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Effect of temperature on the photobehavior of Rose Bengal associated with dipalmitoylphosphatidyl choline liposomes

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ABSTRACT

The association and photobehavior of Rose Bengal (RB) in the presence of dipalmitoylphosphatidyl choline (DPPC) small unilamellar liposomes is determined by the temperature. At temperatures above the main phase transition of the bilayer, the incorporation of the dye is ca. 2.5 times more efficient than that taking place when the bilayer is in the gel state. In both temperature ranges, adsorption isotherms show a noticeable anti-cooperativity that can be related to electrostatic repulsion between bound molecules. The photophysics and the photochemistry of the bound dye molecules also depend on the bilayer status. In particular, in the liquid crystalline state the surrounding of the dye is more polar and production of singlet oxygen is less efficient ($\Phi \sim 0.1$). This reduced singlet oxygen production is partially due to a low triplet yield ($\Phi_T = 0.35$) and triplet self-quenching due to a high local RB concentration. In spite of these, tryptophan is efficiently photobleached when RB is associated to liposomes in the liquid crystalline state, probably due to a Type I mechanism favored by its high local concentration in the sensitized surroundings.

1. Introduction

Dyes are extensively employed in photodynamic therapy [1–3]. The efficiency of this procedure, aimed to the selective killing of undesired cells, relies on the localization of the photosensitizer and its capacity to generate singlet oxygen, a reactive oxygen species considered as the main damaging agent [2]. It is relevant then to evaluate how the dye is distributed between aqueous environments and relevant biological targets, such as proteins, DNA, and membranes.

Rose Bengal (RB; Scheme 1) is frequently employed as a singlet oxygen source, mostly due to the high quantum yield of the process (ca. 0.76 in water) [4]. Its photophysics in homogeneous solvents and bound to proteins has been extensively studied [5]. In particular, several studies have been aimed to the evaluation of its binding to albumins and the photochemistry and photophysics of the bound molecules [6–10].

On the other hand, the binding of RB to membrane-like structures has been less investigated considerably [11,12]. As a first step to get an insight on the factors determining the association of the dye to biological membranes, we report in this communication the extent of RB binding to dipalmitoylphosphatidyl choline (DPPC) small unilamellar liposomes (SUVs) over a temperature range that covers the gel and liquid crystalline states. Furthermore, the microenvironment of the adsorbed dye and its capacity to generate singlet oxygen are briefly discussed.

2. Experimental section

2.1. Chemicals

Rose Bengal (RB), L-Tryptophan (Trp), dipalmitoyphosphatidylcholine (DPPC), deuterium oxide, glycerol, NaCl, $NaHPO_4$, and Na_2HPO_4 (> 99%) were Sigma–Aldrich products. Chloroform, HPLC quality, was obtained from Merck. Water employed was purified through a Milli-Q purification system. All the experiments were carried out in 100 mM phosphate buffer saline (PBS) pH 7.4 or in deuterated buffer, pD 7.4.

2.2. Small unilamellar liposomes (SUVs) preparation and characterization

SUVs were prepared by sonication of a suspension of multilamellar vesicles, according to the standard procedure [13]. Multilamellar liposomes were prepared by solvent (chloroform) evaporation of a DPPC solution, followed by re-suspension in PBS. The multilamellar-formed liposomes were sonicated (sonicator Mixomix 3000) employing 10 cycles (1 min on, 3 min off). After

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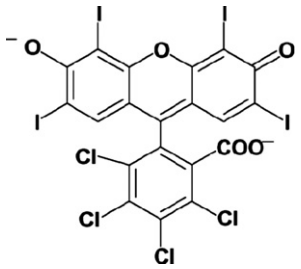

Scheme 1. Chemical structure of Rose Bengal (RB).

sonication, samples were centrifuged to eliminate titanium particles coming from the tip of the sonicator.

Lipid concentration in the SUVs suspensions was determined by titration with ammonium ferrothiocyanate, according to the procedure described by Marshall [14]. Finally, SUVs sizes were determined by dynamic light scattering in a Malvern nano-sizer S90 equipment. The mean diameter of the number-averaged distribution was 30 nm, with a mean width of ± 8 nm.

2.3. RB binding to SUVs

Addition of SUVs to a RB solution in PBS produces a red shift of the maximum of the visible absorption spectrum, initially located at 545 nm. The absorbance of the sample at 567 nm, measured as a function of the lipid concentration, was employed to quantify the amount of dye associated to the liposomal pseudophase [6–7,11,15]. The visible spectrum at high DPPC concentrations, where most of the dye is bound to the liposomes, was considered to estimate the microenvironment of bound RB molecules [7–8,16].

2.4. Tryptophan consumption photosensitized by RB molecules

The consumption of Trp sensitized by RB was evaluated in the absence and in the presence of SUVs. Solutions comprising RB (6 μM) and Trp (300 μM) were irradiated with visible light from a W-filament lamp at temperatures below (25 °C) and above (52 °C) the main phase transition for DPPC–SUVs [17]. Trp consumption was followed by the decrease of its fluorescence intensity (excitation 290 nm, and emission 330 nm) as a function of irradiation time. Experiments were performed in the absence and presence of SUVs (0.2 mM DPPC).

2.5. Photophysics of liposome-bound RB molecules

Transient Rose Bengal triplet absorption decay at 620 nm was recorded in an m-LFP 111 laser-flash photolysis system (Luzchem Inc., Ottawa, Canada), employing the second harmonic from a Surelite II, Nd-YAG laser (532 nm, ca. 10 ns, 10 mJ/pulse) as excitation source. Less than 10% of photodegradation was observed in all the experiments. The triplet quantum yield was evaluated employing the following Equation: (1)

$$\Phi_{\text{RB-Liposome}} = \Phi_{\text{RB-Water}} \frac{(\Delta \text{OD}_{\text{RB-Liposome}}/A_{532\text{nm-Liposome}})}{(\Delta \text{OD}_{\text{RB-Water}}/A_{532\text{nm-Water}})} \quad (1)$$

where ΔOD values correspond to changes in the optical density measured immediately after the excitation laser pulse. These values were obtained by extrapolating to zero time the transient absorption, fitted to a monoexponential decay. Rose Bengal triplet quantum yield in buffer (0.9) was taken as Ref. [18].

Singlet oxygen quantum yields were evaluated in deuterated buffer solution by recording its phosphorescence emission decay at 1270 nm with a Hamamatsu NIR detector (peltier cooled at 62.8 °C operating at 900 V, coupled to a grating monochromator). Excitation was performed employing a Surelite II, Nd-YAG laser (532 nm, ca. 10 ns, 10 mJ/pulse). The primary data were acquired and processed with a customized Luzchem Research LFP-112 system, and the singlet oxygen quantum yields were calculated according to the equation:(2)

$$\Phi_{\text{RB-Liposome}} = \Phi_{\text{RB-D2O}} \frac{(\text{Intensity at} 1270\,\text{nm}_{\text{RB-Liposome}}/A_{532\text{nm-Liposome}})}{(\text{Intensity at} 1270\,\text{nm}_{\text{RB-D2O}}/A_{532\text{nm-Water}})} \quad (2)$$

The intensity at 1270nm corresponds to that obtained immediately after the laser pulse. Singlet oxygen quantum yield of Rose Bengal in buffer (pH=0.8) was employed as Ref. [18].

3. Results and discussion

Addition of DPPC SUVs to an RB solution produces noticeable changes in its absorption spectra. Typical results obtained when, at a fixed temperature, aliquots of a concentrated SUVs suspension are added to a RB solution in buffer (pH=7.4) are shown in Fig. 1. These changes indicate adsorption of the dye by the liposomes [8,19]. This type of data allows an evaluation of the dye distribution (from the change in absorbance at a given wavelength with DPPC concentration). This procedure has been previously employed to evaluate the incorporation of RB to macromolecules [6,7,20], micelles [21–24], and liposomes [11]. Furthermore, since the absorption of RB is determined by the characteristics of the solvent [7,8,16], the microenvironment of the bound dye can be estimated from the wavelength of maxima absorbance when most of the dye is bound to the dispersed microphase [7,8,25,26].

3.1. Adsorption isotherms

Fig. 2 shows the changes in the RB absorbance at 567 nm as a function of DPPC concentration at temperatures below and above DPPC SUVs main phase transition (42 °C) [17].

The data given in Fig. 2 show that, at both temperatures, the absorbance tends to reach a plateau, indicative of total association of the dye to DPPC SUVs [11]. In order to obtain the value of the absorbance of the bound species (A_{∞}), the data of Fig. 2 were fitted to a sigmoidal function and extrapolated to infinite lipid concentration. This allows a representation of the adsorption isotherm as [RB $_{\text{bound}}$]/[lipid] vs. [RB_{free}]. The free and bound concentrations were obtained considering that

$$[\text{RB}_{\text{bound}}] = [\text{RB}] - [\text{RB}_{\text{free}}] \quad (3)$$

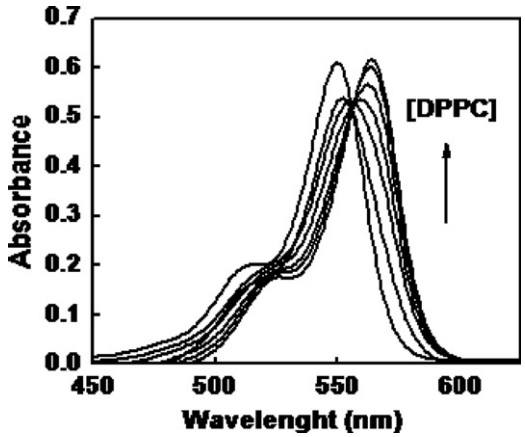

Fig. 1. Absorption spectra of RB (6 μM) in PBS buffer (pH 7.4) as a function of the added DPPC concentration (up to 0.47 mM). Data obtained at 53 °C. The arrow indicates the increasing DPPC concentration.