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An investigation of the triplet state of pheophorbide a using laser flash photolysis

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Abstract

The triplet properties of pheophorbide a (PPa) have been investigated in benzene using nanosecond laser flash photolysis. The absorption maximum at 460 nm, which decays with a lifetime of 9.1 μs, has been assigned to the triplet–triplet (T–T) absorption of PPa. The extinction coefficient of this absorption is 75,900 dm^3 mol^{-1} cm^{-1} and the triplet quantum yield is 0.65±0.03 (versus benzophenone). © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Pheophorbide a; Triplet state; Laser flash photolysis

1. Introduction

Pheophorbide a (PPa; Fig. 1) is derived from chlorophyll-a by the removal of Mg^{2+} and the phytyl group by the action of *chlorophyllase* under acidic conditions. It is known as a photocytotoxic agent [1,2]. It causes photocytotoxicity to murine myeloma cells in tissue culture [3] and human bladder cancer cells in vitro [4]. Treatment of cells with PPa and light irradiation significantly alters the subsequent binding properties of both cytokines and antibodies to cell surface receptors. As a consequence of this photodynamic action, cells may become refractory to extracellular signals, resulting in impaired cellular function, cytotoxicity or cell death [5]. PPa was also incorporated into immunoliposomes coated with a monoclonal antibody directed against the T-24 bladder tumour cell line and the results demonstrated that PPa uptake by target cells and its subsequent delivery to the lysosomes caused photoactivated death of tumour cells [6]. Compared to hematoporphyrin derivative (HPD), PPa has advantages such as high chemical purity and photostability [7], and a stronger absorption in the red region of the visible spectrum. Since the photosensitizing efficacy of PPa on human neutrophils and Ehrlich-ascites cells is comparable to that of HPD, it has been proposed as a new photosensitizer for photodynamic therapy (PDT) [8].

Results of prior studies show that PPa is capable of converting O_2 to $^1O_2$ in the presence of light, which is mainly responsible for cell death by excited PPa [9,10]. It is believed that the generation of $^1O_2$ by exciting PPa occurs by energy transfer from triplet PPa to O_2. Since many other reactions involving photosensitized PPa are also believed to proceed via its lowest triplet state, it is of considerable interest to study this species. With this in mind, the lowest triplet state of PPa was studied

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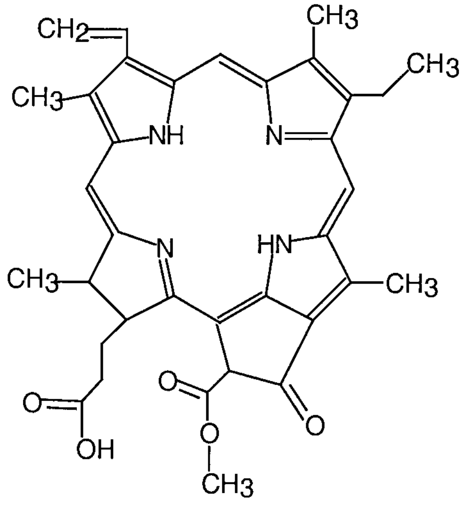

Fig. 1. Chemical structure of pheophorbide a.

using nanosecond laser flash photolysis. The associated absorption spectra, triplet lifetime, extinction coefficient (ε_T), and quantum yields (ϕ_T) were determined.

2. Experimental details

2.1. Reagents

PPa was obtained from Porphyrin Products (Logan, UT) and β-carotene was obtained from Merk. These and all other compounds were obtained at the highest available purity.

2.2. Laser flash photolysis

Laser flash photolysis experiments were performed using an excimer laser that provided a 337 nm pulse with a 20-ns duration. The maximum energy was 3 mJ per pulse. The analyzing light source was a 500 W xenon lamp, the intensity of which was increased about 100 times during the detection of transient absorption. The laser and xenon light beams passed perpendicularly through a quartz cell with an optical path length of 10 mm. The transmitted light entered a monochromator equipped with an R955 photomultiplier. The resultant signals were collected using a HP54510B digital oscilloscope processed with a Sun 586 personal computer.

All samples were prepared by deaerating each solution with high purity nitrogen (99.99%) for 30 min before photolysis.

3. Results

3.1. Transient absorption spectra

Fig. 2 shows the time-resolved absorption spectra of the transient species arising from laser flash photolysis of a dilute solution (~10^{-5} mol dm^{-3}) of PPa in deaerated benzene. The spectra show transient absorption maxima at 340 and 460 nm, which decayed quickly in air-saturated solutions. A negative absorption is observed in the 380–420 nm region, since the absorption due to the transient species produced is less than the absorption loss due to depletion of the parent.

To confirm that the short-lived PPa species arises from the triplet state, a mixture of PPa and β-carotene was subject to laser flash photolysis. It is well known [11] that β-carotene has a strong triplet absorption between 500 and 580 nm with a lifetime of ~ 5 μs and that its intersystem crossing efficiency is very low (~0). Furthermore, the triplet energy level for β-carotene is about 90 kJ mol^{-1}, indicating that the sensitization of the β-carotene triplet by another transient species provides

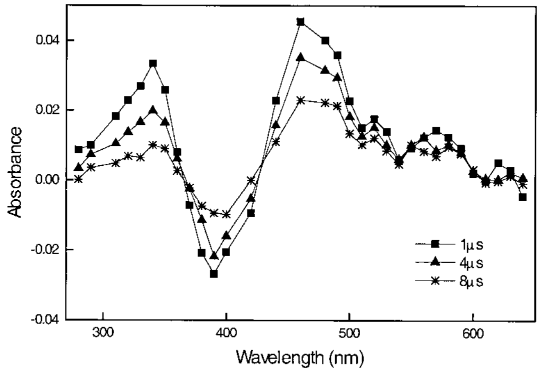

Fig. 2. Time-resolved spectra of the transients formed by laser flash photolysis of PPa (2×10^{-5} mol dm^{-3}) in benzene saturated with N_2 1 μs (■), 4 μs (▲) and 8 μs (*) after the laser flash (337 nm).

an excellent way of confirming that the transient species is a triplet state. Fig. 3 shows the time-resolved absorption spectra recorded after 337 nm laser photolysis of a benzene solution containing 4×10^{-5} mol dm^{-3} PPa and 1×10^{-5} mol dm^{-3} β-carotene. For this solution, the T–T absorption of β-carotene (515 nm) developed immediately after the laser flash. In this case, the absorption spectrum was essential identical with that of the T–T absorption of β-carotene reported previously [12]. With the increase of its intensity, the band at 460 nm decayed rapidly, while the decay of the band at 340 nm seemed to be influenced slightly. Since a β-carotene T–T absorption was not observed in the absence of PPa, the band at 460 nm could be assigned to the T–T absorption of PPa [12]. Quenching of the PPa triplet by β-carotene also allowed us to estimate the rate constant for energy transfer from PPa triplet to β-carotene. The value obtained was about 10^{10} dm^3 mol^{-1} s^{-1}, which corresponds to energy transfer at or near the diffusion-controlled limit.

The addition of β-carotene had little effect on the decay the band at 340 nm, indicating that that this band was not due to the PPa triplet. It is probably due to radical formation, and this hypothesis is presently under investigation.

The band at 460 nm, which is due to the T–T absorption of PPa, decays monoexponentially. From its decay curve, we determined that its half-life is about 9.1 μs.

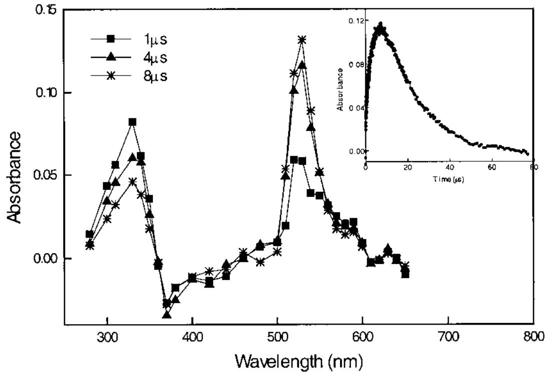

Fig. 3. Transient absorption spectra from photolysis of 4×10^{-5} mol dm^{-3} PPa in benzene containing 1×10^{-5} mol dm^{-3} β-carotene saturated with N_2 1 μs (■), 4 μs (▲) and 8 μs (*) after the laser flash (337 nm). Inset: growth-decay trace of the T–T absorption of β-carotene at 515 nm.

3.2. Extinction coefficient for the T–T absorption of PPa

The T–T molar extinction coefficient (ε_T^{PPa}) was evaluated using the energy transfer method [13]. β-carotene (ε_T of 1.87×10^5 in benzene] at 515 nm) was used as energy acceptor [14]. According to the mechanism of competing reactions, three reactions can occur in a solution containing a mixture of PPa and β-carotene:

$$\mathrm{PPa} + h\nu \rightarrow {}^1\mathrm{PPa}^* \rightarrow {}^3\mathrm{PPa}^* \tag{1}$$

$${}^3\mathrm{PPa}^* + \beta\text{-C} \xrightarrow{k_q} {}^3\beta\text{-C}^* + \mathrm{PPa} \tag{2}$$

$${}^3\mathrm{PPa}^* \xrightarrow{k_d} \mathrm{PPa} \tag{3}$$

The growth of the β-carotene triplet is shown in Fig. 3. The inset curve represents the apparent change in the absorption of the β-carotene triplet at 515 nm, as a function of time after the laser flash. After making corrections due to ^{3}[β-carotene] decay [12], we found that the growth of the absorbance value follows first order kinetics. The value of the slope of the growth curve is the apparent rate constant of β-carotene triplet formation (k_{app}). By varying the concentration of β-carotene (4×10^{-6}–1.7×10^{-5} mol dm^{-3}), a series of k_{app} were obtained. The dependence of k_{app} on the concentration of β-carotene is a straight line (Fig. 4), the slope of which corresponds to the rate constant (k_q) for energy transfer from the PPa triplet to β-carotene . The values for this rate constant was 2.04×10^{10} dm^3 mol^{-1} s^{-1}.

The rate constant for PPa triplet decay (k_d) was determined to be 7.64×10^4 s^{-1}. The PPa triplet to β-carotene energy transfer probability (P) was calculated according to Eq. (4):

$$P = k_q[\beta\text{-C}]/\big(k_q[\beta\text{-C}] + k_d\big) \tag{4}$$

In this case, 71% of the PPa triplet energy was transferred to β-carotene to form β-carotene triplet. The extinction coefficient (ε_T^{PPa}) for the PPa triplet was found to be 75,900 dm^3 mol^{-1} cm^{-1}.

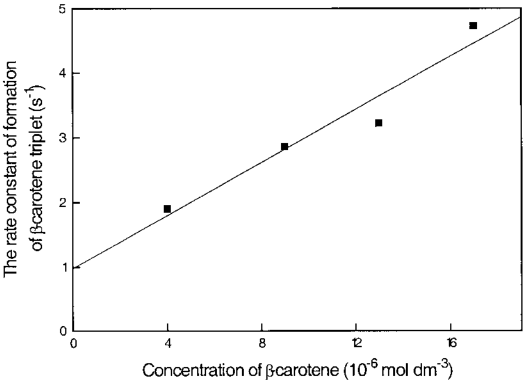

Fig. 4. Plot of the apparent formation rate constant of ^{3}[β-carotene]* vs. the concentration of β-carotene.

3.3. Quantum efficiency of triplet formation (Φ_T^{PPa})

For these experiments, we used the comparative technique for triplet quantum yield (ε_T^{PPa}) determinations, employing benzophenone in benzene as the actinometer ($\Phi_T^S = 1.0$, $\varepsilon_T^S = 7220$ mol^{-1} cm^{-1} at 530 nm) [15]. Under the conditions in which the unknown (PPa) and standard (benzophenone) solutions absorb the same number of photons at the excitation wavelength (337 nm), Φ_T^{PPa} was calculated using Eq. (5) [16]:

$$\Phi_T^{PPa} = \Phi_T^S(\Delta OD_T^{PPa} \times \Delta\varepsilon_T^S / \Delta OD_T^S \times \Delta\varepsilon_T^{PPa}) \qquad (5)$$

where ΔOD_T^{PPa} and $\Delta\varepsilon_T^{PPa}$ are the triplet–singlet optical density and triplet–singlet extinction coefficient respectively, at the wavelength at which the ε_T^{PPa} value was measured for PPa, and ΔOD_T^S and $\Delta\varepsilon_T^S$ are the corresponding values for the standard (benzophenone). The experimental value for Φ_T^{PPa} was 0.65±0.03.

4. Conclusions

The Φ_T^{PPa} for PPa was found to be 0.65±0.03, which is much greater than the fluorescence quantum yield of PPa (0.3) [8]. This indicates that intersystem crossing from S_1 to T_1 is the dominent process in the photophysics of PPa, which is advantageous to the photosensitizing reactions involving PPa. Although, the quantum yield for T_1 formation (0.65) is lower than that reported for HP (>0.8) [16], it has been demonstrated experimentally that the photodynamic action of PPa on cells in vitro and in vivo is comparable to HPD, which is the most effective PDT agent among the porphyrins. This suggests that the photosensitizing efficacy of a sensitizer is not absolutely proportion to the quantum yield of T_1 formation, although the photosensitized reactions proceed via the lowest triplet state. Other properties of a sensitizer (e.g. hydrophilicity) may influence its intracellular distribution and, in turn, influence its photosensitizing efficacy.

Acknowledgements

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