Photodegradation of antibiotic 5-sulfaminouracil in the presence of vitamin B2: A kinetic study

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Objective: This paper studies the kinetics and mechanism of the sensitized photodegradation of 5-sulfaminouracil (SFU). This compound is a synthetic bacteriostatic belonging to the sulfa drugs, usually detected in surface water and effluent wastewater.

Methods: SFU interacts with electronically excited singlet and triplet states of riboflavin (Rf), $^1\text{Rf}^*$ and $^3\text{Rf}^*$, respectively. The rate constants for these processes were determined in MeOH–H₂O by stationary fluorescence spectroscopy (for $k_{Q(1)}$) and by laser flash photolysis (for $k_{Q(3)}$).

Results: SFU is photodegraded by visible light irradiation in the presence of the natural sensitizer (Rf). In competitive processes, $^3\text{Rf}^*$ generates the reactive oxygen species superoxide radical anion ($\text{O}_2^{\bullet-}$) and singlet molecular oxygen [$\text{O}_2(^1\Delta_g)$], which are involved in SFU photodegradation. In aqueous solutions, where SFU adopts different forms depending on the pH of the medium, the participation of $\text{O}_2(^1\Delta_g)$ is predominant. Therefore, the $\text{O}_2(^1\Delta_g)$ -mediated mechanism was evaluated at pHs 7 and 12, employing perinaphthenone as a synthetic photosensitizer.

Discussion: The results indicate that SFU is photodegraded through a relatively efficient process in a neutral environment, whereas it is quickly degraded in alkaline media. This is attributed to the ionization of the sulfamino- group, a substituent in the uracil molecule that exerts an activating power on the molecule. Thus, sensitized photodegradation may be an important tool to reduce the environmental impact of SFU.

Keywords: 5-Sulfaminouracil, Riboflavin, Singlet molecular oxygen, Superoxide radical anion

Introduction

The importance of uracil derivatives in biological processes, as well as their bioactivity, has been considered by several authors.^{1–3} In particular, the 5-substituted uracils, such as the 5-halogenated uracils, exhibit antibacterial activity and have been widely used in the treatment of cancer.⁴

5-Sulfaminouracil (SFU) (Fig. 1) belongs to a family of synthetic antibiotics known as sulfonamides or sulfa drugs (SDs). These compounds have played an important role in both human and veterinary medicine.^{5,6}

It is well known that some of these compounds are poorly metabolized and so excreted unchanged, causing their subsequent incorporation into the environment. Therefore, they become potential micro-pollutants of living organisms in aquatic ecosystems.

SDs have been detected in all kinds of environmental waters,^{7,8} not only in wastewater effluents⁹ but also in surface and groundwater at concentrations up to a few micrograms/liter.^{10–12}

Once antibiotics reach an environmental system, they may undergo different degradation processes, which is very important to limit their presence in the environment. For example, biodegradation and non-biotic elimination processes such as absorption, hydrolysis, oxidation, reduction, and photodegradation have been documented.¹³ SDs present on surface waters do not absorb visible light. However, there are daylight-absorbing substances called photosensitizers, like riboflavin (Rf), which contribute to the process of photodegradation. Rf is a pigment found in practically all types of natural waters.¹⁴

Previous studies have demonstrated that different SDs can be degraded through direct photolysis (by exposure to UV light^{15,16}) or through indirect photolysis (via sensitized photo-processes). This degradation happens in the presence of either dissolved organic

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Figure 1 SFU structure.

matter or other reactive species that can be formed in natural surface waters.^{17–19}

Regarding the uracil molecule, which is part of the SFU structure, it has been reported by our group²⁰ that its sensitized photodegradation takes place with the involvement of reactive oxygen species (ROS) such as singlet molecular oxygen [$O_2(^1\Delta_g)$] and superoxide radical anion ($O_2^{\cdot-}$), both photogenerated from the natural sensitizer Rf.

We also have demonstrated in previous research work into the interaction of ROS with some SDs derived from sulfanilic acid that sulfisoxazol is efficiently photodegraded by indirect photolysis in the presence of natural and synthetic dyes.²¹

In this work, we study the kinetics and mechanism of the sensitized photodegradation of SFU under conditions frequently found in nature: in aqueous environments, in the presence of natural photosensitizers, and under visible light illumination. This research is a contribution towards the possible elimination of this bacteriostatic compound from aquatic environments.

Materials and methods

Chemicals

SFU, Rf, D_2O (99.9%), superoxide dismutase (SOD) from bovine erythrocytes, L-tryptophan (Trp), and sodium azide (NaN_3) were purchased from Sigma Chemical Co. (St Louis, USA), perinaphthenone (PN) was purchased from Aldrich (Steinheim, Germany), and furfuryl alcohol from Riedel deHaën (Buchs, SG Switzerland). Water was triple distilled, and MeOH (high-performance liquid chromatography quality) was purchased from Sintorgan (Buenos Aires, Argentina). In some experiments, water as a solvent was replaced with 1:1 (v/v) MeOH– H_2O in order to increase the solubility of SFU. D_2O was employed instead of H_2O in time-resolved phosphorescence detection (TRPD) experiments to lengthen $O_2(^1\Delta_g)$ lifetime.²² The pHs/pDs 7 and 12 buffered solutions were prepared according to a reported procedure.²³

All experiments were carried out at room temperature and with freshly prepared solutions.

Methods

SFU stationary photolysis in aqueous solutions with PN or Rf sensitizers was performed in a homemade photolyser 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 sensitizers.

Ground-state absorption spectra were recorded in a Hewlett-Packard 8452A diode array spectrophotometer. Stationary fluorescence experiments were performed with a Shimadzu RF5301-PC spectrofluorimeter at 25°C in air-equilibrated solutions. Excitation and emission wavelengths for Rf were 445 and 515 nm, respectively. The interaction of the excited singlet state, $^1Rf^*$, with SFU was evaluated using the Stern–Volmer treatment: $I_0/I = 1 + K_{SV}[SFU]$, where K_{SV} (Stern–Volmer constant) can be obtained by determining the static fluorescence intensity of Rf in the presence (I) and in the absence (I_0) of SFU.

Laser flash photolysis was used to determine the quenching rate constant, $k_{Q(3)}$, of $^3Rf^*$ –SFU interaction (process 7) by means of a previously reported apparatus.²⁴ Nitrogen-saturated aqueous solutions of Rf (0.01 mM) were photolyzed using the same technique. The disappearance of $^3Rf^*$, generated by laser pulse (355 nm), 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 of $^3Rf^*$ was measured at low Rf concentration and at low laser energy to avoid undesirable effects such as self-quenching and triplet–triplet annihilation. $^3Rf^*$ lifetime was evaluated in the presence (τ_3) and in the absence (${}^\circ\tau_3$) of SFU. The $k_{Q(3)}$ was obtained from the Stern–Volmer expression: $1/\tau_3 = 1/{}^\circ\tau_3 + k_{Q(3)}[SFU]$.

In order to obtain SFU concentrations higher than those allowed by the solubility of SFU in water, a solvent mixture, MeOH– H_2O (1:1, v/v), was employed to evaluate the interactions of the bacteriostatic with singlet and triplet excited states of Rf.

TRPD was used to determine the overall quenching rate constant of $O_2(^1\Delta_g)$ by SFU, k_t , in the system described below. Basically, $O_2(^1\Delta_g)$ phosphorescence at 1270 nm, generated by excitation with the emission (355 nm) from a Nd:YAG laser (Spectron), was detected at right angles using an amplified Judson J16/8Sp Germanium detector, after passing through suitable filters. The detector output was coupled to a digital oscilloscope and to a personal computer for signal processing. To obtain a good signal-to-noise ratio, 16 shots were usually averaged, from which decay times were calculated. Within this context, sensitizer PN displayed an $A_{355} \approx 0.4$.

$O_2(^1\Delta_g)$ lifetimes were evaluated in the presence (τ) and in the absence (${}^\circ\tau$) of the quencher, SFU, and the data were plotted as a function of SFU concentration according to the Stern–Volmer treatment: ${}^\circ\tau/\tau = 1 + k_t {}^\circ\tau[SFU]$.

The relative rates of oxygen consumption (V_{ox}) upon PN-sensitized irradiation were determined from

the initial slopes in graphs that show oxygen consumption vs. irradiation time, employing a specific oxygen electrode (Orion 97-08).

The values of the reactive rate constants (k_r) were determined by a comparative method,²⁵ with PN as photosensitizer and Trp as reference. Basically, solutions containing PN ($Abs_{364} = 0.5$) and SFU (0.4 mM) or Trp (0.4 mM) were irradiated (150 W quartz-halogen lamp) under identical conditions using a cut-off filter (400 nm) in order to assure that radiation was only absorbed by PN. Consumption of substrate was followed by spectroscopy. Under these conditions, k_r values were determined from the expression $k_r/k_{rR} = \text{slope}/\text{slope}_R$, where slope and slope_R are the respective slopes of first-order plots showing absorbance (or fluorescence emission) decrease vs. irradiation time of SFU and Trp, assuming that only the reaction of SFU with $O_2(^1\Delta_g)$ leads to SFU consumption.

Results

Difference spectra (SFU + Rf) vs. Rf showed no modifications within a concentration range used for the photochemical experiments, indicating that there is no ground-state association between SFU and Rf.

After visible light irradiation of solutions containing SFU 0.3 mM and Rf 0.02 mM under aerobic conditions, changes in the absorption spectra of both compounds were observed (Fig. 2).

Oxygen consumption was detected when solutions of MeOH–H₂O 1:1 (v/v) containing Rf 0.04 mM and SFU 0.5 mM were irradiated under aerobic conditions. The rate of oxygen consumption clearly diminished in the presence of the enzyme SOD 14 mg/ml (see Fig. 3). This decrease in the rate of oxygen consumption strongly suggests the involvement

Figure 2 Spectral changes of SFU (0.3 mM) vs. Rf (0.02 mM) in MeOH–H₂O 1:1 (v/v) solutions upon irradiation under aerobic conditions. (A) Absorption spectra of Rf and SFU vs. MeOH:H₂O. (B) Spectral changes of SFU (0.3 mM) + Rf (0.02 mM) in MeOH–H₂O (1:1, v/v) solutions upon irradiation under aerobic conditions. Numbers above the spectrum indicate irradiation time in minutes.

Figure 3 Consumed oxygen vs. irradiation time in MeOH–H₂O (1:1, v/v) solution. Solutions were irradiated with visible light (>400 nm) under air-saturated conditions in the presence of 0.04 mM Rf: (a) with SFU 0.4 mM and (b) with SFU 0.4 mM plus 14 μg/ml SOD.

Table 1 Rate constants for the quenching of $^1Rf^*$, $k_{Q(1)}$ ($M^{-1} s^{-1}$) and $^3Rf^*$, $k_{Q(3)}$ ($M^{-1} s^{-1}$) by SFU in MeOH–H₂O 1:1 (v/v) and relative rates of oxygen consumption by SFU upon Rf sensitization of solutions in the absence [$V_{ox}(Rf)$] and in the presence of SOD 14 μg/ml [$V_{ox}(Rf + SOD)$]

Medium	$k_{Q(1)} \times 10^9$	$k_{Q(3)} \times 10^9$	$V_{ox}(Rf)$	$V_{ox}(Rf + SOD)$
MeOH–H ₂ O 1:1	7.40 ± 0.10	2.06 ± 0.11	1	0.51

of $O_2^{\cdot-}$ in the process. SOD is frequently employed as an auxiliary reactive in order to confirm or deny the participation of $O_2^{\cdot-}$ in a certain reaction.^{26,27}

The relative rates of oxygen consumption of SFU under Rf sensitization in the absence and presence of SOD are shown in Table 1.

The photolysis rate of solutions containing Rf 0.02 mM, in the absence of oxygen, diminished in the presence of low SFU concentration (0.05 mM). Spectral changes are shown in Fig. 4, suggesting the existence of an interaction of SFU with $^3Rf^*$, the excited triplet state.

On the basis of the results described above, after visible light irradiation of SFU solutions with sensitizer Rf, three experimental facts can be established: (a) Rf and SFU are degraded; (b) such degradation involves ROS; and (c) SFU interacts with Rf-electronically excited states.

Different processes involved in the generation of ROS can be described as follows:

Rf absorbs light and initially generates the singlet excited state, $^1Rf^*$ (process 1), which can decay to the ground state (process 2) or which can be quenched by SFU (process 4). Also, through a process of inter-system crossing, $^1Rf^*$ can generate $^3Rf^*$ (process 3), which decays to the ground state or yields products (process 5). $^3Rf^*$ can be quenched by ground-state oxygen, $O_2(^3\Sigma_g^-)$, dissolved in the medium, generating

Figure 4 Evolution of the 445 nm band of Rf in MeOH–H₂O (1:1, v/v) (deareated) upon irradiation with visible light. (a) Rf (0.02 mM) not irradiated, (b) Rf (0.2 mM) + SFU (0.05 mM) irradiated for 6 minutes, (c) Rf (0.02 mM) irradiated for 6 minutes. *Inset:* Stern–Volmer plot for the quenching of Rf triplet state by SFU in argon-saturated solutions of MeOH–H₂O 1:1 (v/v).

O₂(¹Δ_g) by energy transfer (process 6), or it can be quenched by SFU, yielding the semi-reduced species Rf^{•−} (process 7).

Rf^{•−} can generate O₂^{•−} by electron transfer to O₂(³Σ_g[−]) (process 8). At physiological pH, Rf^{•−} can be protonated to form the radical RfH[•] (pK = 8.3).²⁸ The bimolecular decay of RfH[•] is known to proceed through a disproportionation reaction, yielding Rf and fully reduced Rf (RfH₂) (process 9). In the presence of O₂(³Σ_g[−]), RfH₂ is reoxidized, giving rise to radicals RfH₂^{•+} and O₂^{•−} and, eventually, Rf and H₂O₂ (process 10).^{29,30} The semiquinone radical RfH[•] has been detected as a product of electron transfer processes to ³Rf* from different electron-donor substrates of environmental and biological importance.^{28,31,32}

The O₂^{•−} generated through process (8) or/and (10) can react with SFU yielding oxidized products (process 11).

Interaction between SFU and Rf-electronically excited states

To obtain SFU concentrations higher than those allowed by the solubility of SFU in water, the solvent mixture MeOH–H₂O (1:1, v/v) was employed to determine the interactions of the bacteriostatic with singlet and triplet excited states of Rf. Likewise, in order to study the interaction between ¹Rf* and SFU (process 4), the fluorescence spectra were evaluated. Rf exhibits a fluorescence emission band centered at 515 nm, with a fluorescence quantum yield of 0.25.³³ In the presence of SFU concentrations much higher than 1 mM, the stationary Rf-fluorescence emission intensity decreased, but the shape of the emission spectrum remained unchanged, discarding the possible formation of a fluorescent complex. Assuming that the fluorescence inhibition is a consequence of the quenching of ¹Rf* by SFU, the bimolecular rate constant, *k*_{Q(1)}, was obtained from *K*_{SV}, the Stern–Volmer constant for the interaction.

The *K*_{SV} value, which was determined graphically (Fig. 5), equals the ratio *k*_{Q(1)}/¹*k*_d, where ¹*k*_d is the rate constant for ¹Rf* deactivation processes towards the ground-state Rf. The value of *k*_{Q(1)} reported in Table 1 was calculated according to a literature value³⁴ 5.75 ns for 1/*k*_d.

Laser flash photolysis experiments of argon-saturated solutions of Rf in the absence and presence of sub-millimolar SFU concentration display qualitative results similar to those described for the SDs, in previous work by our research group,²¹ where transient absorption spectra of Rf in the absence and in the presence of SDs were obtained after the laser pulse. The

Figure 5 Stern–Volmer plot for the static fluorescence quenching of Rf (0.01 mM) by SFU in MeOH:H₂O (1:1, v/v) solution.

Scheme 1 (A) Ionization equilibrium in SFU; (B) ionization equilibrium in uracil.

shape of these spectra was in good agreement with that of the semiquinone radical $\text{RfH}^\bullet$, previously reported.^{31,32} These results allow confirmation of the interaction of $^3\text{Rf}^*$ and SFU. The monoexponential decay curves clearly indicated that $^3\text{Rf}^*$ lifetime decreased in the presence of SFU. The value of the bimolecular rate constant, $k_{Q(3)}$, was obtained from the classical Stern–Volmer treatment of time-resolved data (Fig. 4 inset, Table 1).

$\text{O}_2(^1\Delta_g)$ -mediated photodegradation of SFU

The species $\text{O}_2(^1\Delta_g)$ produced by process (6) can react with SFU generating oxidation products (process 13), or it can also react with Rf generating other oxidation products (process 14). Also, it can interact physically with SFU (process 12) or it can decay by collision with solvent molecules (process 15).

Although our main aim was to study SFU photodegradability at physiological pH values, it is also interesting to investigate the possible influences of pH media on the kinetics of photodegradative processes.

Msagati and Nindi³⁵ have reported a pK_a value of 11.5 for SFU, which corresponds to hydroxyl ionization in the sulfamic group of the molecule (Scheme 1A). The kinetic experiments were performed at pHs 7 and 12 where the non-ionized SFU and more than 50% of its ionized form were present, respectively.

In addition, the behavior of the uracil molecule in aqueous solution is described in Scheme 1B (being the values reported^{36,37} for $\text{pK}_{a1} = 9.5$ and for $\text{pK}_{a2} > 13$).

Assuming that the presence of the sulfamino- group does not vary the distribution of the species described for uracil substantially, in aqueous reaction media at pH 12 the ionized species *b* of Scheme 1B will also be present in the medium.

Initially, Rose Bengal (RB), an efficient generator of $\text{O}_2(^1\Delta_g)$,³⁸ was selected as a sensitizer in order to evaluate the involvement of this oxidative species in SFU degradation. Spectral changes were observed in aqueous solutions with SFU and RB, which may prove the association between the substrate and the xanthenic dye in the ground state of both selected pHs. However, since the aim of the current study is not to assess the magnitude of this association, PN was selected as a sensitizer, which generates singlet oxygen species in water with a quantum yield close to unity.³⁹

Fig. 6 shows changes in the absorption spectrum upon irradiation of SFU-PN solutions at pH 12. These changes were inhibited by the presence of 1 mM NaN_3 , a selective quencher of the species $\text{O}_2(^1\Delta_g)$ with reported k_t values of 5×10^8 – $15 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ in water,²² suggesting singlet oxygen participation. Dark reactions between PN 0.01 mM and SFU 0.1 mM, as well as thermal oxidation by molecular oxygen, were discarded because the spectra of solutions stored in darkness and of air-saturated solutions had not changed during irradiation time.

It was observed that oxygen was consumed in SFU-irradiated aqueous solutions with PN at pHs 7 and 12, and it was inhibited by the presence of 1 mM NaN_3 .

These experiments, in addition to the absence of reactivity in N_2 saturated solutions and the inhibition of $\text{O}_2(^1\Delta_g)$ phosphorescence emission at 1270 nm,

Figure 6 Time evolution of the absorption spectrum of SFU 0.5 mM upon PN-sensitized irradiation vs. PN in H_2O (pH 12).

Figure 7 (A) Stern–Volmer plots for the quenching of $O_2(^1\Delta_g)$ phosphorescence by SFU in D_2O at (a) pH 7 and (b) pH 12. PN ($Abs_{355} = 0.40$) as a sensitizer. (B) First-order plot for the concentration changes of SFU 0.4 mM or Trp 0.4 mM, under irradiation with visible light (cutoff > 400 nm) in the presence of PN ($Abs_{355} = 0.5$).

Table 2 Rate constants for the reactive (k_r , $M^{-1} s^{-1}$) and overall quenching (k_t , $M^{-1} s^{-1}$) of $O_2(^1\Delta_g)$ by SFU, ratio k_r/k_t and relative rates of oxygen consumption (V_{ox}) upon PN-sensitized irradiation of SFU

Medium	$k_t \times 10^8$	$k_r \times 10^8$	k_r/k_t	$V_{ox}PN$
H ₂ O pH/pD 7	0.79 ± 0.01 (0.005)	0.60 ± 0.01 (NR*)	0.75	0.77
H ₂ O pH/pD 12	3.09 ± 0.05 (0.14**)	2.97 ± 0.01 (0.15**)	~ 1	1

Parentheses indicate bibliographic data for uracil, included for comparative purposes.

*No reaction was observed.

**Determined at pH/pD 11.

indicate the participation of $O_2(^1\Delta_g)$ as a primary oxidizing agent in PN-sensitized photooxidation of SFU aqueous solutions.

The overall rate constant, $k_t (=k_Q + k_r)$ (process 12 plus process 13), was determined by TRPD at pDs 7 and 12. The results are displayed in Fig. 7A and Table 2.

The reactive rate constant k_r was determined using the method described by Scully and Hoigne⁴⁰ by consumption of substrate to pHs 7 and 12. Absorbance changes were measured at low conversions of substrate (<10 – 15%) in order to avoid eventual interference of photooxidation products absorption in the same spectral zone. Trp was used as reference, where k_r is $3.47 \times 10^7 M^{-1} s^{-1}$ at pH 7 and $30.6 \times 10^7 M^{-1} s^{-1}$ at pH 12, values reported by Criado *et al.*⁴¹ Consumption of amino acid and SFU were followed by fluorescence emission and absorbance spectroscopy, respectively, and k_r values were obtained from first-order graphs that show SFU and Trp consumption upon sensitized irradiation with PN (Fig. 7B, Table 2).

The quantum efficiency of singlet oxygen-mediated oxidation, ϕ_r , is expressed by the following equation⁴²:

$$\phi_r = k_r[SFU]/(k_d + k_t[SFU]) \quad (16)$$

being k_d the rate constant for $O_2(^1\Delta_g)$ deactivation due to interaction with solvent molecules ($2.5 \times 10^5 s^{-1}$ in water⁴³).

Quantum efficiency is difficult to determine due to the fact that the actual concentration of the photooxidizable substrate (represented by SFU in our case) needs to be known beforehand to apply equation (16). Substrate concentration is unknown in many cases, particularly in natural environments. Then a simpler and more useful approach is the evaluation of the k_r/k_t ratio, which indicates the fraction of overall quenching of $O_2(^1\Delta_g)$ by the substrate, which effectively leads to a chemical transformation.

The respective k_r/k_t ratios for SFU at pHs 7 and 12 are included in Table 2. For comparison purposes, the values of uracil also have been included. It is noted that this compound exhibits a very limited reactivity at pH 7, with an extremely low rate constant k_t for overall interaction and without effective oxidation.

At pH 12, where the main species are ionized SFU forms, not only is a marked increase in the rate constants observed, but it is also noticed that SFU– $O_2(^1\Delta_g)$ interaction is completely reactive ($k_r/k_t = 1$), like that registered for uracil.²⁰

Discussion

Interaction between SFU and Rf-electronically excited states

According to the results of our experiments, both singlet and triplet Rf-electronically excited states are quenched by SFU in aqueous solutions represented by equations (4) and (7), respectively. Although $k_{Q(1)}$

reaches a value close to the diffusional limit, a simple calculation based on the Stern–Volmer treatment indicates that $^1\text{Rf}^*$ is practically unaffected by the presence of SFU under the conditions in which our experiments were performed. Thus, using the value from Table 1 for $k_{Q(1)}$, $7.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, only 1.4% $^1\text{Rf}^*$ is quenched in the presence of 0.4 mM SFU, whereas 20 mM SFU is necessary for the quenching of 50% $^1\text{Rf}^*$, about 70 times the concentration used under stationary photoirradiation conditions.

On the other hand, $^3\text{Rf}^*$ can be quenched by $\text{O}_2(^3\Sigma_g^-)$ to produce the oxidative species $\text{O}_2(^1\Delta_g)$ (process 6), or by SFU (process 7) to produce other reactive species, such as $\text{O}_2^{\bullet-}$ via processes (8) and (9)–(10). This sequence has been formerly proposed in the photooxidation of other phenols.^{31,32} Thus, the species $\text{O}_2^{\bullet-}$ should be present, together with $\text{O}_2(^1\Delta_g)$, as indicated by the observed effect of SOD on the relative rate of oxygen consumption (Fig. 3). The enzyme SOD catalyzes the dismutation of $\text{O}_2^{\bullet-}$ according to the process:

SOD has been used as a specific quencher of $\text{O}_2^{\bullet-}$ in a given photooxidation process,^{26,27} in similar concentrations to those employed in this work.

The high rate constant value for $k_{Q(3)}$ (Table 1) and the inhibitory effect of SOD in oxygen uptake experiments strongly support the conclusion that $\text{O}_2^{\bullet-}$ is generated by processes (8) and (10), which are involved in the oxidation of SFU (process 11). The straightforward generation of $\text{O}_2^{\bullet-}$ by $^3\text{Rf}^*$ (equation not shown) is not considered, because its quantum yield is very low: 0.009.⁴⁴

At the same time, processes (8) and (10) regenerate ground-state Rf, a crucial step in living organisms. It is well known that $\text{O}_2^{\bullet-}$ is a key intermediate in oxygen redox chemistry.⁴⁵

The Gibbs energy for the electron transfer represented by process (7) can be evaluated from the Rehm–Weller treatment⁴⁶:

$$\Delta G^\circ = E_{(\text{SFU}/\text{SFU}^+)} - E_{(\text{Rf}/\text{Rf}^-)} - E_{\text{Rf}^*_{+C}} \quad (18)$$

where $E_{(\text{SFU}/\text{SFU}^+)}$ (0.778 V) is the oxidation potential value for SFU,⁴⁷ $E_{(\text{Rf}/\text{Rf}^-)}$ (−0.80 V) is the redox potential of Rf, E_{Rf^*} (2.17 eV) is the Rf excited triplet energy, and C (−0.06 V) is the Coulombic energy term.⁴⁸ Thus, the $\Delta G^\circ = -0.65 \text{ eV}$ deduced from equation (18) indicates that process (7) is thermodynamically feasible. Consequently, species $\text{O}_2^{\bullet-}$ can be formed from Rf^* , but the kinetic competition between processes (6) and (7) will determine the ratio for these two pathways. Using $\sim 1 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ at MeOH–H₂O for k_{ET} , a literature value,⁴⁹ $2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ for $k_{Q(3)}$, a

value from Table 1, and considering equal concentrations of $\text{O}_2(^3\Sigma_g^-)$ and SFU, both reactive steps occur at similar rates to those seen for other SDs studied in previous work.²¹ In other words, the species $\text{O}_2(^1\Delta_g)$ and Rf^* seem to be generated to a similar degree through processes (6) and (7), respectively. Therefore, the kinetic balance indicates that, under working conditions, the generation rate of Rf^* (the $\text{O}_2^{\bullet-}$ precursor species) is similar to the generation rate of $\text{O}_2(^1\Delta_g)$.

$\text{O}_2(^1\Delta_g)$ -mediated photodegradation of SFU

Our results indicate that SFU interacts with $\text{O}_2(^1\Delta_g)$ at pH 7 in its non-ionized form, as well as at pH 12 where the ionized species are present in aqueous solutions. It is interesting to compare the kinetic constants for SFU at pHs 7 and 12 with those of uracil previously published.²⁰ The values of these constants (Table 2) suggest that the presence of the sulfamino- group as a substituent in the uracil moiety exerts an activating power on its nucleus. This is based on the increase of the constants for the global and for the reactive processes in the interaction with SFU compared to the uracil in alkaline medium. Also, we can observe the high ratio k_r/k_t for the bacteriostatic in relation with uracil, which does not suffer oxidation at pH 7 (Table 2). Therefore, it is clear that the combination of primary amino and sulfonic groups as substituent on the uracilic ring confers high pro-oxidative reactivity to the resulting molecule.

In Table 2, it is also important to note that the ratio of relative rates of oxygen consumption $V_{\text{ox}}(\text{pH } 12)/V_{\text{ox}}(\text{pH } 7)$ is 1.3, while the ratio of the reactive rate constants $k_r(\text{pH } 12)/k_r(\text{pH } 7)$ reached a value close to 5 using the same sensitizer. A possible explanation for such value is that k_r was determined following the decrease of SFU concentration during photolysis. Also, oxygen consumption measures the incorporation of oxygen molecules by products. This means that the mole ratio (mol SFU/mol $\text{O}_2(^1\Delta_g)$) at pH 12 is higher than at pH 7, suggesting that some kind of secondary reaction is involved in the degradation process. Products incorporate less oxygen at pH 12 than at pH 7. Therefore, we can conclude that photolysis products are different depending on the pH present in the medium.

Conclusions

Bacteriostatic SFU is degraded under visible light irradiation in the presence of photosensitizers (PN or Rf) through the reaction of reactive oxygenated species generated in aqueous solutions at different pHs. With PN, photodegradation is mediated totally by $\text{O}_2(^1\Delta_g)$ in a relatively efficient process favored by an alkaline medium. In the presence of the natural sensitizer Rf, SFU reacts with oxidative species $\text{O}_2(^1\Delta_g)$

and O_2^- , both generated from the interaction of $^3Rf^*$ with $O_2(^3\Sigma_g^-)$ in competitive processes.

The results indicate that sensitized photodegradation is an efficient pathway to eliminate SFU from natural waters under controlled conditions, especially in an alkaline medium.

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Disclaimer statements

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