Dyes and Pigments 96 (2013) 626–635

Contents lists available at SciVerse ScienceDirect

Dyes and Pigments

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

Photophysics and photobiology of novel liposomal formulations of 2,9(10),16(17),23(24)-tetrakis[(2-dimethylamino)ethylsulfanyl]phthalocyaninatozinc(II)

Noelia C. López Zeballos [a], Julieta Marino [b], María C. García Vior [c], Nicolás Chiarante [b], Leonor P. Roguin [b], Josefina Awruch [c,*], Lelia E. Dicelio [a]

[a] INQUIMAE, Departamento de Química Inorgánica, Analítica y Química Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II, 1428 Buenos Aires, Argentina
[b] Instituto de Química y Fisicoquímica Biológicas (UBA-CONICET), Facultad de Farmacia y Bioquímica, Junín 956, 1113 Buenos Aires, Argentina
[c] Departamento de Química Orgánica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 1113 Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 18 June 2012
Received in revised form
25 September 2012
Accepted 5 November 2012
Available online 15 November 2012

Keywords:
Photodynamic therapy
Phthalocyanine
Liposome
Singlet oxygen
Photocytotoxicity
KB cells

ABSTRACT

Six novel liposomal formulations of 2,9(10),16(17),23(24)-tetrakis[(2-dimethylamino)ethylsulfanyl]phthalocyaninatozinc(II) (Pc9) were investigated to establish how the environments affect their photophysical and photobiological properties. The incorporation efficiency and solubility during a time period were evaluated. All Pc9-liposomal formulations were efficient singlet oxygen quantum yield generators. The photobiological potentials were evaluated on human nasopharynx KB carcinoma cells. None of the formulations studied showed cytotoxic effects in the absence of light, whereas all of them showed good performance after irradiation. The 50% inhibition of cell proliferation (IC_{50}) was in the range of 0.21–0.47 μM for Pc9-loaded liposome formulations. A lysosomal localization was found for S-PEG as well as for S.

1. Introduction

Photodynamic therapy (PDT) involves the generation of highly reactive species capable of destroying cancer tissues. The reactive species are generated by the irradiation of a photosensitizer with visible light in the presence of oxygen [1,2].

Phthalocyanines have been found to be useful photosensitizers for PDT [3–6]. Since phthalocyanines are highly hydrophobic, which make it difficult to administer them parenterally in physiological media, delivery strategies have been developed to increase their solubility [7–9]. Moreover, the tendency of phthalocyanines to aggregate has a strong negative effect on their photophysical properties [10].

Nanocarriers, such as liposomes, micelles, nanoemulsions, polymeric nanoparticles and many others, are widely used as carrier systems for water-insoluble drugs in pharmaceutics [11].

In particular, liposomes with various lipid compositions have been reported as carrier systems for water insoluble phthalocyanines [12,13].

We have recently studied the photophysical properties, size and stability of different formulations of liposomes containing the lipophilic 2,9(10),16(17),23(24)-tetrakis(1-adamantylsulfanyl) phthalocyaninatozinc(II). These formulations revealed to be efficient singlet molecular oxygen generators [13].

Since 2,9(10),16(17),23(24)-tetrakis[(2-dimethylamino)ethylsulfanyl]phthalocyaninatozinc(II) (Pc9) exhibits a better liposomal incorporation than the above-mentioned phthalocyanine, further studies have been initiated to investigate the photophysical parameters of Pc9 incorporated into these lipid environments. In addition, its photobiological properties were evaluated on human nasopharynx KB carcinoma cells.

2. Materials and methods

2.1. Materials

2,9(10),16(17),23(24)-tetrakis[(2-dimethylamino)ethylsulfanyl] phthalocyaninatozinc(II) (Pc9) [14] (Fig. 1) and tetra-*t*-butyl phthalocyaninatozinc(II) [15] were synthesized in our laboratory. L-α-Phosphatidylethanolamine from egg yolk Type III (PEEY); L-α-Phosphatidylcholine from egg yolk, Type XVI-E (PCEY); 1,2-Distearoyl-*sn*-

* Corresponding author. Tel.: +54 11 4964 8252; fax: +54 11 4508 3645.
E-mail address: jawruch@ffyb.uba.ar (J. Awruch).

0143-7208/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.dyepig.2012.11.005

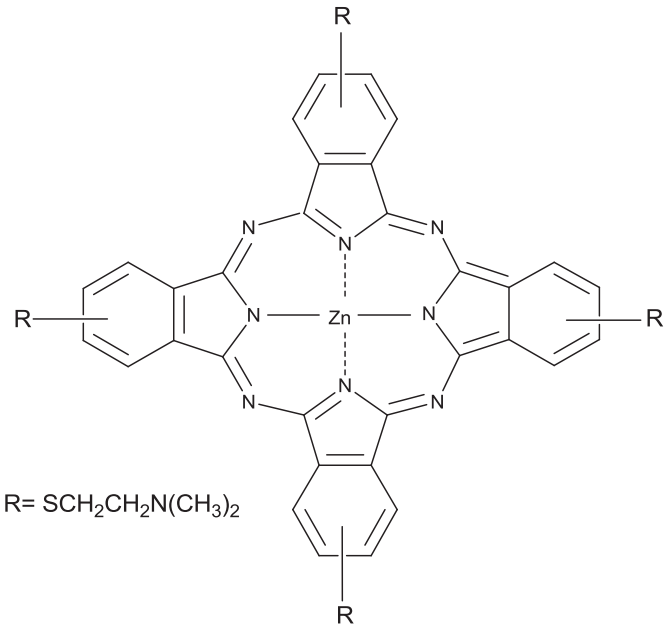

Fig. 1. Chemical structure of phthalocyanine (Pc9).

glycero-3-phosphoethanolamine (DSPE); L-α-Phosphatidylethanolamine distearoyl methoxypolyethylene glycol conjugate (DSPE-PEG); 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC); Cholesterol BioReagent suitable for cell culture (CHOL); HEPES BioUltra for molecular biology; 2′,7′-dichlorofluorescein-diacetate (DCFH-DA) and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma–Aldrich Germany (Schnelldorf, Germany). LysoTracker Green DND-26 and MitoTracker Green FM were obtained from Invitrogen (Carisbad, CA, USA). Sodium chloride was obtained from Mallinckrodt (Phillipsburg, NJ, USA). Imidazole BioUltra and Methylene Blue Hydrate (MB) were from Fluka (Sigma–Aldrich, India); N,N-Diethyl-4-nitrosoaniline 97% and Tetrahydrofuran (THF) spectrophotometric grade were from Sigma–Aldrich (Steinheim, Germany). Diethyl ether was from Carlo Erba (Rodano, Italy), and Chloroform from Merck-Química Argentina (Buenos Aires, Argentina). All chemicals were of reagent grade and used without further purification. Distilled water treated in a Milli-Q system (Millipore) was used.

2.2. Instrumentation

Electronic absorption spectra were determined with a Shimadzu UV-3101 PC spectrophotometer and fluorescence spectra were monitored with a QuantaMaster Model QM-1 PTI spectrofluorometer. pH was measured with a Thermo pH meter Altronix TPX-1. Static light scattering (SLS) experiments were carried out using an SLS 90 Plus/BI-MAS (MultiAngle Particle Sizing Option) equipped with a He–Ne laser operating at 632.8 nm and 15 mW. The sonicator used was MSE Soniprep 150.

2.3. Preparation of unilamellar liposomes

The preparation of unilamellar liposomes was achieved as described elsewhere [13] (See Table 1).

2.4. Sample preparation. Determination of incorporation efficiency (IE)

Stock solutions of Pc9 were prepared in THF, kept at 4 °C, and carefully protected from ambient light. The dye and carrier concentrations are indicated in each experiment.

Table 1
Liposomal composition and Pc9 incorporation efficiency (IE).

Liposome type	Lipid composition	Molar relation	[a]IE (%)
D1	DPPC: CHOL	24:1	83.5 ± 1.1
D2	DPPC	1	92.5 ± 2.1
M1	PCEY: PEEY: CHOL	16:16:1	82.9 ± 8.6
M2	PCEY: PEEY	1:1	88.8 ± 2.9
S	DPPC: PCEY: PEEY: DSPE: CHOL	1:8:7:1:3	90.5 ± 10.0
S-PEG	DPPC: PCEY: PEEY: DSPE-PEG: CHOL	1:8:7:1:3	74.7 ± 0.1

[a] Data represent the mean value ± standard error of three independent experiments.

Liposomes with Pc9 incorporated were disrupted using Triton-X 100 to fully release the dye incorporated into the liposomes, and the absorbance of samples measured after leaving them at room temperature for 24 h. The absorbance of Pc9 at λ_{max} indicated in Table 2 for each sample was measured to determine the concentration of Pc9 incorporated. The IE was calculated by equation (1)

$$IE = \frac{[\text{Incorporated ZnPc}]}{[\text{Initial ZnPc loaded}]} \times 100 \quad (1)$$

2.5. Photophysical properties

2.5.1. Spectroscopic studies

Absorption and emission spectra were recorded with a 10 × 10 mm quartz cuvette with a 500 μL capacity at room temperature.

Emission spectra of Pc9 were recorded at an excitation wavelength (λ_{exc}) of 610 nm (Q-band) between 630 and 800 nm; a cut-off filter was used to prevent the excitation beam from reaching the detector (Schott RG 630).

Emission and absorption spectra of liposomal phthalocyanines were corrected for light scattering by subtracting the spectra from empty liposomes.

Spectroscopic experiments were carried out at concentrations 1×10^{-4} M.

2.5.2. Fluorescence quantum yields

Fluorescence quantum yields (Φ_F) were determined by comparing them with those of tetra-*t*-butyl phthalocyaninato-zinc(II) ($\Phi_F = 0.30$ in toluene) as a reference at an λ_{exc} of 610 nm and calculated as described elsewhere [15].

2.5.3. Quantum yield of singlet oxygen production

The quantum yield of singlet oxygen generation rates (Φ_Δ) was determined using standard chemical monitor bleaching rates [16]. Imidazol (8 mM) and *N*,*N*-diethyl-4-nitrosoaniline (40–50 μM) in HEPES was used for Pc9-loaded liposomes [17]. *N*,*N*-diethyl-4-nitrosoaniline decay was monitored at 440 nm.

A projector lamp (Philips 7748SEHJ, 24 V–250 W) and a cut-off filter at 610 nm (Schott, RG 610) were used to generate polychromatic irradiation and a water filter to prevent infrared radiation. The liposomal samples of Pc9 and the reference (MB: $\Phi_\Delta = 0.56$ in buffer) [16] were irradiated within the same wavelength interval λ_1–λ_2, and Φ_Δ was calculated according to Amore et al. [18].

2.5.4. Aggregation studies of Pc9 in liposomal formulations

The intensity absorption ratio of the two bands corresponding to the monomer and oligomers was calculated. The higher values of the ratio indicated a disaggregated dye form [19,20]. This ratio was calculated for all liposomal formulations using the λ_{max} indicated in Table 2. These values were compared with those obtained in THF, where aggregation was not observed, and with those obtained in HEPES pH 7.4 and 145 mM NaCl.