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On the generation and quenching of reactive-oxygen-species by aqueous vitamin B2 and serotonin under visible-light irradiation

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ABSTRACT

It is well known that endogenous daylight-absorbing compounds produce the sensitized photodegradation of biologically relevant substrates. In this context the photostability of a mixture of the indole neurotransmitter serotonin (Sero) and vitamin B2 (riboflavin, Rf) upon visible-light irradiation and the possible role of Sero and related compounds as generators or deactivators of reactive oxygen species (ROS) was investigated through a kinetic and mechanistic study. The work was done at pH 7 and under experimental conditions in which only the vitamin absorbs photoirradiation. Tryptamine (Trpa) and 5-hydroxyindole (OHIn) were included in the study as model compounds for the neurotransmitter. The visible light irradiation of aqueous Rf in the individual presence of Sero, Trpa and 5-OHIn, under aerobic conditions, induce degradative processes on the indole derivatives (In-der). At least two different mechanisms operate. Our analysis shows that the main reaction pathway is an electron-transfer-mediated quenching of triplet excited Rf ($^3Rf^*$) by the In-der. It produces the species $Rf^{\cdot-}$/RfH$^\cdot$ and the In-der radical cation that could react to form phenoxy and α-amino radicals. In a further reaction step the species $O_2^{\cdot-}$ and OH$^\cdot$ could be produced. In parallel, energy transfer from $^3Rf^*$ to dissolved oxygen would generate $O_2(^1\Delta_g)$.

Within the frame of the proposed mechanism, results suggest that Rf-sensitized degradation of Sero occurs *via* the mentioned ROS and non-oxygenated radical-mediated processes.

The indole compound quenches $O_2(^1\Delta_g)$ in a dominant physical fashion. This fact constitutes a desirable property in antioxidants, provided that the quenching process practically does not eliminate the scavenger.

Sero exerts a photoprotective effect towards tryptophan through the combined quenching of $O_2(^1\Delta_g)$ and $^3Rf^*$, the latter excited species responsible for the generation of ROS. The amino acid can be taken as a target model of oxidizable biological substrates, particularly proteins.

1. Introduction

Photoprocess in living organisms are still being investigated in relation to substrates of relevance in biology, pharmacology and medicine [1,2]. Some of these events in mammalians and particularly in human beings are believed to increase the oxidative damage of cells, tissues and different organs, caused by reactive oxygen species (ROS) through a process currently known as oxidative stress [3]. In many cases the ROS are photogenerated by endogenous light receptors. The knowledge of kinetic and mechanistic aspects of these oxidative events can contribute to the understanding and prevention of degradative biological processes.

The objectives for the above mentioned studies comprise the conjunctive evaluation of three important aspects of the oxidative stress: (a) the potentiality of ROS photogeneration in a given environment, (b) the antioxidant activity of biologically relevant substrates and (c) the extent of possible photo-damages as a result of the action of ROS. These substrates can undergo oxidation, decreasing the activity of their specific biological/therapeutic roles or quenching photo-generated species. The prevalence of physical quenching of ROS is an interesting possibility, since in this manner the oxidative species are eliminated without any chemical transformation in the scavenging substrates.

Photooxidations in biological media constitute a crucial point, particularly when they involve endogenous generators and quenchers of ROS that may occupy the same or close microenvironments. This could be the case of vitamin B2 (Rf, Riboflavin) and Serotonin (Sero).

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Sero, a biomolecule derived from tryptophan, is a neurotransmitter with a number of known biological roles [4]. Its chemistry has been extensively studied, whereas its photochemical behavior has received less attention. Although Sero only absorbs UV light, it has been reported that it can undergo photooxidation upon visible light irradiation in the presence of a sensitizer [5]. In other words, the neurotransmitter could be photodegraded *in vivo*, through interaction with ROS generated by the combined action of a visible light absorber and environmental light.

A decrease in the level of serotonin in mammalian organisms may cause different disorders [4]. Important findings in this line were recently reported [6,7].

Rf is a naturally occurring endogenous daylight-absorbing pigment of particular interest. Rf is endogenously present in living organisms (1), and it is a well known sensitizer for the light-promoted oxidation of different substrates [8,9]. To account for the aerobic photooxidative processes in Rf-sensitized events in solution, the participation of the species superoxide radical anion ($O_2^{\cdot-}$), generated by electron transfer with quantum yield $\Phi O_2^{\cdot-}$ = 0.009 and singlet molecular oxygen [(O_2 ($^1\Delta_g$)] generated by energy transfer with quantum yield Φ_Δ = 0.47, have been demonstrated [10,11]. The processes are represented by reactions 1–3 where $^1Rf^*$ and $^3Rf^*$ are the electronically excited singlet and triplet states of Rf and $O_2(^3\Sigma_g^-)$ is the dissolved ground state molecular oxygen.

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

$$^3Rf^* + O_2\left(^3\Sigma_g^-\right) \rightarrow Rf^{\cdot+} + O_2^{\cdot-} \quad (2)$$

$$^3Rf^* + O_2\left(^3\Sigma_g^-\right) \rightarrow Rf + O_2(^1\Delta_g) \quad (3)$$

rate constant *k*ET

The simultaneous presence of Rf, Sero and visible light in aqueous medium acceptably mimics a natural biological scenery. In this context, the aim of the present work was to develop an integral kinetic and mechanistic study on the potential interaction of Rf and Sero under visible light-irradiation. The interest was focused on the elucidation of the oxidative steps that could account either for the decrease in the available concentration of Sero or the vitamin. For comparative purposes the structurally serotonin-related substrates tryptamine (Trpa) and 5-hydroxy indole (OHIn) were also included in the study. All three indole-derivatives will be generically named In-der in the following and their respective structural formulas are shown in Table 1.

2. Materials and methods

2.1. Chemicals

Serotonin, Tryptamine, 5-hydorxy indole, L-tryptophan (trp), Riboflavin, Rose Bengal (RB), sodium azide (NaN_3), catalase (CAT) from bovine liver, superoxide dismutase (SOD) from bovine erythrocytes and D-mannitol (Mann) were purchased from Sigma Chem. Co. Furfuryl alcohol (FFA) was from Riedel de Haën. The solvent employed was triply distilled H_2O. Phosphate buffer was used to regulate pH.

2.2. Instrumentation and methods

Ground state absorption spectra were registered in a Hewlett Packard 8452A diode array spectrophotometer. Stationary aerobic photolysis of aqueous solutions containing In-der 0.1–0.5 mM and Rf *ca.* 0.04 mM were carried out in a PTI unit (150 W Xe lamp) with a high pass monochromator, irradiating with 440 ± 10 nm, or in a home-made photolyzer for non-monochromatic irradiation (150 W quartz-halogen lamp), using a cut-off filter of 400 nm in order to ensure that the light was only absorbed by Rf. Rf-ensitized photooxygenation rates of In-der were determined from the initial slopes of the plots oxygen consumption *vs.* irradiation time, employing a specific oxygen electrode (Orion 97-08). The reactive rate constant, k_r, for the reaction of $O_2(^1\Delta_g)$ with In-der was determined as described in the literature [12] using the expression slope/slope$_R$ = k_r [In-der]/k_{rR} [*R*], for which the knowledge of the reactive rate constant for the photooxidation of a reference compound, *R*, at similar concentration, is required. Slope and slope$_R$ are the respective slopes of the first-order plots of In-der and *R* consumption, or oxygen consumption by In-der and *R*, assuming a stoichiometry 1:1 for substrate – oxygen in the second case.

Argon-saturated aqueous solutions of Rf 0.04 mM were irradiated with a flash photolysis apparatus. A ns Nd:YAG laser system (Spectron) at 355 nm was used for excitation, employing a 150-W Xenon lamp as a source for the 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 via a HPIB parallel interface to a PC where it was analyzed and stored. The disappearance of $^3Rf^*$, a species generated by the 355 nm pulse, 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 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 $^3Rf^*$- In-der (process 4) was determined by the Stern–Volmer expression $1/^3\tau = (1/^3\tau_0) + {}^3k_q$ [In-der], where $^3\tau$ and $^3\tau_0$ are the experimentally determined lifetimes of $^3Rf^*$ in the presence and in the absence of In-der, respectively.

3. Results

3.1. Stationary photolysis

The visible light irradiation (cut off >400) of a pH 7 aqueous solutions containing 0.05 mM Rf and *ca.* 0.1 mM of individual Sero, Trpa or OHIn produces modifications in the respective absorption spectra of the mixtures, attributed to chemical changes in both the In-der and the sensitizer. The case of Sero is shown in Fig. 1, as a typical example. The absorption band in the 275-nm region decreases with the irradiation time. All solutions were stable when stored for several hours in the dark.

From parallel experiments on similar photoirradiated solutions, oxygen consumption was observed. Relative rates of oxygen uptake for the In-der studied are shown in Table 1. The solutions did not consume any oxygen before photoirradiation.

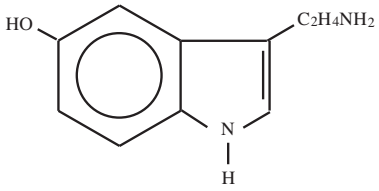
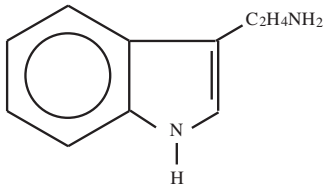
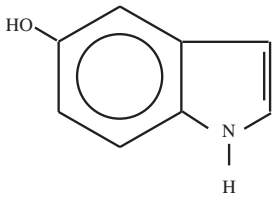
Finally, minor spectral changes in the absorption spectrum of the vitamin were also observed when deaerated solutions of Rf plus individual In-der in the sub-mM concentration range were photoirradiated.

This set of preliminary qualitative results suggests that the electronically excited states of Rf and/or ROS generated from these states react with the In-der. On this basis we carried out a systematic kinetic study in order to establish the mechanism and kinetic parameters involved in the mentioned photopromoted interaction.

3.2. Interaction of In-der with electronically excited states of Rf

The quenching of Rf fluorescence by indole auxins is well known [13,14]. No quenching of $^1Rf^*$ has been detected up to mM-concentration range of the indole derivative. The decrease in the Rf fluorescence intensity in the presence of indoles is due to

Table 1
Rate constants for overall k_t (M^{-1} s^{-1}), and reactive k_r (M^{-1} s^{-1}), quenching of $O_2(^1\Delta_g)$, k_r/k_t ratio, rate constant for the quenching of Rf triplet excited state ^3k_q (M^{-1} s^{-1}), and relative rates of oxygen uptake upon Rf-(V_{ox}Rf) and RB-photosensitization (V_{ox}RB) for Sero, Trpa and OHIn in pH 7 aqueous solution.

Indole derivative	$k_t/10^7$	$k_r/10^7$	k_r/k_t	$^3k_q/10^9$	V_{ox}Rf	V_{ox}RB
 Serotonin (Ser)	2.7 + 0.1[a]	0.49 ± 0.02	0.18	2.7 ± 0.2	0.51	0.18
 Tryptamine (Trpa)	25 + 0.5[b]	2.3 ± 0.3	0.09	4.3 ± 0.2	1.00	0.85
 5 - Hydroxyindole(OHIn)	15 + 0.3[a]	2.7 ± 0.2	0.18	2.7 ± 0.3	0.58	1.00

[a] Ref. [5].
[b] Ref. [24].

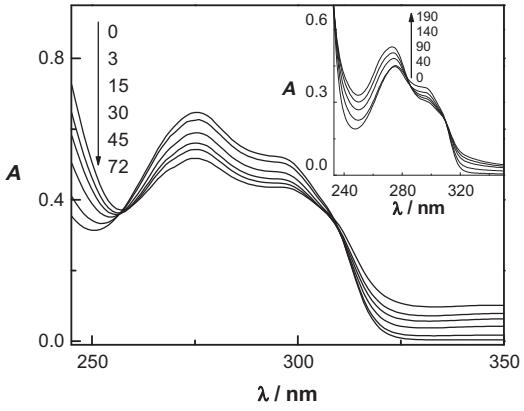


Fig. 1. Changes in the UV–Vis absorption spectrum of a pH 7 aqueous solution of 0.1 mM Serotonin plus 0.05 mM Rf, upon irradiation (>400 nm) under air-saturated conditions. Inset: changes in the UV–Vis absorption spectrum of a pH 7 aqueous solution of Serotonin 0.1 mM plus RB (A_{560} = 0.4), upon irradiation (>400 nm) under air-saturated conditions. Rf and RB components were subtracted out from the main and inserted spectra, respectively.

a weak dark complexation with the vitamin, driven by charge transfer interactions and hydrophobic forces [14]. Nevertheless, even assuming a diffusional value for the eventual rate constant of a dynamic quenching of $^1Rf^*$ and considering [In-der] = 5 mM (*ca.* ten times higher than the concentrations employed in the Rf-sensitized experiments in this work) the eventual interaction In-der–$^1Rf^*$ would produce a decrease in the lifetime of $^1Rf^*$ lower than 2%, being the lifetime for ^1Rf *ca.* 5 ns [15]. Hence, we suggest that the participation the $^1Rf^*$ in the photodegradation of In-der can be disregarded. $^3Rf^*$ lifetime was neatly reduced by the presence of In-der, demonstrating the occurrence of an interaction with the triplet excited pigment. The bimolecular rate constants ^3k_q (process 4) were obtained through a Stern–Volmer treatment (Fig. 2) and the respective values are included in Table 1.

In order to further explore the type of interaction $^3Rf^*$–In-der involved, transient absorption spectra were recorded. Fig. 2 shows the known spectrum of $^3Rf^*$ obtained after the laser pulse [16–18]. The shapes of the long-lived absorption, in the presence of In-der in a concentration able to quench *ca.* 95% $^3Rf^*$, are in good agreement with that of the semiquinone radical, RfH·, previously reported [19] (reactions 4 and 5 and Fig. 2). The transient absorbance of the oxidized radical of Inder (In-der$^{\cdot+}$), could not be clearly

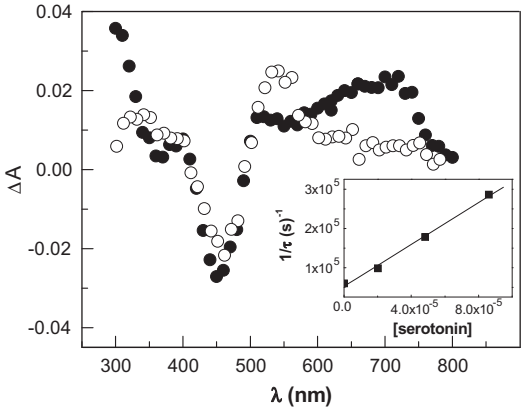


Fig. 2. Transient absorption spectra of 0.01 Mm Rf 10 μs after the laser pulse in argon-saturated pH 7 aqueous solution in the absence (●) and (○) in the presence of 0.5 mM Sero. Inset: Stern–Volmer plot for the quenching $^3Rf^*$ (b) by Sero in pH 7 aqueous solution.

observed. For Sero$^{\cdot+}$ two absorption bands, centred at 340 and 460 nm have been reported [20]. At 340 nm a slight absorption could be attributed to Sero radical (Fig. 2), but the band of 460 nm precisely overlaps the spectral region of ground state Rf bleaching.

At pH 7, the neutral radical RfH$^{\cdot}$ (pK = 8.3) has been detected as a product of electron transfer processes to ^{3}Rf* from different electron-donor substrates of environmental and biological importance [21,22].

$$^3Rf^* + In-der \rightarrow Rf^{\cdot-} + In-der^{\cdot+} \quad (4)$$

rate constant ^3k_q

$$Rf^{\cdot-} + H^+ \rightleftarrows RfH^{\cdot} \quad (5)$$

3.3. The interaction of In-der with Rf-photogenerated ROS

In order to evaluate the potential participation of Sero and related compounds in the generation and quenching of Rf-photogenerated ROS, oxygen consumption experiments in the presence of specific ROS interceptors were carried out. Results for Sero are shown in Fig. 3, being ±5% the estimated error in the slope values. The rates of oxygen uptake in runs employing pH 7 aqueous solutions of Rf 0.04 mM plus 0.5 mM In-der decrease in the individual presence of 10 mM NaN_3, 10 mM mannitol and the enzyme SOD in a concentration of 1 μg/ml. Practically no changes in the rate of oxygen consumption were observed in the presence of 1 μg/ml CAT.

The enzyme SOD dismutates the species $O_2^{\cdot-}$ (reaction 6), whereas CAT decomposes H_2O_2 (reaction 7), mannitol deactivates the species OH$^{\cdot}$ (reaction 8) and NaN_3 quenches $O_2(^1\Delta_g)$ (reaction 16 with NaN_3 instead of In-der, see below).

$$2O_2^{\cdot-} + 2H^+ + SOD \rightarrow O_2\left(^3\Sigma_g^-\right) + H_2O_2 \quad (6)$$

$$2H_2O_2 + CAT \rightarrow 2H_2O + O_2\left(^3\Sigma_g^-\right) \quad (7)$$

$$OH^{\cdot} + \text{mannitol} \rightarrow \text{deactivation} \quad (8)$$

Practically no effect was observed in the presence of the enzyme CAT in concentration 1 μg/ml. In all cases the higher inhibition of oxygen consumption was obtained in the presence of 10 mM NaN_3. Similar experiments with the specific ROS-interceptors have been formerly employed to confirm/discard the participation of

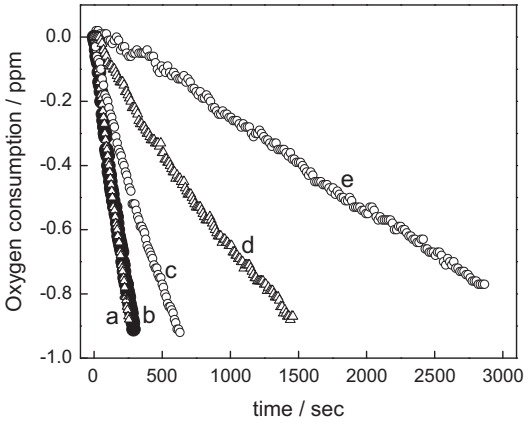


Fig. 3. Oxygen consumption in 0.5 mM Serotonin + Riboflavin (A_{445}) = 0.5 as a function of photoirradiation time (cut-off 400 nm), in pH 7 aqueous solution (a) in the absence and in the presence of (b) 1 μg/ml catalase, (c) 10 mM mannitol; (d) 1 μg/ml SOD and (e) 10 mM NaN_3. All in pH 7 aqueous solution.

$O_2(^1\Delta_g)$, $O_2^{\cdot-}$, H_2O_2 and OH$^{\cdot}$ respectively in a given oxidative event [21–23]. Results suggest that all three In-der behaved in the same qualitative fashion through reactions 9 and 10.

$$O_2^{\cdot-} + In-der \rightarrow Products_9 \quad (9)$$

$$OH^{\cdot} + In-der \rightarrow Products_{10} \quad (10)$$

Regarding the interaction of In-der with $O_2(^1\Delta_g)$ (reactions 11 and 12), the work by Matuszak et al. [5] demonstrated that a series of indolic derivatives, including Sero and 5-OHIn quench the RB-photogenerated oxidative species. The rate constant values k_t accounting for the overall interaction of the substrate with the oxidative species, are shown in Table 1. The k_t value for Trpa was reported by Sanramé [24].

$$O_2(^1\Delta_g) + In-der \rightarrow O_2\left(^3\Sigma_g^-\right) + In-der \quad (11)$$

rate constant K_q

$$O_2(^1\Delta_g) + \text{In-der} \rightarrow \text{products}_{12} \quad (12)$$

rate constant K_r

Being $k_t = k_r + k_q$

The mentioned authors [5,24] observed effective photobleaching of the In-der as a contribution to the overall quenching of $O_2(^1\Delta_g)$ (reaction 16), but the respective k_r values were not evaluated. Nevertheless the authors [5] emphasize on the small contribution to chemical quenching exerted by other substituted indoles, on the basis of literature reports [25].

In Fig. 1, inset, we are shown the evolution of the absorption spectrum of ca. 0.1 mM Sero upon photoirradiation with visible light in the presence of RB (A_{560} = 0.4) in pH 7 aqueous solution. The absorbance of the 275-nm absorption band increases, in opposition to the behavior above described for the Rf-sensitization.

In the same solutions, oxygen consumption upon photoirradiation was observed. For comparative purposes the relative rates of oxygen uptake of Sero, Trpa and OHIn upon RB-photosensitization are shown in Table 1. RB was used as a photosensitizer instead of Rf, in order to eliminate possible interferences due to radical-mediated reactions. The xanthenic dye is the photosensitizer most commonly employed in $O_2(^1\Delta_g)$ reactions and predominantly generates the oxidative species with a quantum yield of *ca.* 0.7 in aqueous solution [26,27].

In biologically relevant $O_2(^1\Delta_g)$-mediated photoreactions the photooxidation quantum efficiency (φ_r) of a given substrate Q, ($\varphi_r = k_r\,[Q]/(k_d + k_t\,[Q])$), constitutes highly valuable information in order to estimate the extent of oxidative photo-damage [28]. This value is not easy to determine, particularly in natural environments, because its evaluation includes the knowledge of the actual concentration of Q. A simpler and useful approach is the calculation 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.

Following we determined the respective rate constants (Table 1) k_r (process 13) for the reactive interaction In-der–$O_2(^1\Delta_g)$, employing the already described actinometric method [12]. Results are shown in Fig. 4, and the respective k_r/k_t values are included in Table 1.

3.4. Photoprotective effect of serotonin towards biological targets

The behavior of biologically relevant compounds such as Sero, under photogenerated oxidative stress, constitutes meaningful information. In this context two aspects must be particularly considered: (a) the ability of Sero to inhibit oxidative processes with the ultimate consequence of protection for surrounding molecules

and (b) the possible photofading of Sero, followed by the concomitant side effects of development of anomalous biological effects. Following, the eventual photoprotective effect exerted by Sero was tested in relation to the aromatic amino acid trp, taken as an oxidizable biological target. The amino acids trp, tyrosine, methionine, histidine and cysteine constitute a family of photooxidizable biological targets [29]. In many cases one or more of these amino acids have been employed to mimic the photodynamic action in order to understand problems in more complex systems such as polypeptides or proteins [30]. Our work was done through oxygen consumption experiments. In Fig. 5 is shown the effect of 0.5 mM Sero on the rate of oxygen uptake by 0.5 mM trp upon photosensitization in separate experiments by 0.04 mM Rf (panel A) and RB A_{560} = 0.5 (panel B), both in pH 7 aqueous solution. For comparative purposes the rates of oxygen consumption by RB and Rf alone and RB and Rf plus Sero are also shown. The rate of oxygen consumption constitutes a measure of the effective overall oxidability of the different mixtures. Results clearly show that the rate of the oxidative process of trp upon photosensitization is decreased. The extent of this effect depends on the photosensitizer employed.

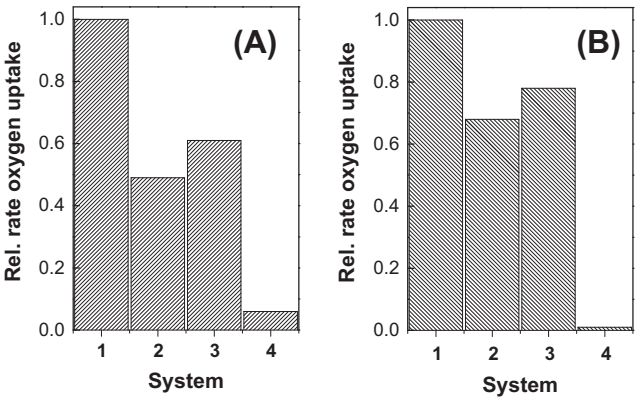


Fig. 5. Relative rates of oxygen uptake in the following systems, all in pH 7 aqueous solutions: Panel A, riboflavin 0.04 mM as a photosensitizer in the presence of: (1) 0.5 mM tryptophan; (2) 0.5 mM serotonin; (3) 0.5 mM tryptophan plus 0.5 mM serotonin; (4) without additives. Panel B, Rose Bengal A_{560} = 0.4 as a photosensitizer in the presence of: (1) 0.5 mM tryptophan; (2) 0.5 mM serotonin; (3) 0.5 mM tryptophan plus 0.5 mM serotonin; (4) without additives.

4. Discussion

There are two evidences that strongly suggest different photodegradation mechanisms of In-der in employing Rf and RB as sensitizers. The first is the absence of parallelism observed in the values of the relative rates of oxygen uptake by the substrates upon sensitization with Rf and RB (Table 1). The second is the different qualitative evolution of the respective absorption spectra of photolyzed In-der solutions, employing both sensitizers individually. The RB-sensitized mechanism mainly involves a $O_2(^1\Delta_g)$-mediated pathway whereas the Rf-sensitized process apparently includes radical-mediated reactions in addition to the $O_2(^1\Delta_g)$ pathway.

Experimental results indicate that $^3Rf^*$ can be quenched either by $O_2(^3\Sigma_g^-)$ (reaction 3) or by In-der (reaction 4). The prevalence of one of these processes depends on the respective concentration of the quenchers and on the rate constant values for reactions 3 and 4. Considering the $k_{ET} = 9 \times 10^8\ M^{-1}\ s^{-1}$ in H_2O [31] (process 3) and a mean ^3k_q value for the In-der of $3.4 \times 10^9\ M^{-1}\ s^{-1}$ (Table 1), it can be deduced that for the same concentrations of In-der and dissolved $O_2(^3\Sigma_g^-)$, the rate constant value for the initial generation of $Rf^{\bullet-}$ (*vía* process 4) is *ca.* 4 times higher than the corresponding one for $O_2(^1\Delta_g)$ generation (reaction 3). In consequence, both processes could operate under work conditions.

The thermodynamic feasibility of the electron transfer (process 4) can be evaluated by means of the Gibbs free energy for electron transfer. $\Delta_{ET}G_0 = N_A\ \{e\ [E^0(\text{In-der/In-der}^{\bullet+}) - E^0(\text{Rf/Rf}^{\bullet-})] + w(\text{In-der}^{\bullet+}\ \text{Rf}^{\bullet-}) - w(\text{In-der Rf})\} - \Delta E_{0,0}$, where e is the elementary charge, N_A is the Avogadro constant, $E^0(\text{In-der/In-der}^{\bullet+})$ is the standard electrode potential of the donor, (0.94 V for Ser) [32], $E^0(\text{Rf/Rf}^{\bullet-})$) is the standard electrode potential for the acceptor (−0.80 V) [33], the difference $w(\text{In-der}^{\bullet+}\ \text{Rf}^{\bullet-})$–$w$(In-der Rf) is the coulombic energy term (−0.06 V) [33] and $\Delta E_{0,0}$ is the vibrational zero energy of the excited partner (2.17 eV) [33]. The so-calculated $\Delta_{ET}G_0 = -0.93$ eV indicates that process 4 may be operative and, consequently, that the species $O_2^{\bullet-}$ could be formed through a radical-mediated mechanism from $^3Rf^*$.

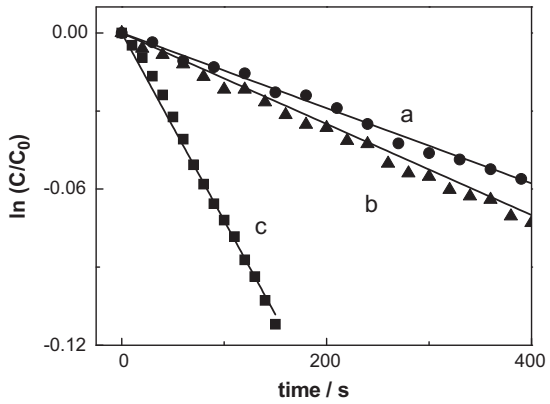


Fig. 4. First order plot of oxygen uptake in the Rose Bengal (A_{560} = 0.4)-sensitized photooxidation of (a) tryptamine 0.5 mM; (b) 5-hydroxy Indole 0.5 mM and (c) furfuryl alcohol 0.5 mM.

The quenching of $^3Rf^*$ by In-der initially produces the species $Rf^{\bullet-}$, $RfH^{\bullet}$ and In-der $^{\bullet+}$. These radicals could react with neutral compounds starting chain reactions or forming phenoxy radicals in the cases of Sero and OHIn, and the corresponding α-amino radical in the case of Trpa (reactions 13 and 14). The occurrence of reaction 14 has been already proposed by Lu and Liu [34] and by Görner [35] in studies on flavin–photosensitization in the presence of different electron donors.

$$\text{Flavin radicals} + \text{In} - \text{der}^{\bullet+} + \text{neutral molecules} \rightarrow \text{radical chain reactions} \quad (13)$$

$$\text{Flavin radicals} + \text{In} - \text{der}^{\bullet+} \rightarrow \text{Phenoxy/}\alpha - \text{amino radicals} \quad (14)$$

Although in a less probable reaction, the biomolecular decay of $RfH^{\bullet}$ is known to occur through the disproportionation reaction 15, to yield Rf and the fully reduced Rf (RfH_2) [34]. In the presence of dissolved oxygen and under favourable conditions RfH_2 may start a series of reactions that include the generation of the ROS $O_2^{\bullet-}$, hydrogen peroxide (H_2O_2) and hydorxy radical ($OH^{\bullet}$) as follows:

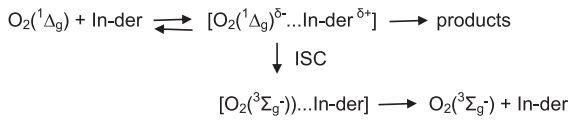
$$2RfH^{\bullet} \rightarrow Rf + RfH_2 \quad (15)$$

$$RfH_2 + O_2\left(^3\Sigma_g^-\right) \rightarrow RfH_2^{\bullet+} + O_2^{\bullet-} \quad (16)$$

$$RfH_2^{\bullet+} + O_2^{\bullet-} \rightarrow Rf + H_2O_2 \quad (17)$$

$$H_2O_2 + O_2^{\bullet-} \quad OH^{\bullet} + H_2O \quad (18)$$

The reaction sequence 15–18 justifies the decrease in oxygen uptake rates exerted by SOD and catalase on the proposed oxidative reactions 9 and 10. These results are in line with several reports on the scavenging activity of Sero and other indole



Scheme 1. Possible formation and evolution of the excited encounter complex.

derivative towards thermally generated radical species, including $O_2^{\cdot-}$ and $OH^{\cdot}$, where the main oxidation products of Sero are oxygenated tryptamine derivatives [36–39].

The kinetics of the $O_2(^1\Delta_g)$-mediated sensitized photooxidation of indoles, aromatic amines and hydroxyaromatic compounds has been extensively studied [25,28,40]. Nevertheless only in a few cases, the quotient k_r/k_t has been evaluated [28,40]. Indoles are efficient overall quenchers of $O_2(^1\Delta_g)$, and generally speaking, the kinetic data found for these three families of compounds can be interpreted in a similar way, which includes the intermediate formation of an excited encounter complex of the type $[O_2(^1\Delta_g)\ldots$ In-der] with partial charge-transfer character (Scheme 1).

Reports indicate that the process of $O_2(^1\Delta_g)$ quenching is essentially physical in nature for aromatic amines, indole and non-ionized hydroxyaromatic compounds [40], in agreement with our findings for all three In-der herein studied (Table 1). The ratio k_r/k_t for the $O_2(^1\Delta_g)$–In-der interaction shows values lower than 20% for Sero and OHIn whereas reaches less that 10% for Trpa (Table 1). It is worth noting that although the k_t for Sero, Trpa and OHIn are quite different, the respective k_r/k_t ratios are fairly close each other. These results must be interpreted in terms of evolution of the excited encounter complex, that can undergo deactivation or chemical reaction. The physical quenching can be regarded as a photophysical deactivation step, yielding the unstable ground state complex whereas the reactive pathway depends on an irreversible charge transfer process which is determined by the redox potential of the quencher. The presence of either an isolated hydroxy- or an isolated amino-group in positions 5 and 3 of the indole ring respectively, increases the k_t value by more than one order of magnitude as compared to indole (k_t indole = 2.5×10^5 M^{-1} s^{-1} in acetonitrile) [25].

The observed decrease in the overall rate of oxygen consumption by the mixture trp + Sero as compared to those of trp, in the individual presence of Rf or RB as photosensitizers (Fig. 5) shows, in principle, a photoprotective effect by Sero on the degradation of the amino acid. The photooxidation of trp seems to occur through a combination of $O_2(^1\Delta_g)$-driven and radical-driven mechanisms[30,2]. Silva et al. reported on the generation of $O_2^{\cdot-}$; H_2O_2; $OH^{\cdot}$ and $O_2(^1\Delta_g)$ in the Rf-sensitized photodegradation of trp, and we determined a $k_r = 3.5 \times 10^7$ M^{-1} s^{-1} for reaction 13 with trp instead of In-der [2,30].

The photoprotection of Sero towards trp apparently operates through two parallel processes: (a) the deactivation of $O_2(^1\Delta_g)$, provided that more than 80% of the collisions Sero-$O_2(^1\Delta_g)$ produce physical quenching of the oxidant and (b) the competitive quenching of $^3Rf^*$ by Sero, that although contributes to the generation of ROS through the radical-mediated mechanism, concomitantly reduces the yield of $O_2(^1\Delta_g)$ production through a competitive pathway. The photoprotection seems to be some more pronounced in the case of Rf- than RB-sensitization possibly due to the double action of Sero on the quenching of $^3Rf^*$ and $O_2(^1\Delta_g)$ deactivation.

5. Conclusions

The Rf-sensitized photoirradiation of aqueous Sero produces the degradation of the neurotransmitter through radical-mediated reactions and oxidation by ROS. $O_2(^1\Delta_g)$ is deactivated in a dominant physical fashion. Sero exerts a photoprotective effect towards trp, taken as model oxidizable target in proteins, through the combined quenching of $O_2(^1\Delta_g)$ and $^3Rf^*$.

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