NITROXIDE REDUCTION BY ELECTRON TRANSFER FROM THE EOSIN TRIPLET STATE: ELECTRON PARAMAGNETIC RESONANCE AND FLASH PHOTOLYSIS STUDIES

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Summary

The paramagnetism loss of nitroxide, photosensitized by eosin, was analysed in basic aqueous and micellar media using electron paramagnetic resonance (EPR) spectroscopy. In cetyltrimethylammonium bromide micelles and sodium bis(2-ethylhexyl)sulphosuccinate–*n*-heptane microemulsions, the paramagnetism decay curves are monoexponential. This is not the case in neat water and sodium dodecylsulphate micelles, for which more complex decays were recorded. It is concluded from flash photolysis measurements that the eosin triplet state is physically quenched by nitroxide and that the latter can be reduced by electron transfer either from the triplet state or from the semireduced form of eosin. This sequence of reactions was confirmed by experiments in micellar media and leads to an understanding of the EPR results.

1. Introduction

The deactivation of electronically excited molecules by paramagnetic species such as oxygen, nitric oxide, metal ions and nitroxide is a phenomenon which has received widespread attention for some time [1 - 7]. This interaction is interesting from a mechanistic standpoint because several possible pathways exist for quenching. Even though the radicals can be reduced or oxidized, most studies have concluded that the quenching of singlets and triplets is the photophysical process

$$S^* + Q \longrightarrow S + Q \qquad (1)$$

where S^* represents an excited electronic state, S a lower energy electronic state and Q the paramagnetic radical. Several mechanisms, such as exchange-

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induced intersystem crossing, vibrational quenching and Förster transfer, have been reported [3, 8].

Besides these physical deactivation mechanisms, some workers have shown that irradiation of porphyrins [9, 10] and furocoumarin derivatives [11] leads to the loss of paramagnetic absorption of nitroxide. Two different mechanisms were proposed: reduction of nitroxide by photoejected electrons [10, 11] and reduction of nitroxide by hematoporphyrin anion radical [9]. Recently, it has been suggested that the destruction of nitroxide induced by stationary irradiation of eosin could be the result of triplet–nitroxide collision [12].

The aim of this work was to reinvestigate carefully the interaction between nitroxide and photoexcited eosin. Flash photolysis experiments and stationary irradiation of the dye were performed in the presence of nitroxide. Flash photolysis was very suitable for this study. Indeed, the semireduced (R) and semi-oxidized (X) species of xanthene derivatives are characterized by transient absorptions which can be easily monitored by this time-resolved technique [13, 14]. It should be pointed out that X and R can also be produced by dye–dye interaction (triplet–triplet and triplet–ground singlet) [13, 14]. In order to suppress these photochemical processes of eosin triplet relaxation which could lead to misinterpretation, we have undertaken a systematic study of nitroxide–dye interaction in micellar solutions. We have previously reported that the solubilization of eosin in cetyltrimethylammonium bromide (CTAB) micelles prevents dye–dye interaction on the triplet lifetime scale (microsecond to millisecond) [15]. Such a protection of excited dye molecules was also observed in inverted micelles. Fremy's salt, $K_2(SO_3)_2NO$, was used as the nitroxide. Its ionic and hydrophilic characteristics allow difficulties which may arise when the location of the probe is not well defined to be avoided. The oxygen concentration and the location, mobility and concentration of the nitroxide were obtained from EPR spectra [16]. Ab(R) or Ab(X) is the maximum absorbance arising from the radicals and T is the half-life of the radical.

2. Experimental details

Eosin Y (Merck) was carefully purified prior to use [17]. An extinction coefficient of 110 000 M^{-1} cm^{-1} at 517 nm in K_2CO_3 (5×10^{-2} M) was found for the purified product. CTAB was purchased from Sigma Chemical Company and sodium dodecylsulphate (SDS) was obtained from Janssen Chimica. The concentration of the aqueous surfactant solutions was 0.1 M. Sodium bis(2-ethylhexyl)sulphosuccinate (Aerosol-OT or AOT) was obtained from Fluka A.G. and purified as described by Matheson and Rodgers [18]. Potassium carbonate and potassium ferricyanide were Merck products. Potassium nitrosodisulphonate (PADS or Fremy's salt) and 3-carbamoyl-2,2,5,5-tetramethylpyrrolidin-1-yloxy (carbamoyl) were supplied by Aldrich-Europe and used as received. A PADS stock solution was prepared in water

buffered with 5×10^{-2} M K_2CO_3 (Merck) to reduce the decomposition rate. The concentration of this solution was measured optically by using a molar extinction coefficient of 20.8 M^{-1} cm^{-1} at 545 nm [19]. All the aqueous solutions were prepared with laboratory distilled water.

Absorption spectra were recorded on a Perkin–Elmer 559 spectrophotometer. EPR spectra were recorded by using a Varian E-109 spectrometer operating at the X-band and a modulation frequency of 100 kHz. Irradiation of the samples was performed inside the cavity of the spectrometer with a halogenated tungsten lamp source (Philips 150 W) through a long wave pass filter ($\lambda > 475$ nm).

Flash photolysis experiments were carried out using an Applied Photophysics 200 J capacitor bank and an Applied Photophysics flash cavity. Two xenon flash lamps (half-life, 6 μs) were used to irradiate solutions contained in a 10 cm cylindrical cell. The flash energy was chosen to ensure complete conversion of the dye to its triplet state. The monitoring system consisted of a stabilized 900 W Osram xenon lamp, a Bausch and Lomb UV–visible 33-86-07 monochromator and a Hamamatsu R955 photomultiplier tube. Transient changes of light transmission were recorded with a Philips PM 3311/02 digital storage oscilloscope triggered by the flash pulse via a photodiode. The spectra were transferred to a Graphtec WX 1100 chart recorder. Oxygen was removed from the solutions by purging for 30 min with nitrogen containing less than 3 ppm O_2. Each transient species was characterized by its maximum absorbance and its lifetime. These parameters are given with 10% confidence limits. This error was estimated from the standard deviation obtained for 20 separate measurements of the parameters using a 1.9×10^{-6} M eosin solution in 10^{-2} M KOH. For the triplet state T_1, the maximum absorbance $Ab(T_1)$ is the triplet absorbance recorded at the end of the flash, and the lifetime τ is the reciprocal initial rate constant of the triplet decay [13, 20].

3. Results

The location of the PADS was determined by measuring the ratio of the amplitude of the EPR absorption line at high magnetic field to the amplitude of the line at medium field, which is sensitive to the tumbling rate of the spin probe [16]. The conclusions are reported in the last column of Table 1. Figure 1 shows the time course of the paramagnetism loss during the stationary irradiation of solutions containing eosin and nitroxide in water (Fig. 1(a)), and normal and inverted micelles (Fig. 1(b)). No comparison can be made between the slopes of these curves because the incident light intensities were not the same. Only the shape of the decay curves will be discussed. Figure 2 presents the dependence of the eosin triplet initial decay rate constant *vs.* PADS concentration in H_2O, CTAB and SDS. The variation in the maximum absorbance of the semi-oxidized form is reported in Fig. 3.

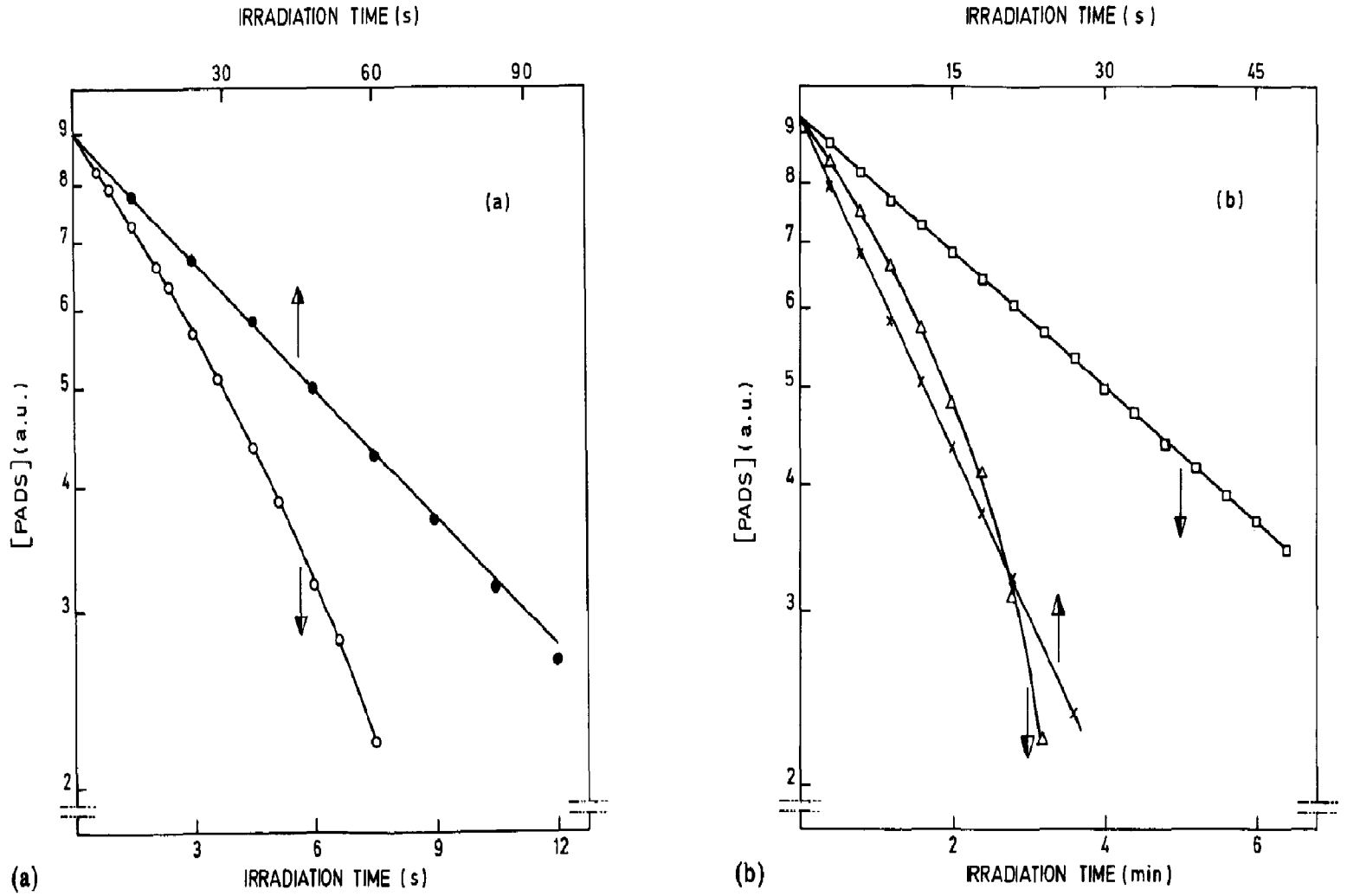


Fig. 1. Semilogarithmic plot of PADS concentration as a function of irradiation time in anaerobic solutions: (a) in 5×10^{-2} M K_2CO_3 aqueous solutions containing 3×10^{-5} M eosin (○) or 10^{-6} M eosin (●); (b) in non-polar surfactant solutions (7×10^{-2} M AOT in 1.7vol.%water–98.3vol.%*n*-heptane) (×), and in aqueous surfactant solutions of SDS (△) and CTAB (□) containing 4×10^{-5} M eosin. The initial concentration of PADS was close to 7×10^{-5} M in each case.

TABLE 1

General properties of the three long-lived transients T_1, R and X produced by flash photolysis of eosin in aqueous and micellar solutions, and the mobility of Fremy's salt in these media

	Eosin triplet state	*Eosin radical species*	*Fremy's salt*
Water	$\tau = 180\ \mu s$	Produced; $T_R = 395\ \mu s$ $T_X = 800\ \mu s$	Free
CTAB	$\tau = 420\ \mu s$ fixed at the micelle interface	Not produced	Partially immobilized; interacts with the micelle interface
SDS	$\tau = 190\ \mu s$ free	Produced; $T_R = 400\ \mu s$ $T_X = 4400\ \mu s$ (protection of X)	Free
AOT–*n*-heptane	$\tau = 190\ \mu s$ located in the aggregate	Not produced	Located in the aqueous core; mobility depends on the amount of solubilized water [16]

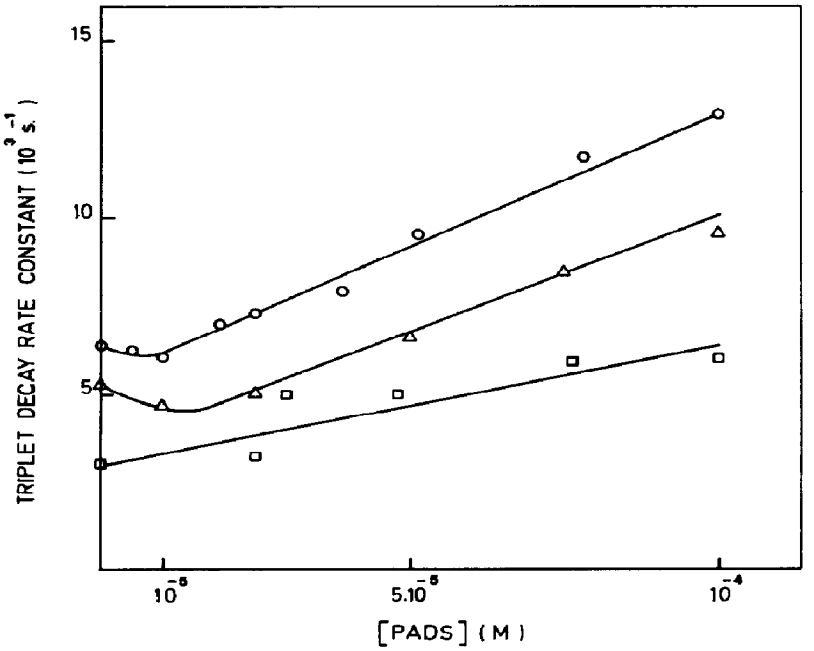


Fig. 2. Evolution of eosin triplet decay rate constant *vs.* PADS concentration in aqueous (○), CTAB (□) or SDS (△) solution ([eosin] = 1.9×10^{-6} M).

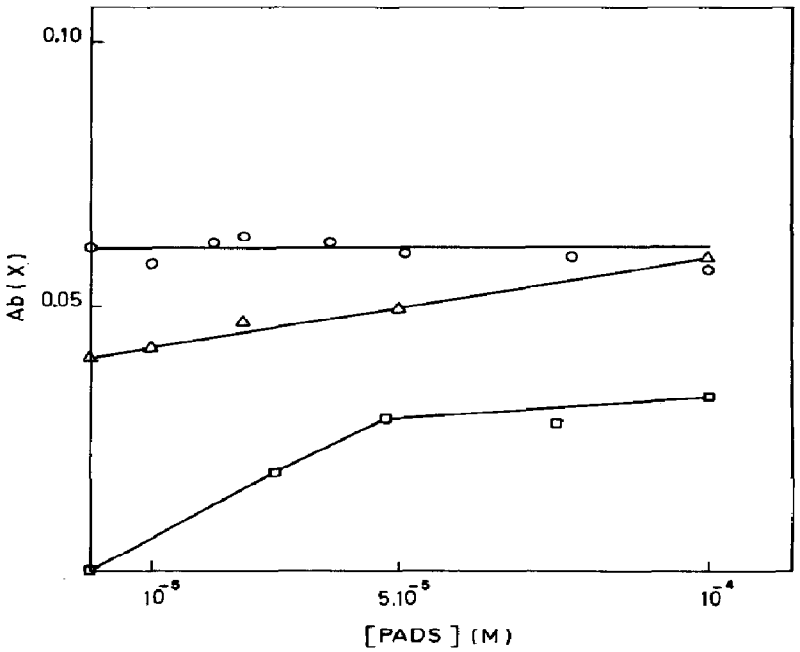


Fig. 3. Dependence of the maximum absorbance Ab(X) of X at 460 nm *vs.* PADS concentration in aqueous (○), CTAB (□) or SDS (△) solution ([eosin] = 1.9×10^{-6} M; optical path, 10 cm).

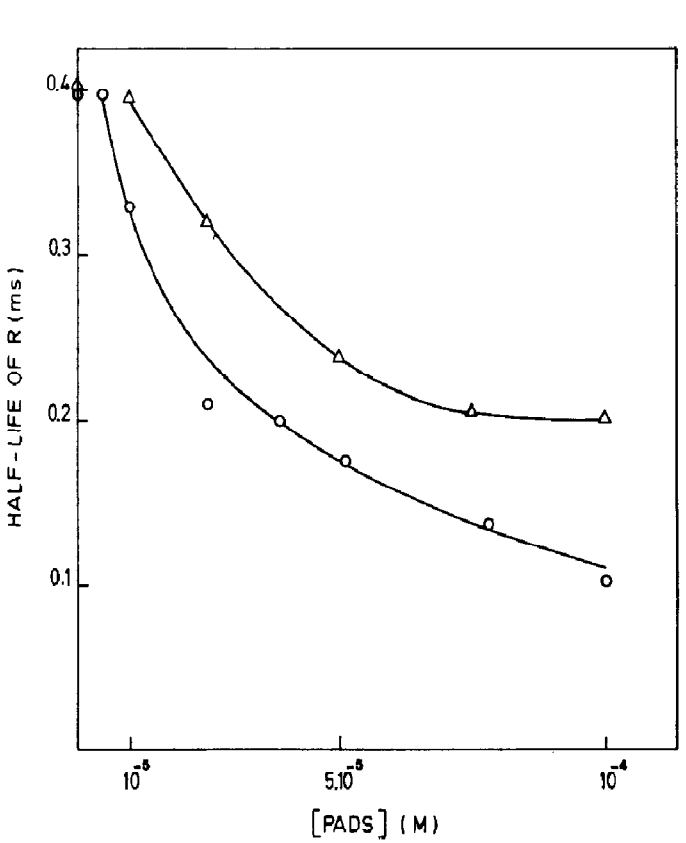
In each case, the maximum absorbance of the eosin triplet state decreases with nitroxide concentration. In H_2O and SDS, the maximum absorbance of semireduced eosin follows the same law. Finally, the modifications of the half-life of semireduced and semi-oxidized forms with different PADS concentrations are plotted in Figs. 4 and 5. We did not perform flash photolysis experiments with PADS in inverted micelles because the introduction of large amounts of this compound leads to the solutions becoming cloudy.

4. Discussion

4.1. Flash photolysis in aqueous solutions

Previous flash photolysis studies [13, 14, 20] have shown that the decay of the eosin dianion triplet state (T_1) is easily followed at 590 nm. The deactivation of the triplet state occurs by intersystem crossing and triplet–triplet and triplet–singlet annihilation [13, 20]. The last two pathways may lead to the formation of dianionic semireduced (R) and dianionic semi-oxidized (X) eosin, which possess maximum absorbances at 405 nm and 460 nm respectively. The formation of these radical species in the presence of reducing or oxidizing agents has been reported on several occasions previously [13, 20 - 23].

Figure 2 shows clearly that nitroxides can quench the eosin triplet state. The bimolecular rate constant k for the quenching of the eosin triplet



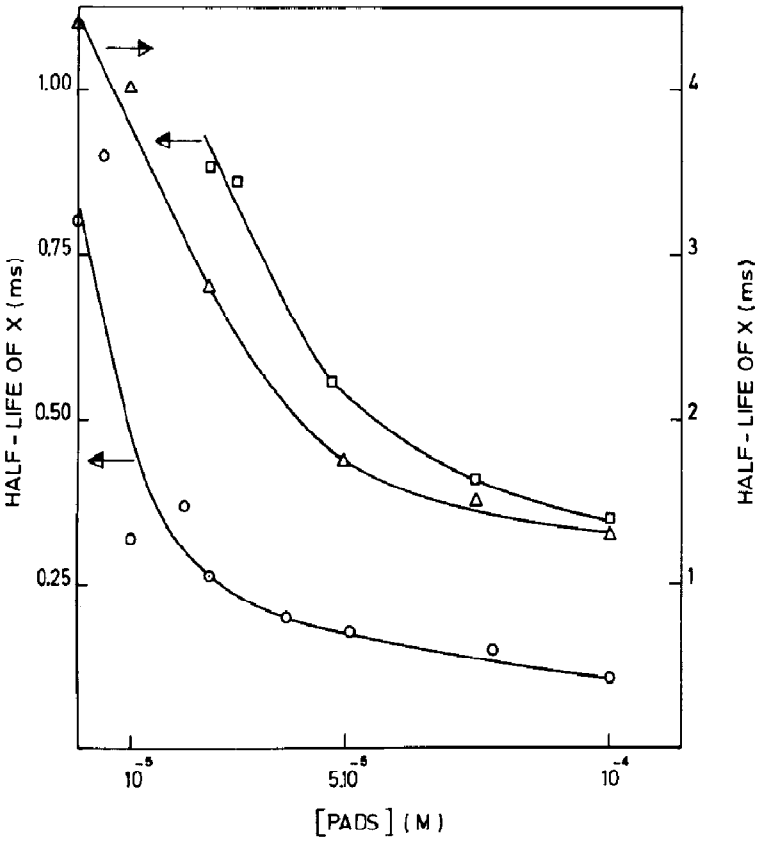


Fig. 4. Influence of PADS concentration on the half-life of R in aqueous (○) or SDS (△) solution ([eosin] = 1.9×10^{-6} M; R is not produced in CTAB solutions).

Fig. 5. Influence of PADS concentration on the half-life of X in aqueous (○), CTAB (□) or SDS (△) solution ([eosin] = 1.9×10^{-6} M). In the absence of nitroxide, X is not produced in CTAB solutions.

state by PADS was calculated as described by Rizzuto and Spikes [20] and a value of 5×10^7 M^{-1} s^{-1} was obtained. For carbamoyl, using the same method, $k = 5 \times 10^8$ M^{-1} s^{-1} was obtained. This significant difference probably arises from the dianionic character of PADS, carbamoyl being a neutral molecule.

The maximum absorbance of X seems to remain constant on increasing the nitroxide concentration (Fig. 3) although the maximum absorbance of R decreases. Since the production of X by dye–triplet reaction is lowered, owing to triplet quenching by nitroxide, this observation indicates that a new mechanism for the production of X exists and that it is influenced by the nitroxide concentration. This conclusion rules out the possibility of photoejection of one electron from T_1. However, electron transfer from T_1 to nitroxide could explain the constant production of X. In order to estimate the efficiency of this photochemical triplet eosin deactivation process, we used $K_3Fe(CN)_6$, a well-known oxidizing agent for the eosin triplet state [13]. We compared the amount of X produced in solutions containing (i) eosin and PADS and (ii) eosin and $K_3Fe(CN)_6$. The PADS and $K_3Fe(CN)_6$ concentrations were adjusted to obtain identical triplet decay rates. In (ii) a very large increase (a factor of 3) of the absorbance of X was detected, confirming the low efficiency of PADS–eosin triplet electron transfer.

Figure 4 shows that nitroxide reacts with semireduced eosin. It seems reasonable to admit that nitroxide is reduced by this species, and that the eosin is restored to its ground state. The occurrence of this reaction is also supported by EPR results, as will be shown below. An identical mechanism has been previously reported to interpret nitroxide reduction by hematoporphyrin anion radical [9]. The high reactivity of semireduced eosin towards many organic substances is well known [13, 21 - 23].

The half-life of semi-oxidized eosin also decreases in the presence of nitroxide (Fig. 5). This decrease may be explained by either a reaction between X and nitroxide or a reaction between X and a product P of nitroxide reaction. This process was not studied in detail. However, EPR results seem to rule out both electron transfer between nitroxide and X and nitroxide regeneration by a back reaction between X and P, as discussed below.

In the presence of nitroxide, the degree of photodestruction of eosin after one flash is lower than in the absence of the paramagnetic probe. Previously, the photodestruction of eosin has been attributed to an incomplete recombination reaction between eosin radical species [13]. In the presence of nitroxide, the concentration of R available to react with X, whose concentration is constant (Fig. 3), is lower than in the absence of nitroxide. Indeed, the concentration of R produced by T_1–T_1 or T_1–S_0 interactions is lowered and R also reacts with the nitroxide. To explain the higher photostability of eosin in the presence of nitroxide, it appears necessary to admit that the deactivation of X by P leads to ground state eosin. Similar conclusions were proposed for various reducing and oxidizing substances on the basis of xanthene photobleaching [21 - 23].

The following scheme summarizes the possible deactivation pathways of the triplet state and of the radical species of eosin dianion in the presence of the nitroxide $R_2NO^{\bullet}$.

$$T_1 + T_1 \longrightarrow T_1 + S_0 \quad (2)$$

$$T_1 + S_0 \longrightarrow 2S_0 \quad (3)$$

$$T_1 + T_1 \longrightarrow X + R \quad (4)$$

$$T_1 + S_0 \longrightarrow X + R \quad (5)$$

$$T_1 + R_2NO^{\bullet} \longrightarrow S_0 + R_2NO^{\bullet} \quad (6)$$

$$T_1 + R_2NO^{\bullet} \longrightarrow X + P \quad (7)$$

$$X + R \longrightarrow 2S_0 \quad (8)$$

$$R + R_2NO^{\bullet} \longrightarrow S_0 + P \quad (9)$$

The product P of nitroxide reaction was not identified. However, it has been shown [24] that nitroxide paramagnetism loss, induced by flavin irradiation, can result both from one-electron reduction leading to hydroxylamine (R_2NO^-) and from paramagnetism loss that cannot be reversed by ferricyanide oxidation. The other nitroxide (carbamoyl) used in this work led to the same sequence of reactions. Indeed, in basic water, the effects of this nitroxide on the absorbance and lifetime of eosin transients are qualitatively identical. Experiments were not conducted in micellar media because of the partition of the paramagnetic probe between the different microphases.

4.2. Flash photolysis in micellar solutions

It appears from the above discussion that results obtained in water were difficult to analyse because of the simultaneous presence of T_1, X and R and the existence of a back reaction of X. In a recent report [15], we have shown that these difficulties can be avoided by the use of micellar media. Indeed, radical species are not produced in cationic CTAB micelles. Consequently, the triplet lifetime is longer in these micelles than in water. Moreover, eosin is not photobleached even after several flashes in CTAB solution. We have also demonstrated that eosin dianion interacts strongly with cationic micelles. The dye is probably located at the micelle mantle [25]. We have reported that semi-oxidized eosin interacts with SDS micelles. Its half-time is longer in surfactant solutions than in water. All the results are gathered in Table 1, together with data for the behaviour of the eosin triplet state in reverse AOT–*n*-heptane micelles. In these non-polar surfactant solutions radical species are not produced and even after several flashes eosin is not bleached. It can be concluded that eosin is solubilized in the aggregates, probably in the aqueous core of the micelles, preventing dye–dye interactions.

In CTAB surfactant solutions, the absorbance characteristic of X appears in the presence of PADS and grows on increasing the nitroxide

concentration (Fig. 3). This observation confirms unambiguously that deactivation of T_1 by nitroxide can occur via electron transfer. However, the half-life of X decreases on increasing the concentration of Fremy's salt and eosin is not photobleached even after several flashes. This shows clearly that deactivation of X returns the eosin dianion to its ground state.

In SDS micelles, the results are identical with those obtained in water except for the increase in maximum absorbance of X *vs.* PADS concentration (Fig. 3). This is a consequence of the protection of X in this medium and supports the operation of mechanism (7).

4.3. EPR experiments

From column 4 of Table 1 it can be concluded that Fremy's salt is free in aqueous and SDS solutions and is partially immobilized in CTAB micelles. This can be understood on the basis of electrostatic attraction between cationic CTAB surfactant and dianionic PADS.

The other results can be interpreted using the reaction scheme proposed above. The complex kinetics of paramagnetism decay induced by stationary irradiation of eosin in aqueous and SDS solutions are the consequence of the two elementary processes (7) and (9). At very low dye concentrations, the semireduced form is unlikely to be produced by triplet–singlet interaction (5) and only process (7) can lead to the destruction of the spin probe. Indeed, paramagnetism decay curves become more and more exponential as the dye concentration is lowered (Fig. 1(a)). In CTAB micelles, radical species are not formed. Consequently, only one nitroxide reduction, pathway (7), can take place. The EPR curve (Fig. 1(b)) confirms the operation of one process for the reduction of Fremy's salt. In inverted micelles of AOT–*n*-heptane, the monoexponential PADS decay curve (Fig. 1(b)) is also the consequence of dye partitioning which supresses dye–dye interaction and the production of dye radical species. In CTAB and AOT–*n*-heptane surfactant solutions, the fact that the PADS decay curves are monoexponential rules out the possibility either that X can react with nitroxide by electron transfer or that nitroxide can be regenerated by reaction between X and P.

In conclusion, we have shown that the quenching of eosin triplet state by nitroxide is the result of photochemical and photophysical processes. The semireduced form of the dye can also reduce the nitroxide. The occurrence of such reactions is supported by the results of EPR and flash photolysis experiments. Finally, the use of micellar solutions demonstrated unambiguously that these reactions take place.

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