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Reverse saturable absorption in chlorophyll A solutions

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ABSTRACT The dynamic nonlinear absorption of a chloroform solution of chlorophyll A was investigated using the Z-scan technique with picosecond pulses at 532 nm. The nonlinear absorption exhibits a reverse saturation, indicating a strong intersystem crossing (singlet–triplet) process. The time evolution of the optical nonlinearity, modeled by means of a five-level energy diagram, allows the determination of excited-state cross sections and the lifetime of the intersystem crossing based on its absorption characteristics and efficient formation of triplet states. Chlorophyll A was found to be a good candidate for a sensitizer in photodynamic therapy.

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1 Introduction

Macrocyclic organic compounds such as porphyrins exhibit large optical nonlinearities and as a result they have been employed in a number of applications with emphasis in optical limiting devices [1–4] and sensitizers for photodynamic therapy (PDT) [5, 6]. These materials are interesting also because they can be chemically synthesized in a wide variety, with the attachment of functional groups. Fine-tuning of the photo-chemical properties may be achieved with the molecular control of the compound itself or of the way the molecules are assembled. The ever-increasing variety of new available materials requires screening processes to help select the most suitable candidates for a given application. For optical limiting devices, for instance, the important requirement is an efficient reverse saturable absorption (RSA). RSA occurs when the cross section for absorption of the excited state exceeds that of the ground state. Usually, this follows from an intersystem crossing process from a higher excited singlet to an excited triplet state that competes with the direct radiative decay to the singlet ground state. RSA efficiency strongly depends on the properties of excited states such as the intersystem crossing time and cross sections. For PDT, the creation of chromophores in the triplet states is an essential requirement. After that is accomplished, neoplastic (malignant) cells

can be destroyed through the production of a singlet oxygen ($^1\text{O}_2$), resulting from the interaction between the light-excited drug (porphyrin) and the usual triplet oxygen [5, 6].

An obvious way to screen materials for the applications mentioned above is through the knowledge of their excited-state properties, which can be obtained using nonlinear optical techniques. The aim of the present paper is to employ the Z-scan technique with pulse trains [7] to investigate the excited-state properties of chlorophyll A, which is classified as a porphyrin, and demonstrate that this material is a suitable candidate for PDT applications. An interesting characteristic of the pulse-train Z-scan (PTZ-scan) method is the possibility of determining the dynamics of the nonlinear process [8]. In chlorophyll A/chloroform solution, the dynamic absorptive nonlinearity (RSA) is associated with the excitation of the Q-band. A five-level energy diagram is used to calculate the population dynamics and thus the nonlinear absorption-time evolution. This allows spectroscopic parameters of excited states to be determined. Remarkable in our results is the short intersystem crossing time, indicating that chlorophyll derivatives may be used as sensitizers for PDT.

2 Experimental details

Chlorophyll A was extracted from fresh spinach leaves via solvent extraction followed by separation in a sucrose chromatographic column, according to the method described in [9]. It was then dissolved in chloroform to a concentration of 1.9×10^{17} molecules/cm³ and characterized in a Hitachi U2001 UV–Vis spectrophotometer. The optical nonlinearity was investigated with the PTZ-scan technique, which transforms changes in the beam-curvature wavefront induced by the sample nonlinear response into transmittance changes measured by a detector placed behind an aperture in the far field [10, 11]. Another detector, without aperture, is also placed in the far field to detect transmittance changes due to nonlinear absorption processes. In this work we employed the PTZ-scan technique [7], which is an extension of the standard Z-scan method [10, 11], that allows one to study the time evolution of the nonlinear process. The experimental setup is fully described in [7] but a few details are presented here. The excitation source is a frequency-doubled, Q-switched and mode-locked Nd:YAG laser, delivering pulses at 532 nm, in pulse trains containing about 20 pulses (separated by 13 ns)

at a 10-Hz repetition rate. This low repetition rate was used to avoid cumulative thermal nonlinearities. The beam was focused with a lens of focal length $f = 12$ cm onto a quartz cell, resulting in a diameter of $50 \mu\text{m}$ at the focal plane. The pulse train is acquired by a detector placed in the far field and a digital oscilloscope. A data-acquisition system based on a personal computer acquires the pulse train as the sample is scanned through the focus. A set of Z-scan signatures is produced by normalizing each pulse train to the one obtained when the sample is far from the focus. The same type of measurement is performed for the nonlinear absorption by reading the signal from the detector without aperture. This method allows us to map both refractive and absorptive nonlinearities along the Q-switch envelope and to determine fast (sub-nanosecond) and cumulative contributions. It has also been shown as a valuable tool for the understanding of excited-state dynamics because the sample absorbs the energy in small amounts contained in each mode-locked pulse. Relative populations can then be followed during the pulse train. All optical measurements, including those with the Z-scan technique, were carried out with the sample placed in a quartz cuvette with an optical path of 2 mm.

3 Results and discussion

Figure 1 shows the absorption spectrum of a solution of chlorophyll A in chloroform, which is similar to the one reported in the literature [9]. The Q-band at 670 nm is the only transition excited by the 532-nm light used in the nonlinear optical absorption investigation. Chlorophyll A absorbs strongly in the 600–800-nm region, in which human tissues are transparent [12]. Therefore, light can reach the dye molecule adsorbed in the cells and undergo a photoreaction, i.e. chlorophyll A satisfies an important requirement for possible use as a sensitizer in PDT. The emission spectrum at room temperature for excitation at the Q-band [9] presents the stronger fluorescence peak at 669 nm, which means that the

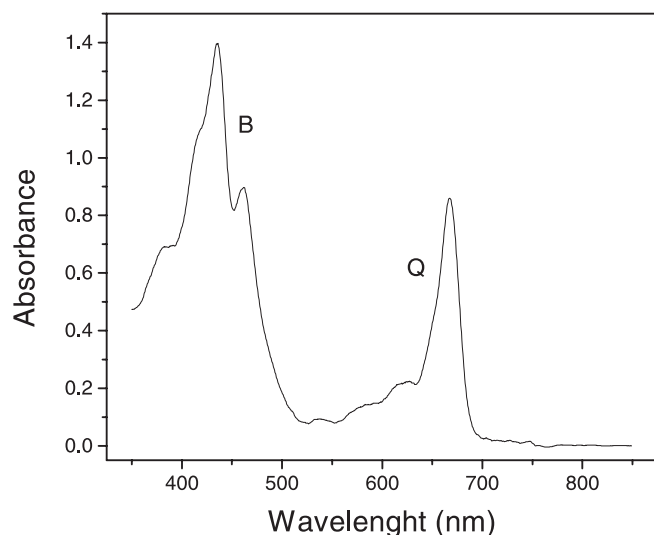


FIGURE 1 Absorption spectrum of a solution of chlorophyll A in chloroform

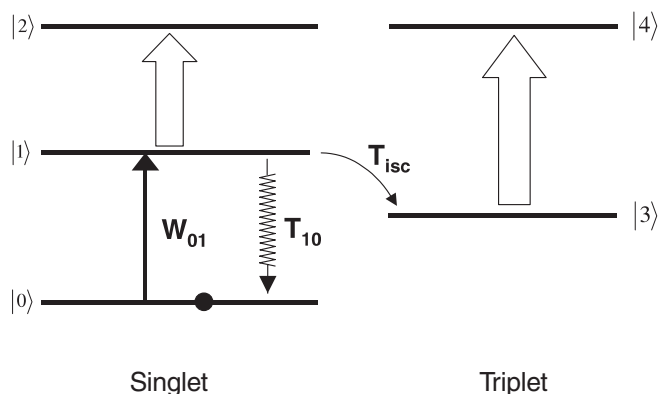


FIGURE 2 Five-level energy diagram used to simulate the experimental results. The optical excitation is represented by upward-pointing arrows and the radiative relaxation by a downward-pointing wiggly line

Q-band is the predominant excitation path. The fluorescence lifetime reported in the literature is 4 ns [9].

Based on the absorption and emission spectra, and in models traditionally used for other porphyrins, we propose the five-level energy diagram illustrated in Fig. 2, where the excitation is achieved with 532-nm light. This will be used to analyze the nonlinear absorption results of Fig. 3.

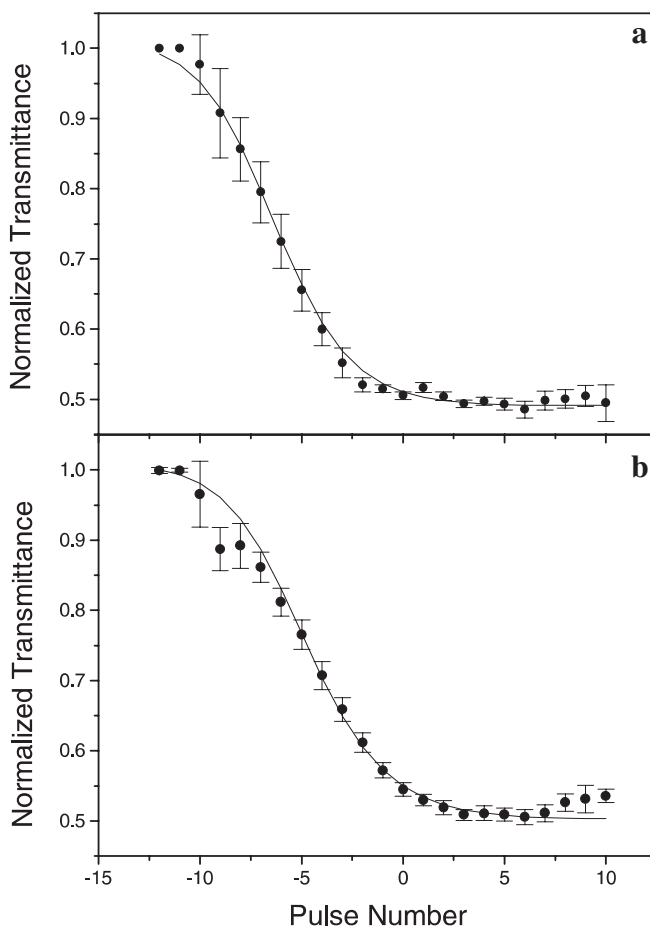


FIGURE 3 Nonlinear absorption along the pulse train for **a** $I^{(0)} = 0.35 \text{ GW/cm}^2$ and **b** $I^{(0)} = 0.68 \text{ GW/cm}^2$. Solid lines are theoretical curves with parameters given in the text

Figure 3a and b show experimental results and theoretical fittings described later for the nonlinear absorption obtained with the PTZ-scan technique at 532 nm, for two laser intensities. The strongest peak in the pulse train was arbitrarily labeled "0". The irradiance is $I^{(0)} = 0.35 \text{ GW/cm}^2$ and $I^{(0)} = 0.68 \text{ GW/cm}^2$ for Fig. 3a and b, respectively. These results may be explained using the five-level energy diagram depicted in Fig. 2. Absorption of laser pulses at this wavelength promotes molecules from the ground state $|0\rangle$ to the excited singlet state $|1\rangle$. Molecules excited to $|1\rangle$ may decay radiatively to level $|0\rangle$ with a characteristic time $T_{10} = 4 \text{ ns}$, or relax to an excited triplet state $|3\rangle$ with the lifetime T_{isc} . The upper excited singlet and triplet levels $|2\rangle$ and $|4\rangle$ are short-lived and thus their populations are neglected. On the basis of this energy diagram, the set of rate equations which describe the fraction of molecules, n_i , at each level are:

$$\frac{dn_0}{dt} = -n_0 W_{01} + \frac{n_1}{T_{10}}, \quad (1)$$

$$\frac{dn_1}{dt} = n_0 W_{01} - \frac{n_1}{T_{10}} - \frac{n_1}{T_{\text{isc}}}, \quad (2)$$

$$\frac{dn_3}{dt} = \frac{n_1}{T_{\text{isc}}}, \quad (3)$$

where $W_{01} = \sigma_{01} I/h\nu$ is the transition rate, T_{10} is the lifetime of level $|1\rangle$ and T_{isc} is the intersystem crossing lifetime. This set of equations was numerically solved using the actual temporal intensity pattern of the Q-switched/mode-locked pulse train of our experiment, yielding the population dynamics, $n_i(t)$. The time evolution of the nonlinear absorption can be calculated according to

$$\alpha(t) = N \{n_0 \sigma_{01} + n_1 \sigma_{12} + n_3 \sigma_{34}\}, \quad (4)$$

where N is the concentration and σ_{12} and σ_{34} are the excited-state cross sections. The ground-state cross section, σ_{01} , was determined by measuring the linear absorption at 532 nm ($\sigma = N[\sigma_{01}]$). This procedure resulted in $\sigma_{01} = 3.1 \times 10^{-18} \text{ cm}^2$. The numerical calculation was carried out with $T_{10} = 4 \text{ ns}$, which is a value obtained from the literature [9] for the chlorophyll fluorescence lifetime. The solid lines in Fig. 3a and b represent theoretical fittings obtained with $\sigma_{12} = 4 \times 10^{-18} \text{ cm}^2$, $\sigma_{34} = 8 \times 10^{-18} \text{ cm}^2$ and $T_{\text{isc}} = 1.5 \text{ ns}$. As expected for materials that exhibit RSA, the cross section for absorption of the triplet state is higher than that of the singlet. Two conclusions can be drawn from these data. (i) Since chlorophyll A molecules have a triplet cross section only two times that of the ground state, it cannot be considered a good candidate for optical limiting devices. A much higher ratio would be required between the

triplet and singlet absorption cross sections. (ii) On the other hand, the intersystem crossing lifetime (1.5 ns) is shorter than the typical values reported for porphyrins and phthalocyanines [1–4, 13, 14]. This short intersystem crossing lifetime indicates an efficient singlet–triplet conversion, which makes chlorophyll A suitable for applications as a PDT sensitizer. This efficient intersystem crossing (singlet–triplet) conversion is consistent with those found for Mg phthalocyanine, which has a yield of triplet formation higher than for most phthalocyanines [13].

4 Conclusions

It has been shown that chlorophyll A solution exhibits a RSA process for Q-switched and mode-locked laser pulses. A five-level energy diagram was used to model the dynamic nonlinear absorption process and to provide a spectroscopic characterization of the excited states. The intersystem crossing time is relatively fast and the triplet-state cross section is twice the value for the singlet, as determined through the Z-scan technique with pulse trains. Chlorophyll A thus has an efficient formation of the triplet state, and is suitable for applications in PDT. However, due to the low triplet–singlet cross-sectional ratio, chlorophyll A is not expected to be efficient as an optical limiter.

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