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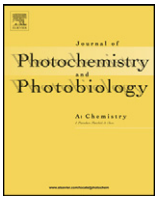

Organized media effect on the photochemical deoxygenation of resazurin in the presence of triethanolamine

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ABSTRACT

The photodeoxygenation of the synthetic dye resazurin in the presence of triethanolamine was investigated in water, CTAC and SDS direct micelles, AOT and BHDC reverse micelles, and soybean lecithin microemulsions (LEC). In all cases the only product observed was the deoxygenated dye resorufin. Triplet and reaction quantum yields were determined in all media. The photoreaction proceeds more efficiently in the microheterogenous systems with positive interface, CTAC and BHDC, while the lower yield is observed in AOT, SDS and LEC. The initial step in the mechanism is the interaction of the triplet state of the dye with the amine, and the effect of the interface is interpreted by a decrease of the recombination rate of the radicals formed in the initial electron transfer step. Negative and zwitterionic interfaces have no effect on the quantum yield.

1. Introduction

In the last years there has been an increasing interest in the study of different photoprocesses occurring in various organized microheterogeneous environments like micelles, reverse micelles, cyclodextrins, liposomes, proteins, etc., because of their numerous applications [1]. Nanometer-sized cavities and cages can be generated in these systems which can serve as “nanoreactors” in which photophysical and/or photochemical reactions can take place, differing significantly from analogous reactions in a homogeneous solution [2]. Synthetic dyes have been frequently employed to characterise organized systems. Owing to electrostatic and/or hydrophobic interactions, organic dyes can be incorporated to the less polar regions of the system, and significant changes occur in their absorption and emission properties and in their photochemical behaviour. [3,4]

Resazurin (RZ, Scheme 1) is a heterocyclic N-oxide dye that is often used to study biological materials [5–8]. The ‘resazurin reduction test’ has been employed for more than 50 years to monitor bacterial and yeast contamination of milk [9], for assessing semen quality [5,10] and for measuring cell proliferation and cytotoxicity [11]. Resazurin, also known as Alamar Blue, is blue and scarce fluorescent, and when used in biological tests it is reduced to resorufin (RF) (pink and highly fluorescent) which is further reduced to hydroresorufin (uncoloured and nonfluorescent). It is still not known how this reduction occurs, intracellularly via enzyme activity or in the medium as a chemical reaction, although the reduced fluorescent form of Alamar Blue was found in the cytoplasm of living cells and nucleus of dead cells. Most of these applications are based on the oxygen atom transfer reaction with the dye as donor. In this way RZ is reduced to RF, which can be used as a target fluorescent probe. The process may be induced by a thermal reaction using organic compounds or enzymes as catalysts [7,12,13] or by light [14].

We have demonstrated that irradiation of RZ at 600 nm in the presence of aliphatic amines leads to deoxygenation of the N-oxide group giving RF [15]. The detailed study of these photoreactions was of interest in our group, in particular the interactions of the excited states of both dyes RZ and RF with amines [16] and aminoacids [17]. It was proved that the photodeoxygenation of RZ occurs from the triplet state of the dye and the efficiency is highly dependent on the amine structure. It is effective only in the presence of tertiary aliphatic amines. This structural effect was ascribed to the redox potential of the amines. The mechanism most likely involves, as an initial step, and electron transfer reaction from the amine to the triplet of the dye [16]. The redox potential of aliphatic amines increases from tertiary to secondary to primary amines [18], making the reaction highly inefficient for primary and secondary amines. This effect of the amine structure on the photoreduction of N-oxides heterocycles was also reported many years ago by Pietra et al. [19] in a study of the photodeoxygenation of 2-nitrophenaxine-10- oxide, where it was found that the reaction occurs quite efficiently in the presence of triethylamine and is much less efficient for other amines. Free radicals are produced during the

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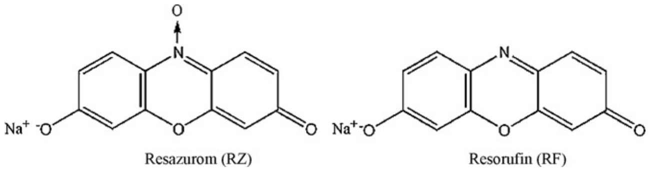

Scheme 1.

photoreduction by amines, making the system RZ-tertiary amine an efficient photoinitiator for vinyl polymerization [20]. Photoreduction was also observed in the presence of aliphatic aminoacids. Although aminoacids quench both, the excited singlet and triplet of RZ, the photoreduction takes place only with aminoacids containing the –SH group [17].

The photophysics of RZ and RF in homogeneous aqueous solution, in direct and reverse micellar solutions and in LEC microemulsions was previously investigated by us [21,22]. The absorption and emission characteristics of the dyes are strongly affected by the medium and the results depend on the charge and type of the interface. In addition, the absorption and fluorescence properties of these dyes are dependent on pH. In particular RF has been used as a probe molecule to study the reorientation of solvent molecules, and has shown interesting spectroscopic properties in protic solvents that strongly depend on temperature, viscosity and structure of the solvent [23].

Due to importance of the use of RZ in biological materials and its photoreduction by biological substrates, it is of interest to investigate the effect of organized media on the photoprocesses of the dye. Here we present a mechanistic study of the photoreduction of RZ by triethanolamine (TEOA) in microheterogeneous systems, comprising direct and reverse micelles and lecithin microemulsions. Quantum yields were determined and the results are discussed in terms of the effect of the medium on the photophysics of the dye and the localization of the amine in the different regions of the organized systems.

2. Experimental

2.1. Materials

The dyes resazurin (RZ), resorufin (RF) and methylene blue (MB) were from Sigma and were used as supplied. Sodium 1,4-bis (2-ethylhexyl) sulfosuccinate (AOT) from Sigma was dried under vacuum over P_2O_5. The surfactant benzylhexadecyldimethylammonium chloride (BHDC) from Fluka was twice recrystallized from ethyl acetate and dried under vacuum. The amines triethanolamine (TEOA) (Aldrich) was purified by vacuum distillation before use. Cetyltrimethylammonium chloride (CTAC) (Kodak) and SDS (Aldrich) were purified by recrystallization. Soybean Lecithin (LEC, Epikuron 200) was obtained from Lucas Meyer and was used without further purification. It has a distribution of fatty acids with a major contribution of $C_{18:2}$. LEC concentrations were estimated using 770 for the average molecular weight [24]. 9,10-Dimethylanthracene (DMA) was from Aldrich and it was also used without further purification. All the orbanic solvents: methanol, benzene, 1-propanol, isooctane and heptane (Sintorgan HPLC grade) were used as received. Water was purified through a Millipore Milli-Q system. The pH was adjusted at pH = 8.5 by the incorporation of a concentrated NaOH solution.

2.2. Measurements

Reverse micelles solutions were prepared with AOT 0.2 M in n-heptane and BHDC 0.1 M in benzene. LEC microemulsions containing the dyes were prepared by the addition of a small amount of the dyes dissolved in propanol to a 0.05 M of soybean lecithin in isooctane-10% 1-propanol solution.

Absorption spectra were determined on a Hewlett Packard 6453E diode array spectrophotometer. Static fluorescence determinations were carried out at room temperature in air equilibrated solutions with a Spex Fluoromax spectrofluorometer. Fluorescence quantum yields were determined from the area under the corrected spectrum, relative to that of cresyl violet in methanol as a standard [25]. Fluorescence lifetime measurements were performed with an OB 900 Edinburgh Instruments fluorometer using the time-correlated-single-photon-counting (TCSPC) technique. All measurements were carried out at $20 \pm 1\,^{\circ}\mathrm{C}$.

Transient absorption measurements were made using a laser flash photolysis equipment previously described [26]. Measurements were performed in samples subjected to a continuous bubbling with high purity argon. Quantum yields of triplet species (Φ_T) were determined using zinc tetraphenylporphyrin (ZnTPP) triplet state as a reference actinometer. Values of $7.3 \times 10^4\,\mathrm{M^{-1}\,cm^{-1}}$ and 0.83 were used for the absorption coefficient and quantum yield of ZnTPP triplet state, respectively [27]. The molar absorption coefficients of triplet dyes were determined by the ground state depletion technique [28]. For both dyes, the negative absorption of the difference transient spectra matched the ground-state band. This is consistent with the lack of photoproduct formation under our conditions of laser experiments, and allows the application of the ground state depletion method to determine the molar absorption coefficients of the triplet state.

The continuous photolysis experiments were carried out with a 150 W Xe lamp coupled to a grating monochromator (Photon Technology International). Irradiation wavelength was 620 nm in all cases. It was chosen with the compromise of overlapping the absorption spectrum of the sample with that of the actinometer and to avoid absorption by the photoproduct. RZ solutions were deoxygenated by argon bubbling. The singlet oxygen mediated photooxidation of dimethyl anthracene (DMA), sensitized by methylene blue (MB) was used as actinometer. Air equilibrated methanolic solutions of MB, with matched absorbances for RZ solutions in the different media, were irradiated at 620 nm in the presence of DMA. The consumption of DMA was measured by the absorption at 397 nm. Quantum yields for the photoreduction of RZ, Φ_R, were determined relative to the photobleaching of DMA in methanol sensitized by MB, Φ_{DMA}, according to:

$$\frac{S_{(RZ)}}{S_{(DMA)}} = \frac{\Phi_R}{\Phi_{DMA}} \tag{1}$$

where $S_{(RZ)}$ and $S_{(DMA)}$ are the initial slopes of the plots of [DMA] and [RZ] vs. time respectively. S_{RZ} was measured at the absorption maximum of RZ in each medium. The quantum yield for DMA photobleaching was calculated by

$$\Phi_{DMA} = \Phi_{\Delta} \frac{k_r[\mathrm{DMA}]_0}{(k_r + k_Q)[\mathrm{DMA}]_0 + k_d} \tag{2}$$

where $[\mathrm{DMA}]_0$ is the initial concentration of DMA (1.2×10^{-4} M), Φ_{Δ} is the singlet oxygen quantum yield of MB (0.50) [29], k_r and k_Q are the reactive and physical quenching rate constant for the interaction of DMA with singlet oxygen, 6.3×10^7 and $5 \times 10^6\,\mathrm{M^{-1}\,s^{-1}}$ respectively, in methanol [30]. k_d is the unimolecular decay rate constant of singlet oxygen in methanol ($1 \times 10^5\,\mathrm{s^{-1}}$) [30]. In this way $\Phi_{DMA} = 0.036$ which is of the same order than Φ_R.

3. Results and discussion

In air equilibrated solutions RZ is stable upon irradiation, even in the presence of amines. When oxygen free solutions of RZ are irradiated in its visible band in the presence of tertiary aliphatic amines, a photobleaching of the dye takes place with the concomitant