# Scavenging of riboflavin-photogenerated oxidative species by uric acid, xanthine or hypoxanthine: A kinetic study

M. Paulina Montaña [a], Walter A. Massad [b], Francisco Amat-Guerri [c], Norman A. García [b],*

[a] *Área de Química-Física, Universidad Nacional de San Luis, Lavalle 1151, 5700 San Luis, Argentina*
[b] *Departamento de Química, Universidad Nacional de Río Cuarto, Campus Universitario, 5800 Río Cuarto, Argentina*
[c] *Instituto de Química Orgánica, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain*

## Abstract

Air-equilibrated aqueous solutions of the purine bases (PBs) uric acid (URA), xanthine (XAN) and hypoxanthine (HXA) have been irradiated with visible light in the presence of riboflavin (Rf) as the only light-absorbing compound, to study to what extent the reactive oxygenated species (ROSs) singlet molecular oxygen, $O_2(^1\Delta_g)$, and superoxide radical anion, $O_2^{\bullet-}$, are involved in the observed photodegradation processes. Both reactive species are generated from triplet excited riboflavin ($^3$Rf*) by well-known processes: energy transfer from $^3$Rf* to ground state oxygen to yield $O_2(^1\Delta_g)$, and electron transfer from each PB to $^3$Rf* to produce the radical anion $Rf^{\bullet-}$ that, after another electron transfer step to ground state oxygen, yields $O_2^{\bullet-}$ and Rf. The kinetics of the involved processes have been studied by polarographic detection of oxygen uptake and time-resolved phosphorescence detection of $O_2(^1\Delta_g)$, at concentrations in the range 0.05–0.5 mM in aqueous solutions at pH values 5, 7, 9, and 12, to compare the reactivity of the neutral and ionic species of each purine base. By means of Rose Bengal photosensitized reactions, it could be shown that $O_2(^1\Delta_g)$ is quenched by the three PBs with an overall rate constant decreasing in the order URA > XAN > HXA, with values ranging from $1.16 \times 10^9\,M^{-1}\,s^{-1}$ (URA at pH 12) to $5 \times 10^6\,M^{-1}\,s^{-1}$ (HXA at pH 7). When Rf is used instead, the quenching process takes place mostly by a chemical mechanism that prevents the oxidation of Rf by $O_2(^1\Delta_g)$.

Experiments with Rf and superoxide dismutase indicate that the PBs also react with $O_2^{\bullet-}$ by a mechanism which is not simple. With URA, largely the most efficient $O_2(^1\Delta_g)$-quencher, the major ROS generated is $O_2^{\bullet-}$, and the process is characterized by the largest rate of oxygen uptake, indicating efficient overall-scavenging and URA oxidation. With HXA, the less efficient $O_2(^1\Delta_g)$-quencher, the process shows a relatively high rate of oxygen consumption, especially in alkaline solution, likely as a result of the efficient generation of $O_2^{\bullet-}$ from $Rf^{\bullet-}$ and its concomitant reaction with HXA. Finally, with XAN the quenching efficiency takes intermediate values. At physiological pH, URA is the most efficient antioxidant, showing the highest overall rate constant for $O_2(^1\Delta_g)$ quenching, the lowest efficiency for the reaction with $O_2(^1\Delta_g)$, and the highest relative rate of oxygen uptake.
© 2007 Elsevier B.V. All rights reserved.

*Keywords:* Photooxidation; Purine bases; Riboflavin; Singlet molecular oxygen; Superoxide radical anion

## 1. Introduction

Day–light-promoted photochemistry involving biologically relevant substrates and, particularly, the consequences of undesired photosensitized reactions occurring in living systems, have been studied in detail in the last decades [1–8]. Riboflavin (Rf, vitamin B$_2$) is one of the endogenous visible-light absorbers which has been postulated as a possible sensitizer for the in

vivo photooxidative degradation of several substrates [9,10]. Rf is synthesized by green plants and participates in a variety of enzyme-catalyzed oxidation–reduction reactions [11], being widely distributed in human tissues, both in free and conjugated form [12]. When Rf absorbs visible light, several reactive oxygenated species (ROSs) species can be formed in water due to the presence of triplet Rf ($^3$Rf*). The most important ones are singlet molecular oxygen ($O_2(^1\Delta_g)$), produced with a quantum yield of 0.49 [13], and superoxide radical anion ($O_2^{\bullet-}$), generated with a low quantum yield: 0.009 [14] (Scheme 1, reactions (1) and (2), respectively). Additional free radicals and products from primary reactions may also partic-

ipate in subsequent steps [15]. In a biological environment, lipids, proteins, DNA and other critical cell components constitute the primary targets for the attack of ROSs generated from Rf-photosensitization [16–19], due to their high reactivity and local concentration. The specific mechanism governing these photo-processes depends on oxygen availability, pH of the medium, type of sensitizer and target molecule, their concentration, etc.

An important group of potentially oxidizable molecules is that of purine bases (PBs), and some aspects of their direct or dye-sensitized photodegradation in the presence of oxygen, including their behavior as $O_2(^1\Delta_g)$ quenchers, have been previously studied [20–22], in most cases in aqueous solution at physiological pH values. Uric acid (URA) appears as the most effective quencher, and the reaction of URA with $O_2(^1\Delta_g)$ has become a kinetic reference for photodynamic reactions [20]. The Rf-sensitized photodegradation of some PBs has also been studied [23], but the kinetic and mechanistic details of the process have not been reported. The hemolysis of blood cells by a combination of Rf, oxygen, visible light and aminophylline, a xanthine compound structurally related to URA, has been explained through the involvement of oxygenated radical species photogenerated in the medium [24]. More recently, it has been shown [25] that URA, hypoxanthine (HXA) and xanthine (XAN) deactivate photogenerated $^3Rf^*$ by an electron transfer process (Scheme 1, process (3)), giving rise to $Rf^{\bullet-}$ and $PB^{\bullet+}$ radicals, with rate constant values $k_{qRf}$ of $2.9 \times 10^9$, $1.2 \times 10^9$ and $0.17 \times 10^9$ $M^{-1} s^{-1}$, respectively, in pH 6.4 aqueous solution. Purine is inert under the same conditions. The values respectively correlate well with the one-

electron oxidation potential values of these PBs. According to all these results, PBs behave as antioxidants, preventing the light-induced formation of ROSs. In the case of URA, this scavenging has been related with the in vivo inhibition of carcinogenesis [26].

In the present work we show the results of a systematic kinetic study on the interaction of Rf-photogenerated ROSs with PBs, under visible-light irradiation, in an effort directed to determine the oxidative mechanisms that could account for reactive and physical interactions of URA, HXA and XAN with the photogenerated ROSs, evaluating their photodegradability and their ability to act as natural photoprotectors in a biological environment.
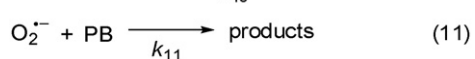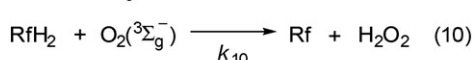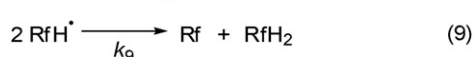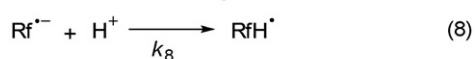
## 2. Materials and methods

### 2.1. Materials

Uric acid (URA), xanthine (XAN), hypoxanthine (HXA), riboflavin (Rf), deuterium oxide 99.9% ($D_2O$), and superoxide dismutase (SOD) from bovine erythrocytes were purchased from Sigma Chem. Co. (St. Louis, MO, USA). Rose Bengal (RB) and furfuryl alcohol (FFA) were from Aldrich (Milwaukee, WI, USA). All these chemicals were used as received. Water was triply distilled. All the measurements were carried out at room temperature and with freshly prepared solutions. Buffered aqueous solutions were prepared [27] with potassium phthalate ($KHC_8H_4O_4$) 0.1 M/NaOH 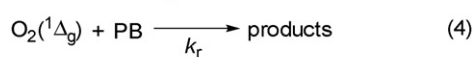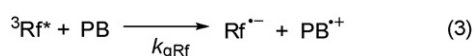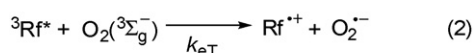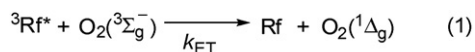0.1 M (pH 5), $KH_2PO_4$ 0.025 M/$Na_2HPO_4$ 0.025 M (pH 7), $Na_2B_4O_7 \cdot 10H_2O$ 0.01 M (pH 9) and NaOH 0.01 M (pH 12).

### 2.2. Methods

Absorption spectra were registered with a Hewlett Packard 8452A or an Agilent 8453 diode-array spectrophotometer. Continuous photolysis was performed in a home-made photolyser with a 300-W quartz-halogen lamp and a cut-off filter at 360 nm, using RB ($Abs_{530} = 0.5$) or Rf (0.03 mM) as sensitizer. The overall quenching rate constant $k_t$ of the $O_2(^1\Delta_g)$-deactivation by each PB (defined as $k_t = k_r + k_q$, with $k_r$ = chemical quenching rate constant, and $k_q$ = physical deactivation rate constant, processes (4) and (5) in Scheme 1) was determined as reported elsewhere [28]. Briefly, Rf was excited with the frequency tripled output at 355 nm, and RB with 532 nm, of a Nd:YAG laser (Spectron). The emitted $O_2(^1\Delta_g)$-phosphorescence at 1270 nm was analyzed by time-resolved phosphorescence detection (TRPD) at right angle using a Judson J16/8Sp germanium detector, after passing through the appropriate filters. The amplified output of the detector was coupled to a digital oscilloscope and to a personal computer to process the decay signal. Usually, averaging 10 laser shots was enough to get a good signal-to-noise ratio. Air-saturated solutions were employed in all the cases. RB ($Abs_{530} = 0.3$) or Rf (0.02 mM) in air-equilibrated solutions were used as sensitizers. In the TRPD determinations, $D_2O$ was used as solvent in order to enlarge the $O_2(^1\Delta_g)$ lifetime [29]. $O_2(^1\Delta_g)$ decay lifetimes were evaluated in the presence ($\tau$) and in the absence ($\tau_0$) of quencher; the data were ana-

$$^3Rf^* + O_2(^3\Sigma_g^-) \xrightarrow{k_{ET}} Rf + O_2(^1\Delta_g) \qquad (1)$$

$$^3Rf^* + O_2(^3\Sigma_g^-) \xrightarrow{k_{eT}} Rf^{\bullet+} + O_2^{\bullet-} \qquad (2)$$

$$^3Rf^* + PB \xrightarrow{k_{qRf}} Rf^{\bullet-} + PB^{\bullet+} \qquad (3)$$

$$O_2(^1\Delta_g) + PB \xrightarrow{k_r} products \qquad (4)$$

$$O_2(^1\Delta_g) + PB \xrightarrow{k_q} O_2(^3\Sigma_g^-) + PB \qquad (5)$$

$$O_2(^1\Delta_g) + Rf \xrightarrow{k_{rRf}} products \qquad (6)$$

$$Rf^{\bullet-} + O_2(^3\Sigma_g^-) \xrightarrow{k_7} Rf + O_2^{\bullet-} \qquad (7)$$

$$Rf^{\bullet-} + H^+ \xrightarrow{k_8} RfH^{\bullet} \qquad (8)$$

$$2 RfH^{\bullet} \xrightarrow{k_9} Rf + RfH_2 \qquad (9)$$

$$RfH_2 + O_2(^3\Sigma_g^-) \xrightarrow{k_{10}} Rf + H_2O_2 \qquad (10)$$

$$O_2^{\bullet-} + PB \xrightarrow{k_{11}} products \qquad (11)$$

$$^3Rf^* \longrightarrow products \qquad (12)$$
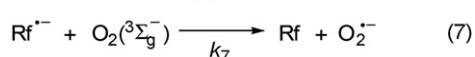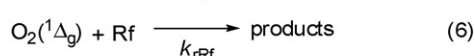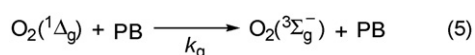
Scheme 1. Major kinetic processes in the visible-light irradiation of an air-equilibrated solution of a purine base (PB) in the presence of riboflavin (Rf).
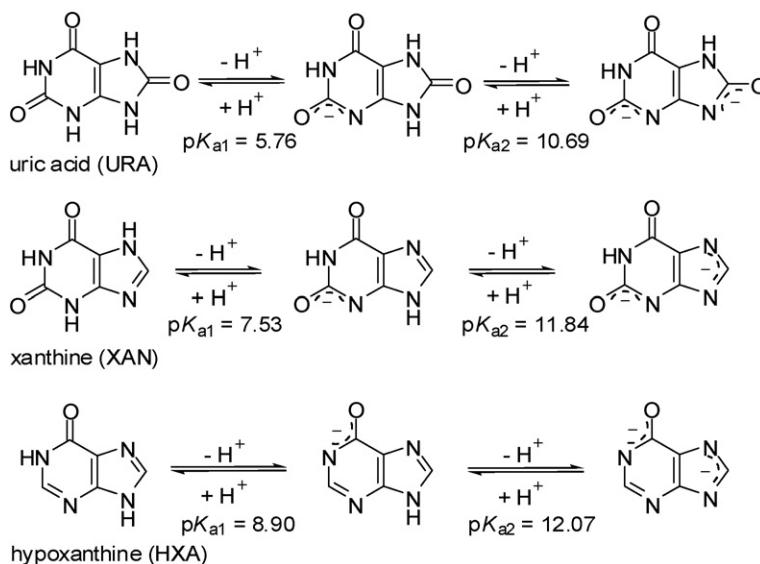
Scheme 2. Ionization equilibria and p$K_a$ values of uric acid, hypoxanthine and xanthine [31–37]. Only main tautomeric species are shown.

lyzed as a function of PB concentration, according to a simple Stern–Volmer expression: $1/\tau = 1/\tau_0 + k_t[PB]$. The value of $k_r$ for the reaction of each PB with RB-generated $O_2(^1\Delta_g)$ was determined by a relative method [30], using the expression: slope/slope$_R = k_r[PB]/k_{rR}[R]$, and assuming that the reaction of $O_2(^1\Delta_g)$ with the quencher (reaction (4)) is the only pathway of oxygen consumption. Briefly, in this method the slope of the first order plot of oxygen consumption by each PB (slope) and by a reference compound R (slope$_R$) are determined experimentally in the same conditions of temperature and concentration. The reference compound used here was furfuryl alcohol (FFA), with a reported pH-independent $k_{rR}$ value of $1.2 \times 10^8 \, M^{-1} \, s^{-1}$ [22]. The relative rate of Rf-sensitized photooxidation of each PB was determined by evaluation of the initial slope of oxygen consumption as a function of the irradiation time, with Orion 97-08 or Orion 810A+ specific oxygen electrodes. Relative photodegradation rates of riboflavin were obtained from the initial slopes of the absorbance decrease at 446 nm, upon irradiation under argon atmosphere (20 min bubbling) in 1 cm × 1 cm cuvettes.

## 3. Results

The influence of pH on the Rf-sensitized aerobic photooxidation of HXA and XAN was studied in aqueous buffers with pH values 7, 9, and 12. In these conditions, the PBs are present in the form of tautomeric and ionized species with a pH-dependent concentration. URA was also studied at pH 5 because the p$K_{a1}$ value of this base is lower than 7. Scheme 2 shows the accepted equilibrium ionizations of URA [31–33], XAN [34–36] and HXA [34,36,37], and the corresponding p$K_a$ values. In pH 5 aqueous solution, a fraction of the molecules of URA is mono-ionized, while XAN and HXA are present as neutral species. At pH 7, URA is mainly mono-ionized, XAN is partially mono-ionized, and HXA is mainly in neutral form. At pH 9, the main species in URA and XAN are the mono-ionized forms, while in HXA equal proportions of neutral and mono-ionized forms must be present. At pH 12, URA is mainly as di-ionized form, while XAN and HXA are present as ca. 1:1 mixture of mono- and di-ionized forms. The ionized forms are expected to be more reactive to oxidation by
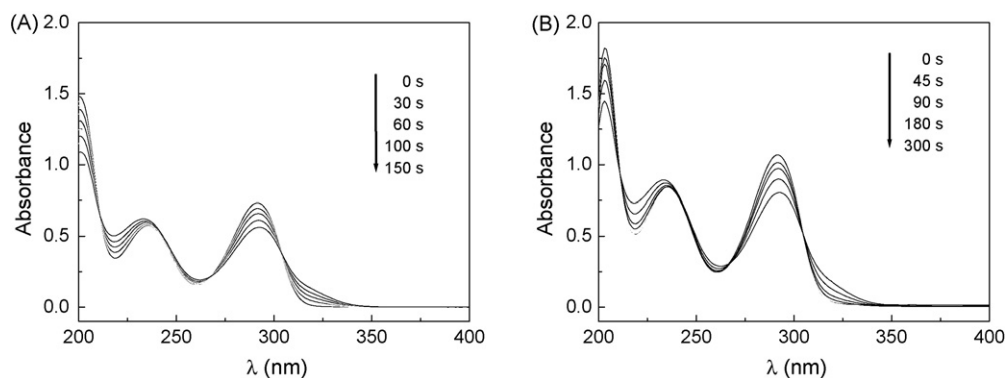


Fig. 1. Spectral changes in the photoirradiation with visible-light of air-equilibrated aqueous solutions (pH 7) of uric acid 0.5 mM in the presence of Rose Bengal (Abs$_{530}$ 0.5) (A), or uric acid 0.8 mM in the presence of riboflavin (0.03 mM) (B).

Table 1
Rate constant values ($M^{-1} s^{-1}$) for overall ($k_t$, in $D_2O$) and chemical ($k_r$, in $H_2O$) quenching of $O_2(^1\Delta_g)$ by purine bases in air-equilibrated aqueous buffer solution

| pH | Uric acid | | | Xanthine | | | Hypoxanthine | | |
|---|---|---|---|---|---|---|---|---|---|
| | $k_t (\times 10^9)$ | $k_r (\times 10^9)$ | $k_r/k_t$ | $k_t (\times 10^9)$ | $k_r (\times 10^9)$ | $k_r/k_t$ | $k_t (\times 10^9)$ | $k_r (\times 10^9)$ | $k_r/k_t$ |
| 5 | 0.18 | 0.01 | 0.05 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 7 | 0.51 | 0.12 | 0.23 | 0.04 | 0.038 | 0.95 | 0.005 | 0.003 | 0.60 |
| 9 | 0.66 | 0.18 | 0.27 | 0.04 | 0.037 | 0.92 | 0.007 | 0.005 | 0.70 |
| 12 | 1.16 | 0.53 | 0.45 | 0.36 | 0.040 | 0.11 | 0.15 | 0.020 | 0.13 |

Rose Bengal as photosensitizer, purine base concentration 0.5 mM in the stationary experiments, room temperature, n.d.: not determined.

electrophilic reagents such as $O_2(^1\Delta_g)$ than the corresponding neutral forms.

The irradiation of mixtures of the sensitizers Rf (0.03 mM) or RB (Abs$_{530}$ = 0.5) in the presence of each PB, URA, XAN, or HXA (0.5–0.8 mM) at pH 12 (RB only), pH 9, pH 7 or pH 5 (URA only) produced spectral changes that could be assigned to the disappearance of the PB and the appearance of photodegradation products (representative cases are shown in Fig. 1). The overall $O_2(^1\Delta_g)$-quenching rate constant $k_t$ was determined in the above solutions by TRPD with Rf or RB as a sensitizer (Table 1, representative plot in Fig. 2). The dye RB is an efficient and almost exclusive $O_2(^1\Delta_g)$ photogenerator, with a quantum yield in methanol of 0.81 [38]. Sensitized photoreactions using Rf could not be carried out pH 12, due the fast photodegradation
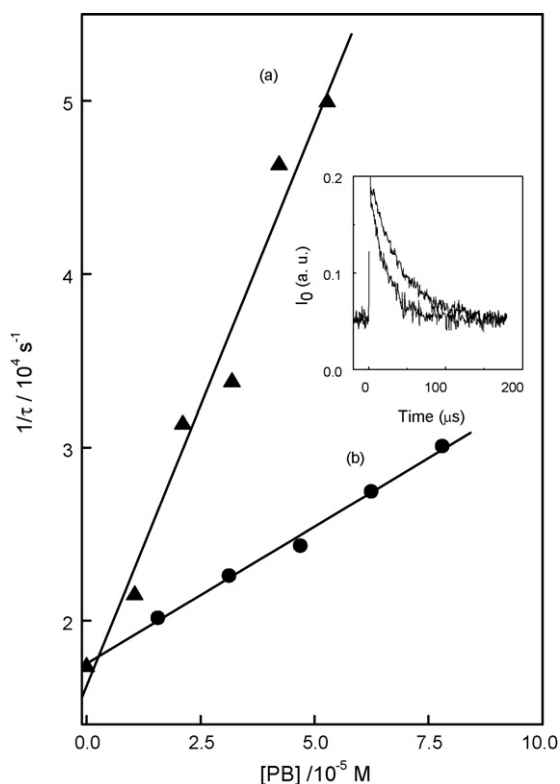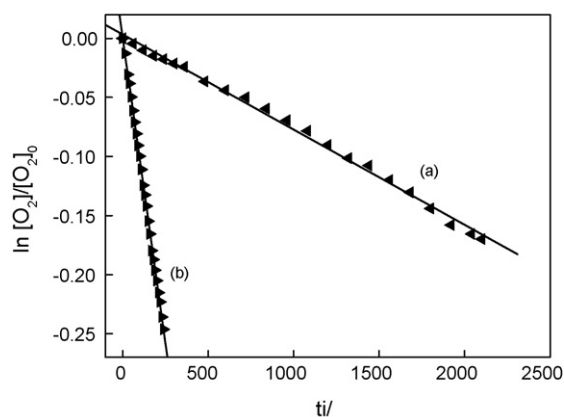


Fig. 3. First order plot of oxygen uptake in the Rose Bengal-sensitized photooxidation of uric acid 0.5 mM (a), and furfuryl alcohol 0.5 mM (b). pH 5 aqueous solution, Rose Bengal as a photosensitizer (Abs$_{530}$ 0.5).

of the vitamin [9]. The reactive rate constant $k_r$ of the chemical reaction of $O_2(^1\Delta_g)$ with each PB was determined by measuring the oxygen uptake, always using RB as a sensitizer (see representative plot in Fig. 3).

The rates of oxygen consumption by PBs, upon Rf-photosensitization (0.04 mM) or RB-photosensitization (Abs$_{530}$ = 0.3) at pHs 7 and 9, were also determined, and expressed as relative values normalized to the highest value for each sensitizer at each pH (Table 2). Additionally, experiments



Fig. 2. Stern–Volmer plot for the quenching of $O_2(^1\Delta_g)$ phosphorescence (analytical wavelength 1270 nm) by: (a) uric acid in $D_2O$ solution (pD 9), with riboflavin 0.02 mM as a photosensitizer; (b) hypoxanthine in $D_2O$ solution (pD 12), with Rose Bengal (Abs$_{532}$ 0.3) as a photosensitizer. Inset: singlet oxygen luminescence transient signal, corresponding to the run (a), at uric acid concentrations of 0.021 mM (upper trace) and 0.056 mM (lower trace).
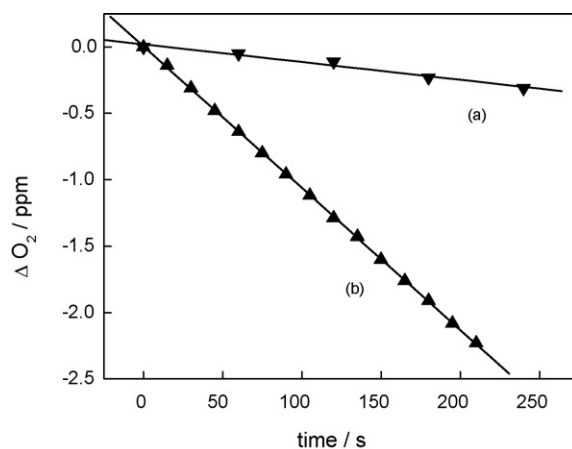


Fig. 4. Oxygen consumption as a function of the irradiation time of hypoxanthine 0.5 mM in aqueous solution (pH 9) in the presence (a) and in the absence (b) of superoxide dismutase (1 µg/mL). Visible light, cut-off at 360 nm, riboflavin 0.04 mM as a photosensitizer.

Table 2
Relative rates of oxygen uptake by purine bases in air-equilibrated aqueous buffer solution upon riboflavin (Rf) (0.04 mM) or Rose Bengal (RB) (Abs$_{530}$ = 0.3) photosensitization

| pH | Relative rate of O$_2$ uptake | | | | | |
|---|---|---|---|---|---|---|
| | Riboflavin photosensitization | | | Rose Bengal photosensitization | | |
| | Uric acid | Xanthine | Hypoxanthine | Uric acid | Xanthine | Hypoxanthine |
| 7 | 1 | 0.22 | 0.02 | 1 | 0.37 | 0.030 |
| 9 | 1 | 0.16 | 0.13 | 1 | 0.24 | 0.025 |

Irradiation with visible light, cut-off at 360 nm, purine base concentration 0.5 mM, room temperature.

indicated that the rate of oxygen consumption was much lower in the presence of SOD (1 μg/mL) (Fig. 4), a specific quencher of the O$_2^{\bullet-}$ species [39,40]. In all the cases, URA exhibits the highest oxygen uptake rate.

The anaerobic photodegradation of Rf under visible-light irradiation proceeds mainly through the triplet state $^3$Rf* (reaction (12)) [9], and the rate of the process can be estimated by absorption measurements (Fig. 5, main and inset). Comparative irradiation of argon-saturated solutions of Rf in the absence and in the presence of URA 0.2 mM showed that this rate is lower in the second case (Fig. 5, main). A curvature is observed in the run of Rf photodegradation in the absence of URA, evidencing the loss of the pigment as the photolysis progresses. The run in the presence of URA is practically linear within the same time range of photolysis, suggesting that in deoxygenated solutions at least some fraction of reaction (3) is a reversible process that regenerates Rf [25].

## 4. Discussion

The experimental evidence shown here strongly supports the presence of specific interactions of O$_2$($^1\Delta_g$) and/or O$_2^{\bullet-}$ with each PB and Rf, resulting in the degradation of both compounds. The interaction of PBs with O$_2$($^1\Delta_g$) has been clearly established by time-resolved phosphorescence detection experiments, whereas the effect of SOD in the rate of oxygen uptake,
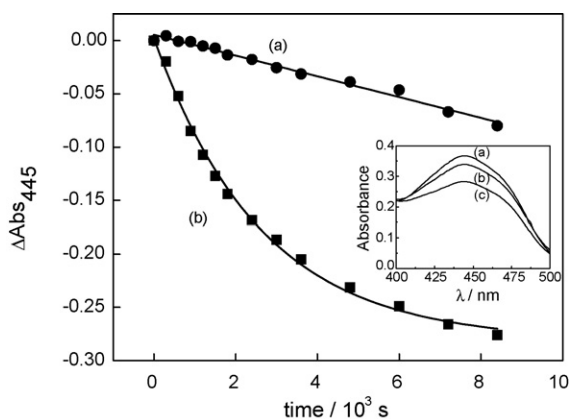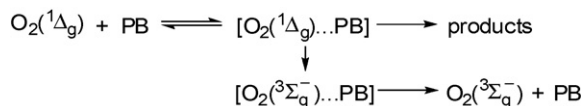


Fig. 5. Absorbance changes at 445 nm in the visible-light irradiation under argon atmosphere of pH 7 aqueous solutions of riboflavin 0.04 mM with (a) and without (b) uric acid 0.2 mM. Inset: spectral changes of the riboflavin absorption spectrum after visible-light irradiation for 0 min (a), 60 min in the presence of uric acid 0.2 mM (b), and 15 min in the absence of uric acid (c). Cut-off at 360 nm.

with Rf as a sensitizer, indicates the involvement of the radical O$_2^{\bullet-}$. Besides, UV–vis absorption and oxygen uptake experiments confirm that some fraction of these interactions occurs through a reactive channel (processes (4) and (11)). The analysis of the kinetic information herein obtained can be of help for determining the operating mechanism. Thus, the data shown in Tables 1 and 2 indicate that (a) the values of the rate constants $k_t$ and $k_r$ increase with the ionization of each PB; (b) for URA, the value of the $k_r/k_t$ ratio shows that the contribution of the reactive quenching component increases as the pH value of the medium increases, whereas in the case of XAN and HXA this ratio is similar at pH 7 and 9, and becomes very low at pH 12; (c) the change in the relative rate of oxygen uptake, upon Rf- or RB-photosensitization, as a function of pH (from 7 to 9), suggests different photooxidation mechanisms for each sensitizer.

The increase of both $k_t$ and $k_r$ values as a function of pH has been already observed in the photooxidation of hydroxyaromatic compounds, such as phenols, hydroxypyridines, hydroxypyrimidines and hydroxyquinolines. This effect has been explained as due to the formation of a charge transfer encounter complex [41–45], a reaction favored by the higher electron–donor ability of the ionized species (Scheme 3). According to this mechanism, both chemical reaction (generation of PB oxidation products) and physical quenching (regeneration of O$_2$($^3\Sigma_g^-$)), can operate, and the relative importance of each process is reflected by the values of the respective rate constants $k_r$ and $k_q$.

In the case of the PBs studied here, this result can be rationalized in accordance with the ionization equilibria shown in Scheme 2. Thus, the increase of both, $k_t$ and $k_r$ values neatly correlates with the larger proportions of mono- and di-ionized PB forms as a function of pH. On the other hand, the values of $k_t$ and $k_r$ do not change in a parallel way, as shown by the respective photodegradation efficiencies represented by the $k_r/k_t$ ratios. This again establishes the importance of the proportion of ionized species in the reactive pathway. A $k_r/k_t$ ratio of 0.60 has been reported previously for HXA at pH 7 [46], which is identical to that determined in the present work (Table 1).

The generation of O$_2^{\bullet-}$ by electron transfer from $^3$Rf* (reaction (2), Scheme 1) is a very low quantum yield process [14].



Scheme 3. Quenching of singlet molecular oxygen, [O$_2$($^1\Delta_g$)], by a purine base, PB.

However, $O_2^{\bullet-}$ can be also generated from $Rf^{\bullet-}$ by the indirect process (7), favored at pH 9 with regard to pH 7 because the radical $RfH^{\bullet}$, formed from $Rf^{\bullet-}$ in the presence of protons (process (8)), has a p$K_a$ value of 8.3 [12,47]. Another oxygen-consuming reaction that gives rise to $H_2O_2$ from $RH_2$, generated *via* the radical $RfH^{\bullet}$ (processes (8) and (9)) is process (10), favored in neutral or acidic media [47]. All these processes can compete with the generation of $O_2(^1\Delta_g)$ (process (1)), and the probability of each reactive channel would depend obviously on the efficiency of $O_2(^3\Sigma_g^-)$ or PB in the $^3Rf^*$ quenching reaction. It is known that the energy-transfer process (1) occurs with a rate constant $k_{ET}$ in water of $7 \times 10^8\,M^{-1}\,s^{-1}$, equivalent to 1/9 of the diffusion-controlled value [48,49]. Taking into account the conditions used in the experiments with continuous irradiation (0.4 mM PB, with a similar value for the oxygen concentration in air-equilibrated water solution [50]), and using the reported $k_{qRf}$ values for these PBs (between $1.7 \times 10^8$ and $2.9 \times 10^9\,M^{-1}\,s^{-1}$) [25], it can be estimated that the rate of $Rf^{\bullet-}$ generation – the main $O_2^{\bullet-}$ precursor species – varies from four times faster (in the case of URA) to four times slower (in the case of HXA) than the rate of process (1). In other words, the rates of the processes generating $O_2^{\bullet-}$ (via $Rf^{\bullet-}$) and $O_2(^1\Delta_g)$ are similar. Assuming that the quantum yield of $O_2(^1\Delta_g)$ generation is not greatly affected by pH, it can be observed (Table 2) a similar trend in the RB- and Rf-sensitized oxygen uptake rates by URA and XAN at the two pH values studied, suggesting the important contribution of the $O_2(^1\Delta_g)$-mediated mechanism. In the case of HXA, the rate of oxygen consumption by the $O_2(^1\Delta_g)$-mediated mechanism takes the lowest value. Therefore, the marked increase of oxygen consumption in the Rf-sensitized process at pH 9 may be due to the contribution of the $O_2^{\bullet-}$ mechanism (reaction (11)), since the oxygenated species is more efficiently generated at this pH than at pH 7 [12,47].

Although Rf is photodegraded by reaction (6) [11], it is regenerated again by the production of $O_2^{\bullet-}$ through reaction (7), with a rate constant value in water of $1.4 \times 10^8\,M^{-1}\,s^{-1}$ [14], and/or the reaction sequence (8)–(10). Both processes, that take place also in natural biological environments, prevent vitamin Rf photodegradation.

## 5. Conclusions

The visible-light irradiation of a mixture of riboflavin and the purine base uric acid, xanthine or hypoxanthine yields $O_2(^1\Delta_g)$ and $O_2^{\bullet-}$. The $O_2^{\bullet-}$ species is generated from the radical anion $Rf^{\bullet-}$, which is formed by electron transfer from triplet-excited riboflavin to the corresponding purine base. A kinetic analysis of these reactions indicates that both $O_2(^1\Delta_g)$ and $O_2^{\bullet-}$ species are quenched by uric acid (largely), xanthine (moderately), and hypoxanthine (weakly). The $O_2(^1\Delta_g)$ quenching process takes place mainly by a chemical channel, and the overall quenching rate constant ($k_r + k_q$, processes (4) and (5)) increases with the pH of the solution. Both uric acid and xanthine prevent riboflavin photodegradation by $O_2(^1\Delta_g)$, whereas subsequent reactions of the radical $Rf^{\bullet-}$ regenerate the vitamin. Thus, these purine bases play the role of sacrificial scavengers of both reactive oxygenated species, with their simultaneous degradation. At physiological pH values, uric acid is the most efficient photoprotector of riboflavin among the three purine bases herein studied, with the highest value of overall rate constant for the quenching of $O_2(^1\Delta_g)$, the lowest proportion of reactive quenching, and the highest rate of oxygen uptake by sensitization with riboflavin. Under anaerobic conditions, the quenching of $^3Rf^*$ by these purine bases partially protects the pigment against photodestruction, likely through a back-electron-transfer mechanism.

## References

[1] I. Fridovich, in: W.A. Pryor (Ed.), Free Radicals in Biology, vol. II, Academic Press, New York, 1976.
[2] L.I. Grossweiner, Curr. Top. Radiat. Res. Q. 11 (1976) 141–199.
[3] N.J.D. Mol, G.M.J. Beijersbergen Van Henegouwen, K.W. Gerritsma, Photochem. Photobiol. 29 (1979) 479–482.
[4] R.C. Straight, J.D. Spikes, Photosensitized oxidation of biomolecules, in: A.A. Frimer (Ed.), Singlet Oxygen, vol. IV, CRC Press, Boca Raton, 1985.
[5] B. Quintero, M.A. Miranda, Ars. Pharmaceutica 41 (2000) 27–46.
[6] A. Posadaz, E. Sánchez, M.I. Gutiérrez, M. Calderón, S. Bertolotti, M.A. Biasutti, N.A. García, Dyes Pigments 45 (2000) 219–228.
[7] A.M. Edwards, E. Silva, J. Photochem. Photobiol. B: Biol. 63 (2001) 126–131.
[8] G. Cosa, Pure Appl. Chem. 76 (2004) 263–275.
[9] P.F. Heelis, Chem. Soc. Rev. 11 (1982) 15–39.
[10] P.F. Heelis, in: F. Muller (Ed.), Chemistry and Biochemistry of Flavoenzymes, 1, Boca Ratón, FL, 1991.
[11] C.H. Winestock, W.E. Plaut, The biosynthesis of coenzymes, in: J. Bonner, J.E. Varner (Eds.), Plant Biochemistry, Academic Press, New York, 1972, p. 424.
[12] C. Lu, G. Bucher, W. Sander, Chem. Phys. Chem. 5 (2004) 47–56.
[13] F. Wilkinson, W.P. Helman, A.B. Ross, J. Phys. Chem. Ref. Data 22 (1993) 113–262.
[14] C.M. Krishna, S. Uppuluri, P. Riesz, J.S. Zigler Jr., D. Balasubramanian, Photochem. Photobiol. 54 (1991) 51–58.
[15] W. Massad, S. Bertolotti, N.A. García, Photochem. Photobiol. 79 (2004) 428–433.
[16] T. Mori, K. Tano, K. Takimoto, H. Utsumi, Biochem. Biophys. Res. Commun. 242 (1998) 98–101.
[17] J.D. Spikes, H.R. Shen, P. Kopecková, J. Kopecek, Photochem. Photobiol. 70 (1999) 130–137.
[18] A. Posadaz, A. Biasutti, C. Casale, J. Sanz, F. Amat-Guerri, N.A. García, Photochem. Photobiol. 80 (2004) 132–138.
[19] S. Miskoski, A.T. Soltermann, P.G. Molina, G. Günther, A.L. Zanocco, N.A. García, Photochem. Photobiol. 81 (2005) 325–332.
[20] F. Fischer, G. Graschew, H.-J. Sinn, W. Maier-Borst, W.J. Lorenz, P.M. Schlag, Clin. Chim. Acta 274 (1998) 89–104.
[21] A. Synytsya, V. Král, M. Blechová, K. Volka, J. Photochem. Photobiol. B: Biol. 74 (2004) 73–84.
[22] F. Wilkinson, W.P. Helman, A.B. Ross, J. Phys. Chem. Ref. Data 24 (1995) 663–677.
[23] J. Newburger, A.B. Combs, Life Sci. 17 (1975), pp. 443–337.
[24] I. Ali, I. Naseem, Life Sci. 70 (2002) 2013–2022.

[25] D.R. Cardoso, P. Homen-de-Mello, K. Olsen, A.B. da Silva, D.W. Franco, L.H. Skibsted, J. Agric. Food Chem. 53 (2005) 3679–3684.

[26] E. Lickl, G. Alth, R. Ebermann, R.H. Beck, K. Tuma, Med. Hypotheses 28 (1989) 193–195.

[27] R.C. Weast (Ed.), Handbook of Chemistry and Physics, 55th ed., CRC Press, USA, 1974/1975.

[28] M. Neumann, N.A. García, J. Agric. Food Chem. 40 (1992) 957–960.

[29] S. Nonell, L. Moncayo, F. Trull, F. Amat-Guerri, E.A. Lissi, A.T. Soltermann, S. Criado, N.A. Garcia, J. Photochem. Photobiol. B: Biol. 29 (1995) 157–162.

[30] F.E. Scully, J. Hoingé, Chemosphere 16 (1987) 681–694.

[31] R.C. Smith, J.Z. Gore, M. McKee, H. Hargis, Microchem. J. 38 (1988) 118–124.

[32] S. Gangully, K. Kundu, Ind. J. Chem. 34A (1995) 47–51.

[33] V. Jiménez, J.B. Alderete, J. Mol. Struct. 755 (2005) 209–214.

[34] J.J. Christensen, J.H. Rytting, R.M. Izatt, Biochemistry 9 (1970) 4907–4913.

[35] F. Bergmann, D. Lichtenberg, Z. Neiman, J. Chem. Soc. C (1971) 1676–1682.

[36] E. Kulikowska, B. Kierdaszuk, D. Shugar, Acta Biochim. Pol. 51 (2004) 493–531.

[37] C.B. Ould-Moulaye, C.G. Dussap, J.B. Gros, Thermochim. Acta 375 (2001) 93–107.

[38] F. Amat-Guerri, M.M.C. López-González, R. Martínez-Utrilla, R. Sastre, J. Photochem. Photobiol. A: Chem. 53 (1990) 199–210.

[39] R.M. Baxter, J.H. Carey, Nature 306 (1983) 575–576.

[40] L.-Y. Zang, H.P. Misra, J. Biol. Chem. 267 (1992) 17547–17552.

[41] N.A. García, J. Photochem. Photobiol. B: Biol. 22 (1994) 185–196.

[42] A. Pajares, J. Gianotti, E. Haggi, G. Stettler, F. Amat-Guerri, S. Criado, S. Miskoski, N.A. García, J. Photochem. Photobiol. A: Chem. 119 (1998) 9–14.

[43] A. Pajares, J. Gianotti, G. Stettler, E. Haggi, S. Miskoski, S. Criado, F. Amat-Guerri, N.A. García, J. Photochem. Photobiol. A: Chem. 135 (2000) 207–212.

[44] E. Haggi, S. Bertolotti, N.A. García, Chemosphere 55 (2004) 1501–1507.

[45] G. Bosio, S. Criado, W. Massad, F.J. Rodríguez Nieto, M. González, N.A. García, D.O. Mártire, Photochem. Photobiol. Sci. 4 (2005) 840–846.

[46] G. Peters, M.A.J. Rodgers, Biochim. Biophys. Acta 637 (1981) 43–52.

[47] C. Lu, W. Lin, W. Wang, Z. Han, S. Yao, N. Lin, Phys. Chem. Chem. Phys. 2 (2000) 329–334.

[48] J.G. Calvert, J.N. Pitts, Photochemistry, Wiley, New York, 1966.

[49] M. Koizumi, S. Kato, N. Mataga, T. Matsuura, I. Isui, Photosensitized Reactions, Kagakudogin, Kyoto, 1978.

[50] S.L. Murov, I. Carmichael, G.L. Hug, Handbook of Photochemistry, 2nd ed., Marcel Dekker, New York, 1993.