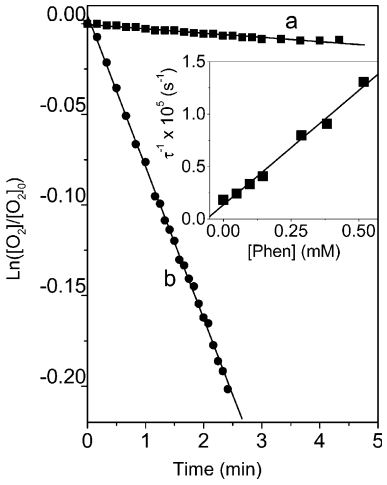


Fig. 5. First order plots of oxygen consumption of aqueous solutions of Rose Bengal ($Abs_{530\,nm} = 0.5$) containing (a) Phen (0.4 mM); (b) FFA (0.4 mM) upon visible-light irradiation (cut-off 400 nm). Inset: Stern–Volmer plot for the quenching of $O_2(^1\Delta_g)$ phosphorescence by Phen in D_2O.

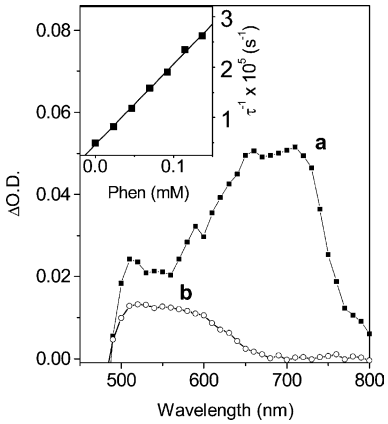


Fig. 6. Transient absorption spectra of Rf (40 μM) in argon-saturated aqueous solution, in the absence (a) and in the presence (b) of Phen (0.70 mM) taken at 1 and 40 μs after the laser pulse, respectively. Inset: Stern–Volmer plot for the ^{3}Rf* quenching by Phen in aqueous solution.

cess (11)), purely reactive (Process (12)) or a simultaneous composition of both mechanisms.

A reactive quenching rate constant value, $k_r = 2.7 \pm 0.2 \times 10^5$ (Reaction (12)) was determined using the method described by Scully and Hoigné [18], employing Rose Bengal as a dye sensitiser, through the first order plots shown in Fig. 5.

3.4. Quenching of ^{1}Rf* by Phen

The fluorescence properties of Rf are well known [7]. The presence of Phen produced a decrease in the intensity of the steady-state emission of ^{1}Rf* without any change in the shape of the emission spectrum. In parallel, the fluorescence decay of Rf was evaluated in the presence and in the absence of Phen by means of the SPC technique. From a classical Stern–Volmer treatment of the steady-state and time-resolved data, the values of $K_{SV} = 35 \pm 2\ M^{-1}$and $^1k_q = 3.33 \pm 0.08 \times 10^9\ M^{-1}\ s^{-1}$, respectively, were obtained (Eqs. (2.4) and (2.5), data not shown). Employing for $^1\tau_0$ the determined value of 4.80 ns, which is in good agreement with literature reports [7], it can be seen that $K_{SV} > {}^1k_q{}^1\tau_0$, a fact attributable to the formation of the dark complex above described.

3.5. Laser flash photolysis experiments and the interaction ^{3}Rf*-Phen

^{3}Rf* lifetime was neatly reduced by the presence of Phen, demonstrating the occurrence of an interaction between the medicament and the triplet excited state of the pigment. A value for the bimolecular quenching rate constant $^3k_q = 1.60 \pm 0.03 \times 10^9\ M^{-1}\ s^{-1}$ (Process (7)) was graphically obtained as shown in Fig. 6, inset. In order to further explore the type of interaction ^{3}Rf*–Phen involved, transient absorption spectra were recorded. Fig. 6 shows the known spectrum of ^{3}Rf* obtained after the laser pulse [25–27]. The shape of the long-lived absorption, obtained in the presence of 0.7 mM of Phen (ca. 95% ^{3}Rf* quenched by Phen) is in good agreement with that of the semiquinone radical, RfH$^{\bullet}$, previously reported [21,22]. At pH 6, the neutral (Rf radical RfH$^{\bullet}$) ($pK_a = 8.3$) would be formed [28] after protonation of the species $Rf^{\bullet-}$ (Process (13)), generated through Process (9).

$$Rf^{\bullet-} + H^+ \leftrightarrows RfH^{\bullet}. \quad (13)$$

RfH$^{\bullet}$ has been detected as a product of electron transfer processes to ^{3}Rf* from different electron-donor substrates of environmental and biological importance [7,25].

A quantum yield of 0.27 ± 0.03 for semireduced riboflavin generation ($\Phi_{RfH^{\bullet}}$) was determined (from 2.8), with a quantum efficiency value of 0.47 ± 0.05, associated with the electron transfer processes (from 2.7).

3.6. Photoprotective effect of Phen towards tryptophan

In order to evaluate the potential photoprotective effect of Phen towards photooxidizable biological targets, the rate of Rose Bengal-sensitised photooxidation of the amino acid Trp, in a concentration 0.5 mM $O_2(^1\Delta_g)$ in water was determined, in the presence and in the absence of 0.5 mM Phen. It is known that the RB-sensitised photooxidative degradation of the amino acid in aqueous solution operates through a composition of $O_2(^1\Delta_g)$ (mainly) and $O_2^{\bullet-}$ – mediated mechanisms [29,30]. Its evolution is often employed to monitor the extent of photooxidation of Trp-containing proteins [31,32].

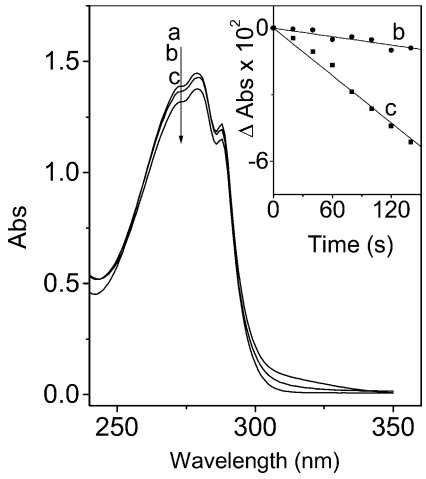


Fig. 7. Changes in the absorption spectra of aqueous solutions of RB ($Abs_{530\,nm}$ = 0.5) + Trp (0.5 mM); (a) non-photolised; (b) photolised 5 min in the presence of Trp (0.5 mM) and Phen (0.5 mM); (c) photolised 5 min in the presence of Trp (0.5 mM). Inset: Δ Absorbance at 260 nm vs. irradiation time (s) of aqueous of RB ($Abs_{530\,nm}$ = 0.5) plus Trp (0.5 mM) in the presence (b) and in the absence (c) of Phen (0.5 mM). Cut-off 400 nm.

Results, in Fig. 7, show that the photooxidation rate of free Trp experiments a neat delay in the presence of Phen.

4. Discussion

Most of the photolysis experiments were carried out in the sub-mM range of Rf and Phen concentrations. The determined value of $K_{ass} = (5.5 \pm 0.5)\ M^{-1}$ denotes a weak dark interaction Rf–Phen and indicates that the fraction of complexed pigment can be ignored under work conditions.

According to the fluorescence quenching data, a Phen concentration of ca. 0.6 mM – superior to those concentrations employed in the photolysis experiments – would produce a diminution in the lifetime of ^{1}Rf*, lower than 1%. Hence, the effects of decrease in the rate of aerobic and anaerobic photodegradation of Rf in the presence of Phen should be purely ascribed to an interaction Phen–^{3}Rf*.

Under aerobic conditions there operates a competence between Phen (step (9)) and $O_2(^3\Sigma_g^-)$ (steps (6) + (7)) towards ^{3}Rf*. With the same concentrations of Phen and dissolved $O_2(^3\Sigma_g^-)$, and employing the ^3k_q value of $1.6 \times 10^9\ M^{-1}\ s^{-1}$ and a k_{ET} value of $7 \times 10^8\ M^{-1}\ s^{-1}$in water (1/9 of the diffusion-controlled rate constant, accounting for Process (6)), the respective rates of $O_2(^1\Delta_g)$ production and ^{3}Rf* quenching by Phen are fairly similar. The interaction of ^{3}Rf* with oxygen should be totally assigned to the $O_2(^1\Delta_g)$ generation pathway due to the reported [8] extremely low quantum yield of direct $O_2^{\bullet-}$ production through Reaction (7). Besides, as demonstrated by flash photolysis results, the generation RfH$^\bullet$, with a quantum yield of 0.28, must be included in the reaction scheme. The quantum efficiency value of 0.47 obtained for Process (9) indicates that the electron transfer processes constitute a considerable fraction of the overall process represented in the interaction between ^{3}Rf* and Phen. In that case, although the species $O_2^{\bullet-}$ would not be formed through Process (14), (reported $k_{14} = 1.4 \times 10^8\ M^{-1}\ s^{-1}$) [34,35], due to the protonation step (13), the oxygenated radical could be generated anyway through step (16).

$$Rf^{\bullet-} + O_2(^3\Sigma_g^-) \xrightarrow{k_{14}} Rf + O_2^{\bullet-}. \quad (14)$$

The species RfH$^\bullet$ has a different and much slower reaction path with $O_2(^3\Sigma_g^-)$ from Rf$^{\bullet-}$[33]. The bimolecular decay of RfH$^\bullet$ is known to proceed through disproportionation reaction to yield equimolar Rf and fully reduced Rf (RfH_2) (Process (15)), which in the presence of $O_2(^3\Sigma_g^-)$ is reoxidised to give Rf radical and $O_2^{\bullet-}$ and finally Rf and H_2O_2 (Process (16)), [33,34].

$$2RfH^\bullet \rightarrow RfH_2 + Rf, \quad (15)$$

$$RfH_2 + O_2(^3\Sigma_g^-) \rightarrow [Rf\ \text{radical} + O_2^{\bullet-}] \rightarrow Rf + H_2O_2. \quad (16)$$

In any case, Reactions (14) and (16) constitute pathways for $O_2^{\bullet-}$ and H_2O_2 production and Rf regeneration. The recovery of the pigment – through Processes (15) and/or (16) – represents a crucial step in living organisms, in which it is well known that $O_2^{\bullet-}$ is a key intermediate in the oxygen redox chemistry [35].

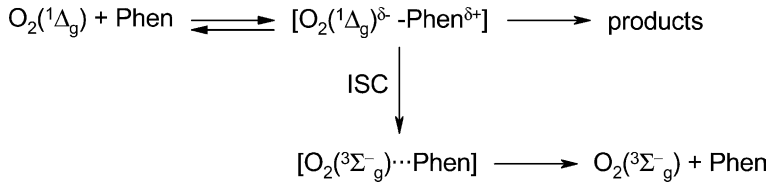
As estimated by oxygen uptake and UV absorption experiments (Figs. 2 and 4) the photo-reaction of Phen under Rf-sensitised photoirradiation is extremely low and, according to experimental evidence, the mechanism should obey to one or more of the following processes: oxidation due to electron transfer mechanism (9), or oxygenation through Reactions (12) and/or (17).

$$O_2^{\bullet-} + Phen \xrightarrow{k_{17}} Products. \quad (17)$$

The fate of Rf$^{\bullet-}$ and Phen$^{\bullet+}$ in the absence of $O_2(^3\Sigma_g^-)$ is possibly back electron transfer, as suggested by the results of Rf photoprotection exerted by Phen upon photoirradiation of the pigment (Fig. 3).

Data shown in Fig. 4 agree with those of absorption spectroscopy, in the sense that limited oxygen is consumed by Phen upon Rf-sensitisation. This scarce Phen degradation, in fact, not only confirms its poor proclivity towards a $O_2(^1\Delta_g)$-mediated oxidation, but also discards, in practise, a substantial chemical reactivity towards $O_2^{\bullet-}$.

Phen molecule presents two centres (Scheme 1) susceptible to attack by the oxidative species $O_2(^1\Delta_g)$: the phenolic moiety and the isopropilamine group. Literature reports indicate k_t values in the order of $10^7\ M^{-1}\ s^{-1}$ for substituted phenols in water [36] and



Scheme 2. Possible reaction pathways in the interaction of $O_2(^1\Delta_g)$ with phenolic compounds.

k_t values up to the order of 10^8 M^{-1} s^{-1} for different aliphatic amines in several solvents [19]. For both families of compounds it is currently accepted that bimolecular reactions with $O_2(^1\Delta_g)$ [36] can take place through a mechanism that involves an exciplex, as illustrated in Scheme 2. Physical quenching results from the intersystem crossing (ISC) within the exciplex. In the case of Phen, the balance between physical quenching and chemical reaction, an event sensitive to spin–orbit coupling and entropy factors [37], seems to be dominated by the ISC pathway, resulting in a practically exclusive physical deactivation of $O_2(^1\Delta_g)$.

The antioxidant properties of the phenolamine moiety in Phen may be estimated by comparison to trolox, a synthetic compound extensively recognised as an antioxidant in processes mediated by $O_2(^1\Delta_g)$ and radical species [17]. Although a k_t value of 3.5×10^8 M^{-1} s^{-1} has been determined in our laboratory for Trolox in water [17], very similar to the corresponding one for Phen, the k_r/k_t quotient, a measure of the degradability of a given substrate due to photooxidation by the species $O_2(^1\Delta_g)$, reaches a value of 0.63 for trolox [17], as compared to the value $k_r/k_t = 0.001$ obtained for Phen. In addition, the photoprotective effect of Phen towards Trp, taken as a case of photooxidisable target, is evident from Fig. 7. A concentration of 0.1 mM Phen reduces ca. 80% the rate of the RB-sensitised photooxidation of the amino acid.

5. Conclusions

Rf forms a dark complex with Phen in aqueous solution, only detectable at relatively high concentrations. Upon visible-light photoirradiation the phenolamine interacts with ^{1}Rf* and ^{3}Rf*, but only the second process is important in the sub-mM range of Phen concentration. The species $O_2^{\bullet-}$ and $O_2(^1\Delta_g)$ are formed under aerobic conditions, through direct and indirect interaction of ^{3}Rf* with Phen and dissolved oxygen, respectively. Experimental evidence indicates that Phen is unreactive towards these oxidative species; even more, it is a very efficient physical deactivator of $O_2(^1\Delta_g)$. Kinetically, the phenolamine structure in Phen appears as an excellent non-sacrificial scavenger of activated oxygen species.

6. Abbreviations

FFA	furfuryl alcohol
OAS	oxygen active species
Phen	phenylephrine
RB	rose Bengal
Rf	riboflavin or vitamin B2
SD	sympathomimetic drugs
Trp	L-Tryptophan
ZnTPP	zinc tetraphenylporphyrine

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