Riboflavin

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Abstract

In recent years, increasing attention has focused in photoredox catalysis using preferentially visible light. As a result of this effort, myriads of different applications of transition-metal based or the alternative metal-free photocatalysts, typically organic dyes, have been developed.[1]

Riboflavin (RF) is a natural dye present in waters of rivers, lakes and seas, responsible for the redox activity of cofactors flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). It has a three-ring isoalloxazine chemical structure, a 7,8-dimethyl substitution and a ribotyl chain at the N 10 position (Figure 1). Its characteristic visible absorption spectrum exhibits two defined bands peaking *ca*. 450 and 350-375 nm, with high molar absorption coefficients ($>10^4$ $M^{-1}cm^{-1}$). For this reason, RF has been considered as the most likely candidate to explain "natural" photodegradation of pollutants in the environment.[2] The photophysical properties of RF have been studied in detail; briefly, RF exhibits in aqueous solution a moderate fluorescence quantum yield of $\Phi_F = 0.26$ (which increases up to 0.47 in CH_3CN), and an intersystem crossing quantum yield of $\Phi_{ISC} = 0.70$. Furthermore, RF is able to sensitize generation of singlet oxygen from its triplet excited state with $\Phi_\Delta = 0.47$.[2] The RF singlet and triplet energies are 2.48 eV and 2.17 eV, respectively. Although the redox potential of RF depends on the solvent, values ranging between -0.80 V and -0.45 V have been reported.[3]

The potential of RF as a metal-free photocatalyst for water remediation, more specifically to promote photooxidation of phenolic or *N*-heteroaromatic pollutants upon solar irradiation, has been extensively reported.[4] Mechanistic studies based on time-resolved techniques, have demonstrated that most of the pollutants are able to quench the singlet and/or the triplet excited states of RF, giving rise to the corresponding pollutant radical cations along with the characteristic RF$^{\cdot-}$, which shows a transient absorption spectrum with a broad maximum in the 500-600 nm region.[5] Further oxidation has been attributed, in many cases, to the reactivity of the radical cations with superoxide anion, which results from the electron transfer process from RF$^{\cdot-}$ to O_2. More recently, RF or related flavin derivatives have also found applications as visible-light redox photocatalysts to produce different transformations with synthetic purposes, such as aerobic photooxidation of

sulfides,[6] oxidative chlorination of arenes[7] or selective removal of benzylic protecting groups,[8] among others. However, no attention has been paid to the potential of RF as a reductant in either water remediation or organic synthesis.

Reductive dehalogenation procedures that are of wide application in many different synthetic contexts have also been optimized using visible-light photocatalysts such as Ru(bpy)$_3$Cl$_2$;[9] however, the improvement of these reductive processes still remains a challenge for synthetic chemists.

Although a deeper mechanistic understanding of the underlying processes could help to broaden the substrate scope of the photocatalyzed organic reactions, most of the recent publications lack a kinetic study of the involved excited states. However, postulated reaction mechanisms should be the result of investigating on the photophysical properties of the employed photocatalysts for determining the kinetic parameters of the competitive pathways they undergo in the presence of different substrates.[4a, 10]

In this context, the goal of the present work is to gain understanding on the overall reactions mechanisms by analyzing the thermodynamic and the kinetic feasibility of all the competing pathways of the photocatalyzed redox processes. For this purpose, the kinetic constants of every step will be determined using fast kinetic techniques, to postulate a plausible mechanism based on the experimentally obtained results. Such a protocol will be applied to investigate on the capability of RF as a metal-free photocatalyst to produce reductive dehalogenation of different bromo-derivatives, upon irradiation using visible light, and in the presence of amines and isopropanol as e$^-$ and H-donors, respectively. Specifically, the selected substrates are bromides such as bromobenzene (**1**), benzyl bromide (**2**) and α-bromoacetophenone (**3**) have been selected (Figure 1).
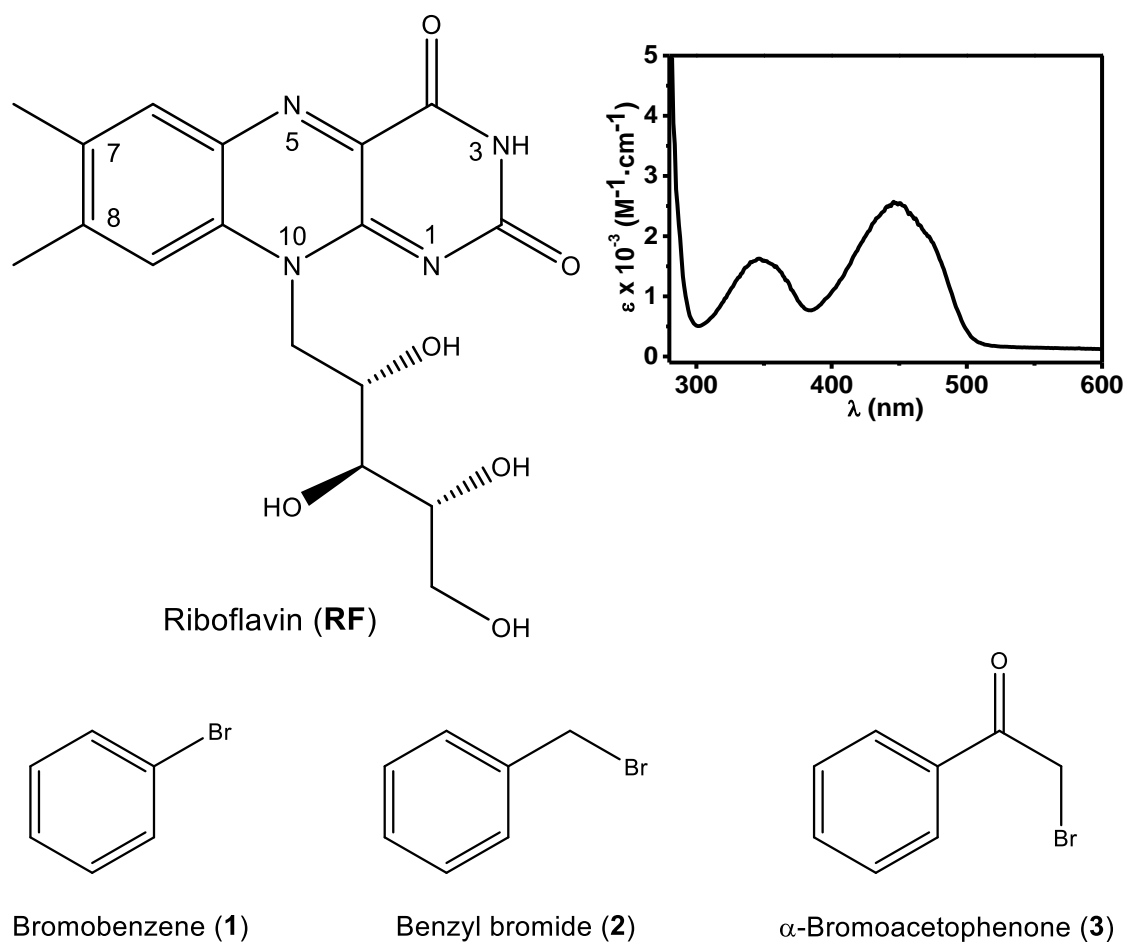
Figure 1. Chemical structure and absorption spectrum of Riboflavin (RF) in dimethylformamide (top); chemical structure of selected bromides (bottom).

Results and Discussion

Photocatalyzed reactions

To be perfomeddddddd

Thermodynamic feasibility of the involved processes

To eventually postulate a plausible reaction mechanism to explain reductive dehalogenation of bromides we analyzed the thermodynamic feasibility of all the potentially involved processes (see Scheme 1).

Scheme 1. Postulated reaction mechanism

Quenching of the excited states of a photocatalyst could, in principle, be due to two different mechanisms: energy trans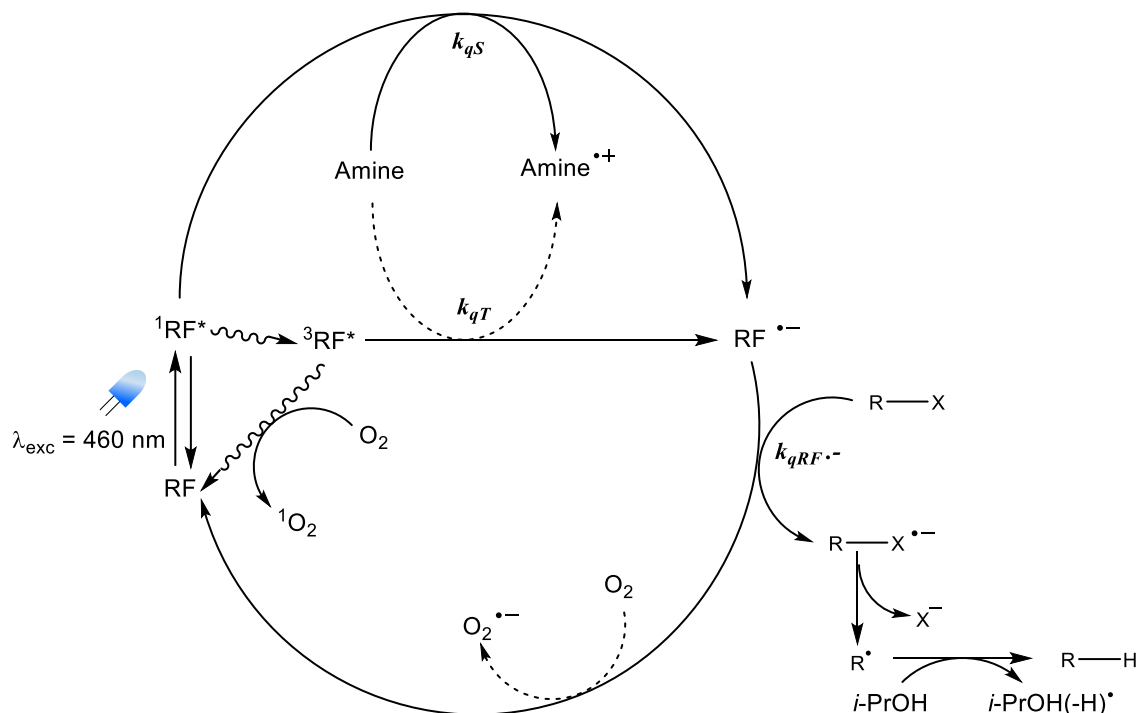fer or electron transfer. By comparing the UV-visible spectra of the photocatalyst and the bromides (See Figure 1 and SI), while RF absorbs up to 500 nm, all the studied bromides absorb at much shorter wavelength, below 300 nm for **1** and **2**, and below 400 nm in the case of **3**. Therefore, one can anticipate much more energetic excited states in the case of the bromides than in the case of RF (singlet and triplet energies for RF are 2.48 eV and 2.17 eV, respectively).[3b] As a result, the energy transfer from RF will be thermodynamically disfavored in all cases and can be safely ruled out.

Next, we studied the feasibility of the photoinduced electron transfer from different amines to the excited states of RF using the Gibbs energy of photoinduced electron transfer equation (formerly known as Rehm-Weller equation):[11]

$$\Delta G_{et}^{o}(eV) = -(E_{red}^{o}(RF/RF^{·-}) - E_{red}^{o}(D^{·+}/D)) - E(RF^*) + \Delta E_{Coulombic}$$

The redox potential of RF has been reported as $E_{red}^{o}(RF/RF^{·-})$ = -0.80 V (*vs.* SCE) in DMSO (see above). The reported redox potential values for the oxidation of different

amines are shown on Table 1. By applying the Gibbs energy of photoinduced electron transfer equation we can conclude that oxidation of the amines from the singlet or the triplet excited state of RF is in every case exergonic.

Then, once the radical anion of RF is obtained, next step would be the reduction of the bromides. The thermodynamics of these processes could be evaluated using the redox potentials of the involved species by applying the "thermal" free Gibbs energy equation:

$$\Delta G^o(eV) = -(E_{red}^o(C - Br/C - Br^{·-}) - E_{red}^o(RF/RF^{·-}))$$

Using this equation the resulting ΔG^o are: +0.76 eV; -0.23 eV and -0.31 eV, for bromides **1**, **2** and **3**, respectively. This array would allow establishing thermodynamically based conclusions.

Kinetic viability of the involved processes

Having analyzed the thermodynamics, the following step was to determine the viability of the different competing processes in the time scale of the corresponding RF excited states. First, the participation of the singlet excited state was investigated by means of steady-state and time-resolved fluorescence and then laser flash photolysis was used to monitor the dynamics of the triplet excited state as well as the radical anion.

Steady-state and time-resolved emission results

Quenching of fluorescence of RF by amines was performed in aerated DMF. In all cases it proceeded without changes of the emission spectral shape; likewise, no changes were found in the absorption spectrum of RF, thus precluding formation of ground state complexes. Nevertheless, an efficient emission quenching was observed in all cases (see Figure 2 and SI). Moreover, time-resolved emission showed a very efficient quenching upon addition of DABCO, DMA or TEA, in agreement with the expectations taking into account the redox potential of RF and those of the employed amines (see Table 1). The corresponding quenching constants were determined from the Stern-Volmer relationships. The obtained data (see Table 1) confirmed the dynamic participation of ^{1}RF* in the electron transfer process, with values close to the diffusion limit.[12] On the other hand, control experiments showed that singlet lifetime of ^{1}RF* remained unchanged upon addition of bromides **1-3**, up to 5×10^{-2} M concentration (see Supporting

Information), indicating that the ^{1}RF* is unreactive in the presence of the bromides, as expected from their redox potentials.
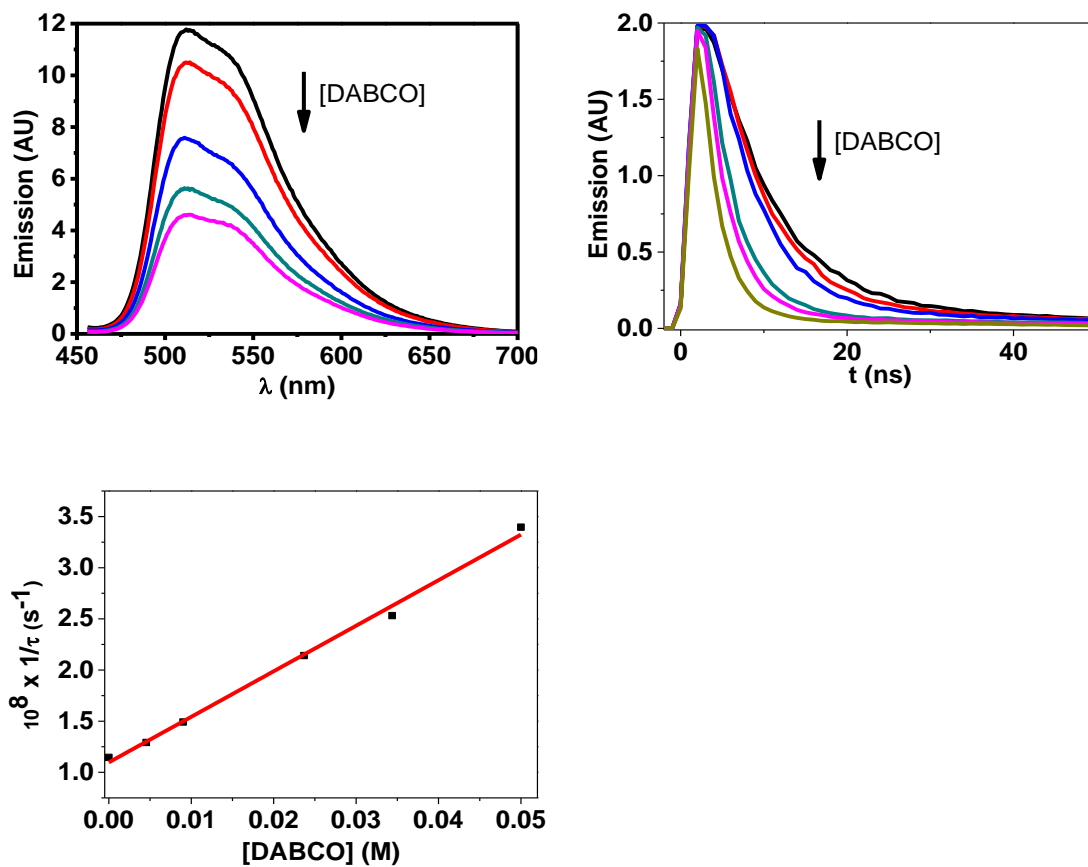
Laser flash photolysis experiments

Additional confirmation of the redox process was obtained from the comparison of the transient absorption spectrum recorded in the absence and in the presence of DABCO.

Table 1

	E ($D^{\cdot+}/D$, V vs SCE)	E ($A/A^{\cdot-}$, V vs SCE)	k_{qS} ($M^{-1}s^{-1}$)	k_{qT} ($M^{-1}s^{-1}$)	$k_{qRF^{\cdot-}}$ ($M^{-1}s^{-1}$)
DABCO	+0.56[12]	-	4.5×10^9		-
DMA	+0.81[12]	-	5.3×10^9		-
TEA	+0.96[12]	-	2.9×10^9		-
DIPA	+1.31[3b]				
1	-	-1.56[13]	-	-	$< 10^6$
2	-	-0.57[13]	-	-	9.0×10^7
3	-	-0.49[14]	-	-	1.5×10^8
i-PrOH	-	-		-	-
O$_2$	-	-0.33[15]	-	$9.0 \times 10^{8[4b]}$	$1.4 \times 10^{8[4b]}$

The quantum yield of the RF$^{\cdot-}$ formation could be calculated due to the quenching of ^{1}RF* or ^{3}RF* can be evaluated from the following equation:

$$Formation\ of\ RF^{\cdot-}\ (\%\%)$$

$$= \frac{k_{qs}[Amine]}{\frac{\Phi_F}{\tau_S} + \frac{\Phi_{ISC}}{\tau_S} + k_{qs}[Amine]} + \frac{\frac{\Phi_{ISC}}{\tau_S}}{\frac{\Phi_F}{\tau_S} + \frac{\Phi_{ISC}}{\tau_S} + k_{qs}[Amine]} \times \frac{k_{qT}[Amine]}{\frac{1}{\tau_T} + k_{qT}[Amine]}$$

Conclusions

Riboflavin (RF), 1,4-diazabicyclo[2.2.2]octane (DABCO), dimethylaniline (DMA), trimethylamine (TEA), bromobenzene (**1**), benzyl bromide (**2**) and α-bromoacetophenone (**3**) were obtained from Aldrich. Dimethylformamide (DMF) was of HPLC quality from Merck.

A homemade set-up photoreactor build with 2.5 m strip blue-LEDs Samsung SMD5630 IP20 of 15 W/m, spiral-shaped and an irradiation centred at 460 nm was used for the photochemical reactions. DMF solutions of 5 mL containing a mixture of one of the pollutants and NMQ$^+$ (3×10^{-5} M each) were irradiated under N$_2$ atmosphere
Aliquots from these reactions were injected into the HPLC (Waters 600 C chromatograph equipped with a Waters 600 Pump and Controller, a Waters in-Line degasser AF and a Waters 996 Photodiode Array Detector). A Teknokroma C18 Mediterranea Sea (25 ×

0.46 mm and 5 μm particle size) analytical column was used as stationary phase, and a mixture of acetonitrile/water was used as a eluent working in isocratic (55% acetonitrile) or gradient mode (10-70% acetonitrile), at a flow rate of 1 mL/min. Thus, 5 μL of 2-nitrobenzoic acid (8.8×10^{-4} M) were added as internal standard to every aliquot (100 μL) before injection The chromatograms were analyzed at 254 nm.

2.3. Photophysical instrumentation

A Shimadzu UV-2101PC spectrophotometer was employed to perform the UV/Vis absorption spectra. Steady-state and time-resolved fluorescence experiments were carried out on a Photon Technology International (PTI) LPS-220B spectrofluorometer and on a EasyLife V spectrofluorometer from OBB, respectively. In the case of time-resolved fluorescence, the apparatus was equipped with a pulsed LED (λ_{exc} = 460 nm) excitation source; residual excitation signal was filtered in emission by using a cut-off filter (50% transmission at 475 nm). Monoexponential decay functions that use a deconvolution procedure to separate them from the lamp pulse profile provided the fitted kinetic traces. A pulsed Nd: YAG SL404G-10 Spectron Laser Systems at the excitation wavelength of 355 nm was employed to carry out the laser flash photolysis (LFP) experiments. The energy of the single pulses (~10 ns duration) was lower than 30 mJ pulse^{-1}. The laser flash photolysis system is formed by the pulsed laser, a pulsed Lo255 Oriel Xenon lamp, a 77200 Oriel monochromator, an Oriel photomultiplier tube (PMT) housing, a 70705 PMT power supply and a TDS-640A Tektronix oscilloscope. Photophysical measurements were run at room temperature under nitrogen using quartz cells of 1 cm optical path length. For the steady-state fluorescence experiments, aerated DMF solutions of RF (absorbance lower than 0.15 at λ_{exc} = 446 nm) were treated with increasing concentrations of amine or bromoderivative (up to 5×10^{-2} M). Transient absorption spectra were obtained from a deaerated solution of RF in DMF (5×10^{-4} M???).

Acknowledgements

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