Short Communication

Effects of micellar adsorption on the photosensitizing properties of xanthene dyes

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The quantum yields of singlet oxygen production of dichlorofluorescein, eosin, phloxin and rose bengal have been measured in micellar solutions. Comparison of these quantum yields with the values reported for homogeneous solutions indicates that when the xanthene dyes, except eosin, are adsorbed on the cationic micellar aggregates they experience a microenvironment similar to that in ethanol. Photoexcited rose bengal produces singlet oxygen in these micellar solutions with the same high efficiency (about 0.8) as in water and ethanol.

1. Introduction

The ability of many halogenated fluorescein derivatives to act as photosensitizers for oxygenation of a wide variety of compounds has been recognized for a long time [1]. The magnitude of the spin–orbit coupling between singlet and triplet states and the long triplet lifetime explain their high photodynamic efficiency [2, 3]. Besides the intramolecular heavy atom effect, others factors, such as the physicochemical solvent properties, influence the deactivation processes of the photoexcited xanthene dyes. The photophysical properties of these chromophores have been shown to be critically dependent on the hydrogen-bonding capacity of their environment [4 - 6]. Several manifestations of this effect, such as a drastic increase in fluorescence lifetime [5, 6], an enhancement of the fluorescence quantum yield [4] and a decrease in the intersystem crossing quantum yield [7, 8], have been observed in going from protic to aprotic homogeneous media.

More recently, xanthene derivatives solubilized in microheterogeneous solutions have been used to produce singlet oxygen [9]. The formation of singlet oxygen confirmed the high efficiency of the energy transfer from the metastable state of the dye to molecular oxygen in these media, in agreement with the remarkable diffusion properties of molecular oxygen [10, 11].

The aim of this work was to determine the quantum yield of singlet oxygen production of several fluorescein derivatives solubilized in aqueous

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surfactant solutions. Previous quantitative studies have been restricted to the xanthene singlet excited states [12 - 14]. Singlet oxygen produced by stationary irradiation of the dyes was detected by indirect chemical methods. When the monitoring compound 1,3-diphenylisobenzofuran is located in micellar aggregates it exhibits a high reactivity towards singlet oxygen, as in homogeneous organic solvents [15, 16]. The dianionic forms of dichlorofluorescein, eosin, phloxin and rose bengal were studied in an aqueous dispersion of cationic cetyltrimethylammonium bromide (CTAB) and in an aqueous dispersion of anionic sodium dodecylsulphate (SDS).

2. *Experimental details*

2.1. *Chemicals*

The dyes employed were 2′,7′-dichlorofluorescein ($FlCl_2$), tetrabromofluorescein or eosin Y ($FlBr_4$) and tetrachlorotetrabromofluorescein or phloxin B ($FlBr_4Cl_4$) from Merck; tetrachlorotetraiodofluorescein or rose bengal (FlI_4Cl_4) was obtained from Eastman Kodak Co.

All these dyes were purified prior to use [17]. CTAB was purchased from Sigma Chemical Company, SDS from Janssen Chimica, 1,3-diphenylisobenzofuran (DPBF) from Aldrich Chemical Co. and (4-^{14}C)-cholesterol from Amersham Belgium.

The concentrations of the sensitizer and DPBF in aqueous micellar SDS (0.1 M) or CTAB (0.1 M) were always kept below 10^{-5} M and 2.5×10^{-5} M respectively. All experiments were carried out at ambient temperature (20 - 23 °C).

2.2. *Irradiation*

Surfactant-containing solutions were excited in contact with air and were mixed manually after each successive polychromatic irradiation. The solutions (about 2.5 cm^3) were contained in a quartz cell (1 cm × 1 cm). Exponential curve fitting (least squares) was used to determine the slopes of the DPBF decays. These values, when corrected for absorption, give a relative measure of the DPBF oxidation, Φ(DPBF). The relative number of photons absorbed by the dyes was determined by a method described by Gandin and Lion [18]. The relative uncertainty of the quantum yields is estimated to be less than 7%.

Previous studies [16, 19] have indicated that Φ(DPBF) can be used as a measure of the relative values of the quantum yields $\Phi(^1O_2)$ of singlet oxygen production. The reaction of singlet oxygen with cholesterol solubilized in the micelles was monitored by the method described by Decuyper *et al.* [20]. Irradiation of these solutions was performed with a krypton laser (Innova 90K, Coherent).

3. *Results and discussion*

Solubilization of the xanthene dyes in the micellar solutions leads generally to a red shift of the visible absorption peak with respect to neat neutral water (fifth and sixth columns of Table 1); $FlCl_2$ and $FlBr_4$ in SDS

TABLE 1

Quantum yields Φ(DPBF) of DPBF oxidation photosensitized by xanthene derivatives in micellar solutions of CTAB and SDS, the ratio r between Φ(DPBF) for the same dye solubilized in SDS and CTAB solutions, the red shifts Δλ of the visible absorption peak of the photosensitizer and the quantum yields $\Phi(^1O_2)$ of singlet oxygen production in ethanol and water

Dyes	Φ(DPBF) *CTAB*	Φ(DPBF) *SDS*	r	Δλ (nm) *for* *CTAB–H_2O*	Δλ (nm) *for* *SDS–H_2O*	$\Phi(^1O_2)$[a] *in ethanol*	$\Phi(^1O_2)$[a] *in water*
Rose bengal	1	1.16	1.16	11.5	7.5	1	1
Phloxin	0.46	0.59	1.29	12.5	12.5	0.46	0.89
Eosin	0.18	0.82	4.5	9	0	0.37	0.79
$FlCl_2$	0.033	0.096	2.9	8.5	0	0.04	0.09

In the second, seventh and eighth columns, a value of unity was arbitrarily attributed to the highest quantum yield.
[a]From ref. 8.

are two exceptions. These absorption peak displacements, which are in good agreement with the values reported previously [9, 12], are evidence of the association of the dye with the micellar aggregates. The magnitude of the bathochromic effect indicates that the binding of the sensitizer is tighter in CTAB than in SDS. The favourable electrostatic interaction between the dianionic compounds and CTAB probably explains this behaviour. Hydrophobic interactions are also very important, as shown by the solubilization of negatively charged dyes (FlI_4Cl_4 and $FlBr_4Cl_4$) in anionic micellar SDS. Fluorescence lifetime data [12 - 14] suggest that the xanthene dyes are located at the hydrophilic mantle of the micelles. Moreover, the fluorescence decay profiles are mono-exponential, providing evidence that all the dye molecules experience the same microenvironment [13, 14].

The kinetics of the decay of DPBF photosensitized by the xanthene dyes were first order in each case, indicating that singlet oxygen is predominantly deactivated by the solvent under our experimental conditions.

Comparison between the relative magnitudes of $\Phi(^1O_2)$ determined in CTAB and in homogeneous solvents (water and ethanol) [8] reveals good agreement between the values in the micellar and alcoholic solutions for $FlCl_2$, FlI_4 and FlI_4Cl_4 (second and seventh columns of Table 1). Consequently, these dyes seem to have the same photophysical properties in both CTAB and ethanolic solutions. This result agrees with the picosecond fluorescence studies of rose bengal in CTAB micellar dispersions [12, 13]. Fluorescence data indicated that rose bengal molecules are located in an environment which has a hydrogen-bonding capacity similar to that of ethanol.

In the case of eosin, the results show that $\Phi(^1O_2)$ is dramatically reduced in the micellar medium. In order to check this point, we performed further photosensitization experiments using a different singlet oxygen trap, the cholesterol. Singlet oxygen oxidation of this compound leads to a specific product, the 3β-hydroxy-5α-cholest-6-ene-5-hydroperoxide (5α chol). The ratio between the quantum yields of 5α chol formation following irradiation of $FlBr_4$ and FlI_4Cl_4 is 0.19. The good agreement between this value and the data given in the Table 1 confirms, on the one hand, the peculiar behaviour of $FlBr_4$ in cationic micellar CTAB and, on the other hand, the specificity of DPBF towards $^1O_2$ in our solutions. On the assumption that the hydrogen-bonding nature of the medium affects the photophysical properties of xanthene in micellar media in much the same way as has been inferred for neat liquids [4 - 6], the data presented above show that eosin in micellar CTAB solutions experiences a microenvironment which has a markedly different protonicity from that probed by the other xanthene dyes. This suggests that eosin is more buried in the CTAB micelle than the other fluorescein derivatives.

The $\Phi(^1O_2)$ values of FlI_4Cl_4, $FlBr_4$ and $FlCl_2$ in SDS micellar solutions are in good agreement with the values previously reported in neat neutral water (third and eighth columns of Table 1). As the absorption measurements show that for both $FlBr_4$ and $FlCl_2$ the peak is at the same wavelength

in neat neutral water as in the SDS system, it is inferred that the absolute $\Phi(^1O_2)$ of these dyes are the same in the two media, *i.e.* 0.07 and 0.57 respectively [7, 8]. Consequently, the absolute $\Phi(^1O_2)$ of FlI_4Cl_4 in SDS is also probably close to the value reported in neutral water (0.75).

The $\Phi(^1O_2)$ for phloxin in SDS is closer to the value found in ethanol. This behaviour of $FlBr_4Cl_4$ can be clarified by comparison of the Φ(DPBF) in the two dispersions. Lindig and Rodgers [19] claimed that the quenching of singlet oxygen by DPBF is 1.5 times larger in SDS than in CTAB. These researchers attribute the difference in DPBF reactivity to various environmental influences of the micelles. Consequently, the ratio r between the Φ(DPBF) in SDS and in CTAB must be close to 1.5 for a sensitizer possessing the same $\Phi(^1O_2)$ in the two dispersions. In the case of phloxin, the r value of 1.29 (fourth column of Table 1) suggests that the properties of the dye's surroundings are identical in the two micellar aggregates. This is confirmed by the visible absorption spectrum of the compound, which shows a maximum at 551 nm in both SDS and CTAB solutions.

The r value of rose bengal (1.16) is also very close to 1.5, indicating that the $^1O_2$ production is almost insensitive to the nature of the surfactant molecules. From the foregoing conclusions, it follows that $\Phi(^1O_2)$ for rose bengal is close to 0.8 whether the medium is homogeneous (water and ethanol) or heterogeneous (SDS and CTAB solutions). The high r values determined for the sensitizers $FlCl_2$ and $FlBr_4$ show that the $\Phi(^1O_2)$ of these dyes are strongly modified by their micellar adsorption. In both cases, hydrophobic and electrostatic interactions lead to a drastic decrease in $\Phi(^1O_2)$, *i.e.* to a reduction in the rate of intersystem crossing. The magnitude of this effect seems to depend on the nature and the number of the heavy atoms introduced into the chromophore. Laser flash photolysis of eosin and its complex with lysozyme has previously shown that the binding of the dye in a hydrophobic location leads to a marked reduction in both the quantum yield and the lifetime of the eosin triplet state [21].

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