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Interaction of eosin and its ester derivatives with aqueous biomimetic micelles: Evaluation of photodynamic potentialities

Bianca Martins Estevão, Diogo Silva Pellosi*, Camila Fabiano de Freitas, Douglas Vanzin, Douglas Santana Franciscato, Wilker Caetano, Noboru Hioka

Departamento de Química, Universidade Estadual de Maringá, Av. Colombo, 5.790, CEP 87.020-900 Maringá, Paraná, Brazil

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ABSTRACT

In this paper, the physicochemical and photophysical properties of xanthene dyes interacting with micelles were evaluated. Xanthenes are potential photosensitizers for photodynamic therapy. The dyes were eosin Y and its ester derivatives with methyl, butyl and decyl alkyl groups.

In aqueous media at physiological pH eosin is present as dianionic protolytic form while its esters are monoanionic. Analysis of the water/octanol partition coefficient showed that the eosin is the most hydrophilic compound and the hydrophobicity increases as the alkyl chain of the ester increases: eosin < methyl ester < butyl ester < decyl ester. Computational calculations of the dipole moments, molecular volume, charge distribution, solvation energy and molecular orbital performed by the B3LYP/DGDZVP method and IEFPCM solvent model, allowed to justify the electronic absorption properties and its dependence with the dye environment. In order to mimic the interaction of these compounds with membranes, it was used micelles of sodium dodecyl sulfate (SDS), cetyl trimethylammonium bromide (CTAB) and polymeric micelles (Pluronics®) of P-123 and F-127. The binding constant between the xanthenes and micelles increased with the hydrophobicity of the dye in all evaluated systems. Furthermore, we observed the formation of pre-micellar aggregates when using cationic CTAB micelles due to electrostatic attraction. The iodide fluorescence quenching showed that the more hydrophobic photosensitizer were located deeply within the micelles. The fluorescence quantum yield values increased in the presence of surfactants, indicating protection against non-radiative deactivation. The dyes singlet oxygen quantum yield values suffered a small decrease when formulated in micelles, however they are still suitable for photodynamic applications. The results show that eosin and its ester derivatives have favorable characteristics for use in photodynamic therapy, particularly formulated in biocompatible polymeric surfactants such as Pluronics®.

1. Introduction

The Photodynamic Therapy (PDT) consists on the use of a photosensitizer (PS), oxygen and visible light involving photo-damage on undesirable cells. The PS locally or systemically administered is irradiated with adequate light that penetrates deeper into the biological tissue. The compound in excited singlet state (^{1}PS*) undergoes intersystem crossing to triplet state (^{3}PS*) followed by two possibilities: (i) it reacts directly with oxygen/biological molecules by electron transfer reaction leading to excited reactive oxygen species (EROS) – type I photochemical mechanism or (ii) it transfers energy to molecular oxygen ($^3O_2$) generating singlet oxygen ($^1O_2$) – type II photochemical mechanism [1,2]. EROS and $^1O_2$ are highly reactive species that cause necrosis and/or apoptosis in tissue where the $^1O_2$ is considered the main species involved in the photo-damage [3]. This technique has also been employing against microorganisms, which is known as Photodynamic Inactivation of Microorganism (PDIMO) [4–6]. Both PDT and PDIMO exhibit high selectivity due to drug localization and light focalization, which avoid health tissue's damage.

Among photosensitizers used in photodynamic treatments, we are interested in halogenated xanthene dyes. These compounds show several advantages such as high singlet oxygen yield, high light absorption in the range of 500–600 nm region (green light), low cost and low toxicity in the dark [7,8]. Xanthenes do not absorb in the phototherapeutic window (600–800 nm region, red light), however green light with xanthenes is applicable in skin and eye

* Corresponding author at: Av. Colombo, 5.790, CEP 87.020-900 Maringá, Paraná, Brazil. Tel.: +55 44 3011 3654; fax: +55 44 3261 4125.
E-mail address: diogo.pellosi@gmail.com (D.S. Pellosi).

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diseases [9] and in the microorganism control [6]. The elected xanthenes investigated in the present work are Eosin Y (**EOS**) and its ester derivatives.

EOS is a high soluble dye in water at physiological pH due its two negative charges (Fig. 1a) [7]. At this medium **EOS** exhibits a wavelength of maximum absorption (λ_{max}) at 517 nm with molar absorptivity (ε) of 96,600 mol cm^{-1} L^{-1}; its singlet oxygen quantum yield (ϕ_Δ $^1O_2$) is 0.57 [10]. The hydrophilic nature of **EOS** can be decreased introducing alkyl groups to the carboxylate leading to esters, which in water at physiological pH show only one negative charge. Therefore, we synthesized derivatives of Eosin Y, the methyl, butyl and decyl ester, named respectively as **EOSMET**, **EOSBUT** and **EOSDEC** (Fig. 1a). The charge decreased and the presence of the alkyl group turn these compounds very hydrophobic. Ideally, the interaction of the PS with biological membranes increases as the hydrophobicity increases. This property is very interesting as membranes are one of the most important targets of photosensitizers, especially in PDIMO [6]. However, hydrophobic PS has low water solubility leading it to self-aggregate. In self-aggregate state there is a decrease in $^1O_2$ yield due to self-collisions that deactivates the excited states of the PS. Hydrophobic PS should be formulated in a convenient aqueous drug delivery system to avoid its self-aggregation.

Aqueous micelles constitute efficient drug delivery systems [11–14]. Among surfactants applied in drug formulation, we highlight the amphiphilic polymeric molecules based on alkylene oxide monomers due to their biocompatibility, low toxicity, large binding sites and protection of the incorporated drugs against the immunologic biological system [15,16].

Beside their uses as drug delivery systems, micelles are very helpful to mimic biomembranes. The determination of the PS physical–chemical properties as a function of the micelle nature helps the understanding of the PS-biomembranes interaction. In this paper the interactions of photodynamic photosensitizers **EOS**, **EOSMET**, **EOSBUT** and **EOSDEC** (Fig. 1a) with ionic surfactant sodium dodecyl sulphate (SDS) and cetyl-trimethyl-ammonium bromide (CTAB) and the non-ionic polymeric P-123 and F-127 Pluronics® (Fig. 1b) are described and evaluated.

2. Materials and methods

2.1. Materials

All solvents employed were analytical grade and were used without further purification. SDS, CTAB, P-123 and F-127 were purchased from Sigma–Aldrich and the solutions were prepared by the weight of the previously dried materials in desiccators under low pressure for 24 h. Fresh stock xanthene solutions were prepared in dimethyl sulfoxide (DMSO, Mallinckrodt) and standardized by UV–vis spectrophotometry. The analysis was performed by UV–vis spectrophotometry with a Varian Cary-50 or by fluorescence spectrometry with a Varian Cary-Eclipse apparatus. All experiments were conducted at 30.0 °C in aqueous solutions at pH 7.25 maintained by a buffer (McIlvaine, [Na_2HPO_4] = [citric acid] = 7.5×10^{-3} mol L^{-1}) and the ionic strength controlled by NaCl addition (0.10 mol L^{-1}). For absorbance experiments/analysis the dye concentrations were 5.0×10^{-6} mol L^{-1} and for fluorescence measurements were 5.0×10^{-7} mol L^{-1} in which the absorbance is lower than 0.05 to avoid internal filter effect.

2.2. Methods

2.2.1. Synthesis of ester derivatives

The synthesis of the eosin ester derivatives followed methodology adapted from ref [17].

2.2.1.1. Synthesis of eosin methyl ester (*EOSMET*).

0.723 mmol of Eosin Y (Reagen) were dissolved in a solvent mixture composed of 10 mL of acetone (Merck) and 5 mL of DMF (Synth). 0.3 mmol of Na_2CO_3 (Synth) and 1.446 mmol of methyl iodide (Vetec) were added to the mixture, which was maintained under agitation for 24 h and protected from light at around 25 °C. After that, the acetone was rota-evaporated under low pressure and the remaining DMF was mixed with ethyl acetate (Merck) and distilled water in acid conditions. The biphasic mixture was separated using a phase separator funnel. The organic portion that contains the synthesized product was rota-evaporated under low pressure obtaining **EOSMET** as solid product.

2.2.1.2. Synthesis of eosin butyl ester (*EOSBUT*) and eosin decyl ester (*EOSDEC*).

For **EOSBUT** and **EOSDEC** synthesis, the previous procedures were employed, however the alkyl bromide was butyl-1-bromide (3.615 mmol, Aldrich) and decyl-1-bromide (1.446 mmol, Aldrich), respectively. The reactions were maintained under agitation and protected from light at 50 °C during 48 h.

2.2.2. Ester derivatives characterization

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz), DEPT (90 and 135), COSY and HSQC spectra were registered in a Varian Mercury Plus BB spectrometer. The samples were prepared in DMSO-d_6 (Merck) with TMS as reference. The FT-IR spectroscopy was performed in KBr using FT-IR Thermo Nicolet Model Nexus 470 apparatus.

2.2.3. Theoretical structural calculations of EOS and its ester derivatives

First of all the structural optimization of eosin compounds was performed using HF/STO-3G, with the scan job rotating some possible dihedral angles of the carboxylic group (**EOS**) and the carboxylate group (**EOSMET**), taking 18 steps of 20 degrees. Once the most stable structure was obtained, a more detailed optimization of **EOS** and its ester derivatives were performed in water and gas phases with B3LYP hybrid functional and DGDZVP basis set in combination with IEFPCM solvent polarizable continuum model. Frequency calculations confirmed the real minimum energy of all structures and it additionally provided the isotropic polarizability at 0.0 hartrees (static calculation) and the Gibbs Free Energy taking the sum of electronic and thermal free energies from the thermochemistry section. The molar volume calculation was carried out using the default Monte Carlo method adding the keyword "volume = tight" in the command line. All the calculations were carried out with Gaussian 09 package [18] at 298.15 K. The Electron Density from total SCF Density, mapped with ESP at 0.0004 isovalue, generates the Molecular Electrostatic Potential maps.

2.2.4. Partition coefficient (K_p)

The PS was added to a 50% (v/v) mixture of water/1-octanol under vigorous stirring. After 48 h resting in the dark, the dye concentrations in the aqueous ($[PS]_{water}$) and organic ($[PS]_{oct}$) phases were determined. The K_p was obtained by Eq. (1) as Log K_P.

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

2.2.5. Binding constant (K_b)

Aqueous dye solutions were titrated with surfactant while monitored by fluorescence spectroscopy. Aliquot of the surfactant from concentrated stock solution was added and shaken, followed by spectrum acquisition. The titration data was fitted through Eq. (2) [19].

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