Dyes and Pigments 99 (2013) 705–712

Contents lists available at SciVerse ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

Photophysical properties of erythrosin ester derivatives in ionic and non-ionic micelles

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ARTICLE INFO

Article history:
Received 4 January 2013
Received in revised form
20 April 2013
Accepted 14 June 2013
Available online 29 June 2013

Keywords:
Erythrosin
Xanthene
Micelle
Photodynamic
Formulation
Pluronic

ABSTRACT

The xanthene dye erythrosin B (ERY) has high molar absorptivity and singlet oxygen quantum yield ($\Phi_\Delta{}^1O_2$), which make it a potential photosensitizer for photodynamic applications. However, ERY is very hydrophilic in water at physiological pH due to its two negative charges, which results in low interaction with biological membranes. In this work we synthesized ester derivatives of ERY: methyl (ERYMET), butyl (ERYBUT) and decyl (ERYDEC) esters. The light absorption process was not greatly affected by the insertion of the alkyl group. The ester hydrophilic–lipophilic balance was evaluated by partition in a 1-octanol/water mixture. The insertion of the alkyl chain and the loss of one charge increased the hydrophobicity and, as expected, ERYDEC was the most hydrophobic dye. The interaction of the esters with the biological membranes was simulated in biomimetic models, micelles of: CTAB (cationic), SDS (anionic) and the polymeric surfactant pluronic F-127® and P-123® (non-charged). The order of the binding constants was ERY < ERYMET < ERYBUT < ERYDEC in all micelles investigated, equal to the hydrophobicity sequence. The localization of the dyes in micelles determined by quenching fluorescence measurements using iodide as a suppressor was consistent with their charge and hydrophobic characteristics. CTAB micelles showed a biphasic interaction due to the association of the cationic monomers of the surfactant and the anionic dyes. The fluorescence quantum yields of the esters in buffered water were equal to those of ERY, with low values of ~0.02 and a slight increase to ~0.1 in micellar media, the value of which was still small. The quantum yields of singlet oxygen of all xanthenes investigated were high in micellar systems and similar to each other, independent of the alkyl group (0.6–0.7). ERYDEC showed low $\Phi_\Delta{}^1O_2$ of 0.38 in buffered water due to the self-aggregation process, but high values in micellar systems. These results in micelles are very important for formulated medications in photodynamic therapy and photodynamic inactivation of microorganisms. Ester derivatives of erythrosin show a potential as photosensitizers in photodynamic clinical applications, especially those formulated with biocompatible surfactants, such as polymeric pluronics.

1. Introduction

In situ activation of a photosensitizer by visible light in the presence of oxygen is the basic principle of two phototherapeutic approaches, photodynamic therapy (PDT) and photodynamic inactivation of microorganisms (PDIMO). The luminous energy absorbed by the photosensitizer is transferred to oxygen, producing highly reactive cytotoxic species [1–3].

Once excited, photosensitizers (PS) can cross from the excited singlet state ($^1PS^*$) to the triplet state ($^3PS^*$) with a lifetime long enough to react with oxygen [4]. The singlet oxygen is acknowledged to be the main photodynamic agent that can lead to apoptosis and tissue necrosis [5,6]. Two requirements are that the compound exhibits high visible light absorption and high singlet oxygen quantum yield ($\Phi_\Delta{}^1O_2$). However, the successful incorporation of a photosensitizer into target cells is another essential requirement.

One of recognized preferential sites of photodynamic action is biological membranes. Therefore, the evaluation of the photosensitizer-membrane interaction is fundamental [7] and it depends on the hydrophobic and electrostatic characteristics of the photosensitizer and the membrane. Since studies on biomembranes are difficult to conduct due to the biological complexity of cells, the use of biomimetic systems, such as micelles that emulate cell membranes, becomes interesting [8].

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0143-7208/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.dyepig.2013.06.026

The hydrophilic–lipophilic balance (HLB), usually estimated by the partition coefficient of photosensitizers in a water/1-octanol mixture [9], furnishes initial information. Aqueous micelle systems allow the investigation of molecule permeation and interaction in organized systems [10]. Ionic micelles, such as SDS (anionic) and CTAB (cationic), are valuable in the study of charge effects on the interaction with a membrane model, while polymeric micelles constituted by hydrophilic polyethylene oxide (PEO) and hydrophobic polypropylene oxide (PPO) forming $(PEO)_x(PPO)_y(PEO)_x$ tri-block micelles, known as Pluronics®, are useful in the investigation of non-electrostatic interactions (non-charged surfactant). In addition to being stable and non-toxic, tri-block micelles provide several sites for the solubilization of hydrophobic drugs and are less affected by biological fluid environments [12]. High amounts of SDS and CTAB are toxic to human beings [11], while Pluronics® can be used as a drug delivery system due to its biocompatibility.

In the present work, xanthene, a dye class, was investigated as a photosensitizer. It has many important applications, for example, as pH-dependent fluorescent probes [13]. The xanthenic and benzoic rings of these molecules are approximately orthogonal to each other, which makes each of the rings almost independent from each other [14]. In light absorption, the excited electron is confined in the xanthene ring (chromophoric part of the molecule), thus all subsequent photochemical and photophysical processes are due to the xanthenic portion [15]. Erythrosin B (Acid Red 51 and C.I. 45430; ERY in Fig. 1), an halogenated xanthene, exhibits high molar absorptivity, $\varepsilon_{532nm} = 96.6 \times 10^3$ L mol^{-1} cm^{-1} [16] and high formation of long-lived triplet states, consequently a high production of $^1O_2$ at physiological pH in water, which corresponds to its dianionic protolytic form [16–18]. Erythrosin B has been successfully tested as a photosensitizer in dental plaque treatment *via* PDIMO [19,20].

Despite its excellent photodynamic properties, ERY is highly hydrophilic thereby has low affinity to cell membranes. The addition of an alkyl substituent to the carboxylate group leads to the formation of esters and increases its hydrophobicity due to the presence of the substituent and the decrease of the charge. In the present work, xanthene esters of ERY, namely: Erythrosin methyl ester (ERYMET), erythrosin butyl ester (ERYBUT) and erythrosin decyl ester (ERYDEC) (Fig. 1) were synthesized and their photophysical properties were evaluated in aqueous micellar systems of SDS, CTAB, and the Pluronics F-127® and P-123® in order to investigate their potential as drug candidates in PDT and PDIMO.

2. Materials and methods

2.1. Materials

All solvents employed were of analytical grade and used without further purification. Erythrosin B (ERY, Vetec) was analyzed and identified by ^{1}H NMR. Erythrosin ester derivatives were synthesized according to the procedure described in the literature [21] and analyzed and identified by H^1 (300 MHz) and ^{13}C (75 MHz) NMR in a Varian Mercury Plus BB spectrometer in DMSO-d_6 (Merck) and by FT-IR spectroscopy performed in a FT-IR Thermo Nicolet Model Nexus 470.

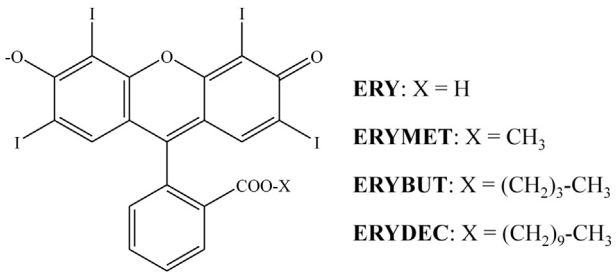

Fig. 1. Erythrosin B structure and its ester derivatives.

SDS ($C_{12}H_{25}O_4SNa$, MM = 288.4 g mol^{-1}), CTAB ($C_{19}H_{42}NBr$, MM = 364.5 g mol^{-1}), F-127 ($(PEO)_{100}(PPO)_{70}(PEO)_{100}$, MM = 12,600 g mol^{-1}), and P-123 ($(PEO)_{19}(PPO)_{69}(PEO)_{19}$, MM = 5750 g mol^{-1}) were purchased from Sigma–Aldrich and the solutions were prepared by weighing materials previously desiccated under vacuum for 24 h. For experiments at fixed surfactant concentration, the following amounts were employed [SDS] = 5.00×10^{-3} mol L^{-1}, [CTAB] = 4.00×10^{-3} mol L^{-1}, [P-123] = 1.73×10^{-3} mol L^{-1}, and [F-127] = 1.59×10^{-3} mol L^{-1}.

Fresh dye stock solutions were prepared in DMSO and standardized by UV–Vis spectrophotometry. All other analysis were performed by UV–Vis or by fluorescence, respectively, spectrophotometer Beckman Coulter DU 800 and Cary-Eclipse spectrofluorimeter. For experiments monitored by absorbance, the dye concentrations were 5.0×10^{-6} mol L^{-1}, and for fluorescence measurements, 5.0×10^{-7} mol L^{-1}. All experiments were conducted in aqueous solutions with pH 7.25 controlled by buffer (McIlvaine, [Na_2HPO_4] = [citric acid] = 7.5×10^{-3} mol L^{-1}) and constant ionic strength controlled by NaCl addition (0.10 mol L^{-1}) at 30.0 °C.

2.2. Methods

2.2.1. Partition coefficient (K_p)

The dyes were added to a 50% (v/v) mixture of 1-octanol/water. After vigorous stirring and resting in the dark for 48 h, the dye concentrations in the aqueous ($[PS]_{water}$) and organic ($[PS]_{oct}$) phases were determined by UV–Vis. K_p was determined from Equation (1).

$$K_P = \frac{[PS]_{oct}}{[PS]_{water}} \quad (1)$$

2.2.2. Binding constant (K_b)

The binding constant (K_b) of the dyes (5.0×10^{-7} mol L^{-1}) to micelles were evaluated by titrimetry, monitored by fluorescence. After aliquots of the concentrated stock solutions of the surfactants were added and vigorously agitated, the fluorescence spectrum was registered. The fluorescence titrimetric data were fitted using Equation (2) [22].

$$F = F_f + \frac{\left(F_0 - F_f\right)}{\left(1/K_b([S] - CMC)^N\right) + 1} \quad (2)$$

where F: fluorescence intensity; F_f: fluorescence of the surfactant-bound PS; F_0: fluorescence in the absence of surfactant; [S]: surfactant concentration; N: number of surfactant molecules per PS molecules; and CMC: critical micellar concentration.

2.2.3. Fluorescence quenching

The fluorescence quenching experiments were performed with iodide ion as a quencher. The amounts of NaI stock solutions were 1.00 mol L^{-1} for water, SDS, F-127 and P-123 solutions, and 0.10 mol L^{-1} for the CTAB system. The Stern–Volmer quenching constant (K_{sv}) was calculated from Equation (3).

$$\frac{F_0}{F} = 1 + K_{SV}\left[I^-\right] \quad (3)$$

where F_0 and F: fluorescence intensities in the absence and in the presence of the suppressor, respectively, and [I^-]: iodide concentration.