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Photophysical properties of diphenyl-2,3-dihydroxychlorin and diphenylchlorin

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Abstract

The photophysical properties of 5,15-diphenyl-2,3-dihydroxychlorin (DPCOH) and 5,15-diphenyl-chlorin (DPC) in organic solution were studied. Absorption, fluorescence, triplet state and photobleaching experiments are reported. The ground states of both compounds show strong absorbance in red region ($\lambda = 638$ nm, $\varepsilon = 35{,}000$ M^{-1} cm^{-1} and $\lambda = 645$ nm, $\varepsilon = 42{,}000$ M^{-1} cm^{-1}, respectively) and the singlet excited states show low fluorescence quantum yields of 0.0802 and 0.150 in benzene and the lifetimes are 7.38 and 10.18 ns, respectively. Absorption spectra of the triplet states were also measured and they have nearly the same triplet state lifetimes of 53 μs (DPCOH) and 50 μs (DPC). The triplet quantum yields are 0.82 and 0.75, respectively. The data of photobleaching quantum yields show that the presence of oxygen does not significantly affect the photobleaching. All the results demonstrate that both diphenylchlorines are good candidates for second-generation photosensitizer in photodynamic therapy.

Keywords: Chlorins; Photophysical properties; Absorption; Fluorescence; Triplet state

1. Introduction

Nowadays, photodynamic therapy (PDT) has been used widely as a powerful therapeutic method for selective treatment of cancerous tumors and many other illnesses [1–3]. PDT is based on that the photosensitizer can be concentrated in tumor cells and upon subsequent irradiation with visible light in the presence of oxygen, specifically destroys the tumor cells [4–6].

Porphyrins and their ramifications are the most popular photosensitizers for PDT. A number of new porphyrin-based photosensitisers have been developed for PDT in recent years, including chlorins, bacteriochiorins and purpurins. In addition to the first-generation photosensitiser "Photofrin" (a hematoporphyrin derivative), the second-generation drug "Benzoporphyrin derivative mono-acid ring A" (BPD-MA, Verteporfin) and "Meso-tetra(*m*-hydroxphenyl)chlorin" (*m*-THPC) have received approval for clinical use. Other second-generation photosensitisers are also undergoing clinical trials for PDT, including "Mono-L-aspartyl chlorin e6" (MACE) and "Tin Etiopurpurin" ($SnET_2$) [7–9].

5,15-Diphenylporphyrin (DPP) is an interesting porphyrin model compound that combines the features of two classic porphyrins, namely 5,10,15,20-tetraphenyl-porphyrin (TPP) and 2,3,7,8,12,13,17,18-octaethyl-porphyrin (OEP) [10]. The characteristics of this molecule had not been explored until a new synthetic method developed recently for the preparation of DPP [11].

In our earlier publications, we described the synthesis and tumor photocytotoxicity of DPP-based 5,15-diphenyl-2,3-dihydroxychlorin (DPCOH) [12]. In vitro, compared

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with HPD (hematoporphyrin derivatives), DPCOH shows 200 times more potent photocytotoxicity to tumor cells, and the dark toxicity is low (dark $IC_{90} > 32$ μg/mL). The quantum yield of $^1O_2$ from DPCOH is 0.7 in organic solution [12].

5,15-Diphenyl-2,3-dihydroxychlorins with different substituents on phenyl groups have also been synthesized. These chlorins can successfully bioconjugate with special proteins. It demonstrates that DPCOH isomers have excellent targeting potential during the PDT applications [13,14]. A similar derivative, 2,3-dihydro-5,15-di(3,5-dihydroxyphenyl) porphyrin, was recently reported and has promising activity in PDT [15].

Since DPCOH has great potential use for PDT agent, it is necessary to investigate the photophysical properties in detail. Herein we report the photophysical properties of DPCOH and 5,15-diphenyl-chlorin (DPC), including the transient state studies of singlet and triplet properties in homogeneous solution.

2. Materials and methods

2.1. Chemicals

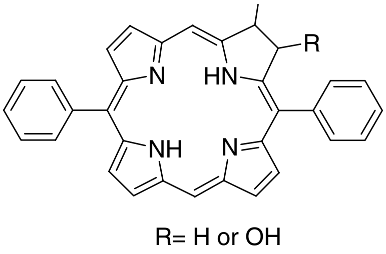
The reagents of spectroscopic grade (chloroform, benzene, acetonitrile, methylene chloride and methanol) were used without further purification. β-Carotene was bought from Fluka Chemie GmbH (Buchs, Switzerland). The photosensitizers (DPC and DPCOH) were synthesized according to the literature methods [12,16] (see Scheme 1).

2.2. Measurement

2.2.1. Singlet state studies

Absorption spectra. The absorption spectra were measured with a Shimadzu 1601 spectrometer in different solvents. In each solvent, molar absorption coefficients were determined from the slope of the Beer–Lambert plots for a series of five dilutions, with each solution having an absorbance value below 1.0.

Fluorescence. Steady-state fluorescence spectra of molecules were recorded with a Hitachi F2500 spectrometer at room temperature in air- or nitrogen-saturated solvents. In each case, the Q (0,0)-band absorption values were below 0.05. Excitation was performed at 510 nm, and the fluorescence emission spectra were corrected for instrument response time and temporary variations in light intensity. Fluorescence quantum yields were determined by comparison with the reference compound tetraphenylporphyrin (TPP) ($\Phi_f = 0.13$ in the nitrogen-saturated benzene solution) [17]. The absorption value of each sample was adjusted to about 0.02.



Scheme 1. DPC (R = H) and DPCOH (R = OH).

The fluorescence lifetimes were measured using the time-correlated single photon counting method with a time resolved transient state fluorescence spectrometer (Edinburgh Analytical Instruments F900). Measurements were performed with 409 nm (for DPC) or 400 nm (for DPCOH) excitation of argon-saturated benzene solutions at room temperature.

2.2.2. Triplet state studies

The laser flash photolysis measurements were carried on a LP-920 pump–probe spectroscopic setup (Edinburgh). The excited source was the unfocused third harmonic (355 nm, 7 ns fwhm) output of a Nd:YAG laser (Continuum surelite II): the probe light source was a pulse-xenon lamp. The signals were detected by Edinburgh analytical instruments (LP900) and recorded on a Tektronix TDS3012B oscilloscope and computer. The photosensitizers were dissolved in benzene with concentration of approximately 10^{-5} mol/L. Samples in 10 × 10 mm path-length quartz cuvettes were bubbled 30 min with argon prior to irradiation.

Triplet absorption coefficient. Ground state depletion method was used to determine the extinction coefficient of the T–T absorption [18–20]. After excitation, the optical density ΔOD can be described as following (ε_T, ε_G are triplet and ground state absorption coefficient, L is light path length (1 cm)):

$$\Delta OD_T = (\varepsilon_T - \varepsilon_G) \times [D]_T \times L \tag{1}$$

At a wavelength λ_1, the triplet absorption has a very strong negative peak, so at this wavelength the triplet absorption can be assumed as zero [18–20]. Then the triplet concentration ($[D]_T$) can be obtained as follows:

$$[D]_T = -\Delta OD_T(\lambda_1)/\varepsilon_G(\lambda_1) \tag{2}$$

$$\varepsilon_T(\lambda_2) = \varepsilon_G(\lambda_2) + \Delta OD_T(\lambda_2)/[D]_T \tag{3}$$

Therefore, the triplet absorption coefficient at any a wavelength λ_2 can be calculated from Eq. (3).

Triplet state quantum yield. Triplet state quantum yields of photosensitizers DPC and DPCOH were determined by following the method of literatures [22,23], benzophenone as the standard substance. The triplet state quantum yields of DPC and DPCOH were calculated using the following equation:

$$\Phi_T^D = \Phi_T^S \times \frac{\Delta OD_T^D \times \Delta\varepsilon_T^S}{\Delta OD_T^S \times \Delta\varepsilon_T^D} \tag{4}$$

where the superscript S refers to the reference sample (benzophenone) and D to the DPC or DPCOH, the subscript T to the triplet state, ΔOD is the optical density