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Quantum yield of triplet formation for indocyanine green

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Abstract

Yields of triplet formation, $\phi_{S_1T_1}$, by S_1–T_1 intersystem-crossing are determined for indocyanine green in DMSO ($\phi_{S_1T_1} \approx 17\%$), methanol ($\phi_{S_1T_1} \approx 16\%$), water ($\phi_{S_1T_1} \approx 14\%$), and aqueous albumin solution ($\phi_{S_1T_1} \approx 11\%$) using a picosecond laser double-pulse fluorescence excitation technique. © Elsevier Science S.A.

Keywords: Triplet quantum yield; Intersystem crossing; Indocyanine green; Laser dye IR 125; Fluorescence quantum yield

1. Introduction

The organic dye indocyanine green (cardio-green, ICG) [1] is widely applied in medical diagnosis [2,3]. It was applied recently to photodynamic therapy studies on hepatocarcinoma transplanted rats [4] and of HaCaT keratinocyte cell cultures [5]. High interests of using ICG in photodynamic therapy come from the fact that the dye has its strongest absorption band around 800 nm. In this region, the penetration depth of light into biological tissues is highest [6]. Indocyanine green is used as laser dye [7–9] and as saturable absorber [10] under the name IR 125.

The absorption behaviour of ICG in liquid solution has been studied in detail ([11] and references therein). Some fluorescence spectroscopic analysis was performed ([11] and references therein, [12,13]). No information about intersystem-crossing and the quantum yield of triplet formation for ICG could be found in the literature. But there is some need of knowing the triplet quantum yield of ICG for photodynamic therapy studies [5], since molecules in the triplet state are able to convert ground-state triplet oxygen to singlet oxygen [6,14–16]. Singlet oxygen is an active agent in destroying cancer cells [6,14–17].

Here we determine the yield of triplet formation for indocyanine green dissolved in DMSO, methanol, water, and aqueous albumin solution at room temperature by a recently developed picosecond laser double-pulse fluorescence excitation technique [18]. The technique is based on the fact that in the case of intersystem-crossing the fluorescence induced by the second picosecond excitation is reduced compared to the fluorescence induced by the first picosecond excitation because of transfer of molecules from the fluorescing S_1-state to the non-fluorescing triplet T_1-state within the fluorescence lifetime.

2. Experimental

The experimental arrangement is shown in Fig. 1. A mode-locked ruby laser (laser wavelength 694.3 nm) is used as excitation source [20]. A single pulse is selected from the pulse train and amplified. The amplified pulse (duration $\Delta t_L \approx 35$ ps, energy $W_L \approx 2$ mJ) is split into two parts (first and second excitation pulse). The second excitation pulse is time delayed by 5 ns, using an optical delay line, DL. The second to first pulse energy ratio was set to $W_2/W_1 = 0.7$ by a beam splitting mirror, M, and a filter, F1. The first and the second pulse are overlapped in the sample, S. The fluorescence in sideward direction is collected, passed through a spectrometer, SP, and detected with a microchannel plate photomultiplier, MCP. The photomultiplier output signal is registered with a fast digital oscilloscope. The excitation pulses are slightly focused to the sample cell (lens L1). The input pulse peak energy density is determined by energy transmission measurement through the saturable absorber dye DDI (1,1′-diethyl-2,2′-dicarbocyanine iodide) in methanol [19]. It was tested by transmission measurements that no

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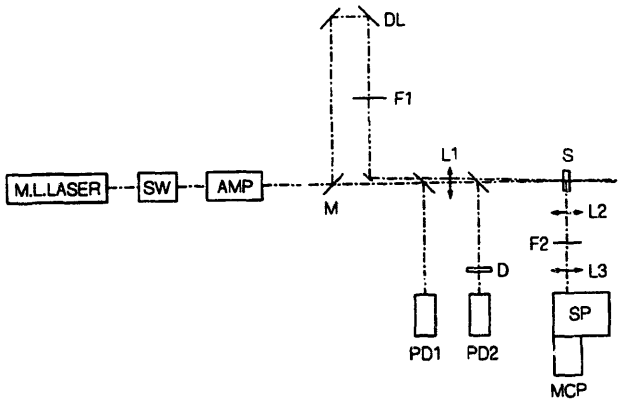

Fig. 1. Experimental arrangement. M.L.LASER, picosecond ruby laser. SW, single pulse selector. AMP, laser amplifier. M, partially reflecting mirror. DL, optical delay line. F1, neutral density filter. L1, lens (focal length f= 1 m). L2, L3, lenses (f= 10 cm and 15 cm). S, sample (length 2 mm). F2, short-wavelength cut-off filter. D, DDI in methanol (small signal transmission 0.03) for energy density detection [19]. PD1, PD2, photodetectors. SP, spectrometer. MCP, microchannel plate photomultiplier (Hamamatsu type R1564U).

degradation of the dye molecules occurred due to laser excitation.

Indocyanine green from Acros Organics, Pittsburgh, PA (IR 125) and indocyanine green sodium iodide from Pulsion Medizintechnik, Munich, Germany (ICG–NaI) were used without further purification. The solvents dimethylsulfoxid (DMSO) and methanol were of analytical grade. H_2O was doubly distilled. The concentration of albumin in water at pH 7.4 was 50 g dm^{-3}. The applied human serum albumin [21] was purchased from Sigma (Fraction V powder, 96–99% albumin). The small signal transmission, T_0, of the indocyanine green samples at the excitation wavelength was set to $T_0 \approx 0.85$.

3. Results

In the experiments the fluorescence signal peaks, $S_{F,m}(1,w_{10})$, caused by the first excitation pulse of peak energy density, w_{10}, and $S_{F,m}(2,w_{20})$ caused by the second excitation pulse of peak energy density, w_{20}, are measured. The ratio $S_{F,m}(2,w_{20})/S_{F,m}(1,w_{10})$ is calculated and compared with numerical calculations of $S_{F,c}(2,w_{20},\phi_{S_1T_1})/S_{F,c}(1,w_{10})$ [18]. The quantum yield of $S_1 \rightarrow T_1$ triplet formation, $\phi_{S_1T_1}$, is found by fitting the theoretical fluorescence peak ratios to the experimental fluorescence peak ratios.

In Fig. 2 the normalised experimental results, $[S_{F,m}(2,w_{20})/S_{F,m}(1,w_{10})]/[S_{F,c}(2,w_{20},\phi_{S_1T_1}=0)/S_{F,c}(1,w_{10})]$, for the investigated dye solutions are plotted versus the normalised first pulse excitation energy density, $w_{10}/w_{sat}^{S_0}$, where $w_{sat}^{S_0}=h\nu_L/\sigma_L$ is the saturation energy density of ground-state depopulation [22] (h, Planck's constant; $\nu_L=c_0/\lambda_L$, laser frequency; σ_L, ground-state absorption cross-section; c_0, vacuum light velocity). Theoretical curves

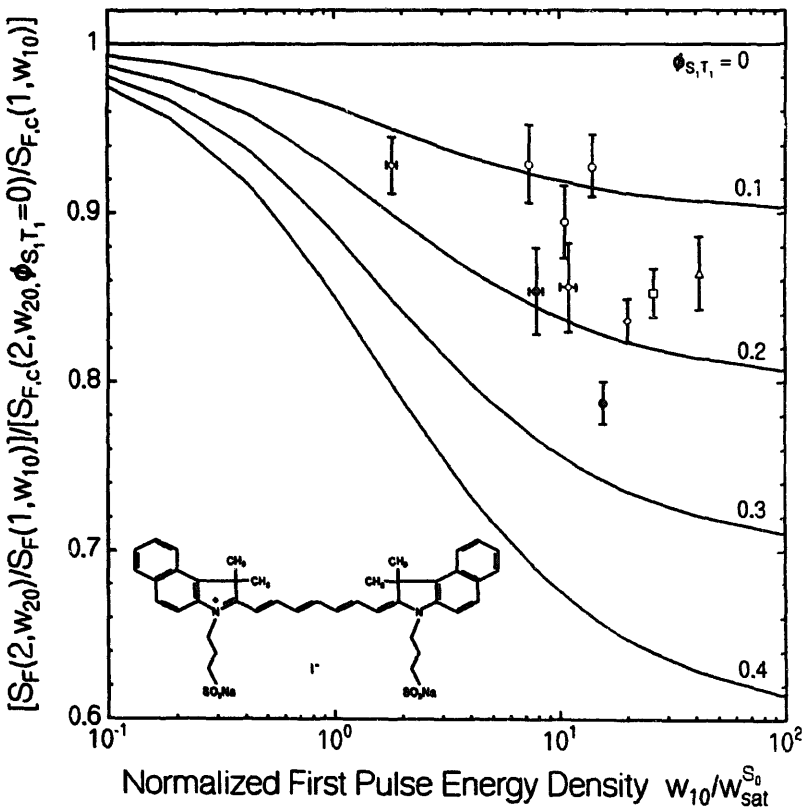

Fig. 2. Fluorescence reduction by intersystem-crossing. Circles, ICG–NaI in aqueous albumin (50 g dm^{-3}). Triangle, ICG–NaI in water. Square, ICG–NaI in methanol. Diamonds, ICG–NaI in DMSO. Dots, IR 125 in DMSO. Error bars indicate standard deviation. The curves are calculated using the theory of Ref. [18]. The structural formula of ICG–NaI is included.

Table 1
Spectroscopic parameters of indocyanine green solutions at room temperature. Wavelength $\lambda_L = 694.3$ nm

Dye	IR 125	ICG–NaI			
Solvent	DMSO	DMSO	Methanol	Water	Aqueous albumin
C [a] (mol dm^{-3})	2.8×10^{-5}	2.8×10^{-5}	1.9×10^{-5}	1.4×10^{-5}	2.7×10^{-5}
σ_L (cm^2)	1.35×10^{-16}	1.35×10^{-16}	2×10^{-16} [11]	2.8×10^{-16} [11]	1.5×10^{-16} [11]
ϕ_F	0.106 ± 0.005	0.106 ± 0.005	0.042 ± 0.002 [11]	0.005 ± 0.001 [11]	0.0038 ± 0.003 [11]
τ_{rad} [b] (ns)	5.5 ± 0.2	5.5 ± 0.2	5.2 ± 0.3 [11]	4.1 ± 0.3 [11]	4.5 ± 0.3 [11]
τ_F [c] (ps)	580 ± 40	580 ± 40	190 ± 10	20 ± 5	200 ± 10
w_{sat}^{So} (J cm^{-2})	2.1×10^{-3}	2.1×10^{-3}	1.4×10^{-3}	1×10^{-3}	1.9×10^{-3}
$\phi_{S_1T_1}$	0.21 ± 0.04	0.17 ± 0.02	0.16 ± 0.02	0.14 ± 0.03	0.11 ± 0.03
$k_{S_1T_1}$ (s^{-1})	$(3.6\pm0.8)\times10^8$	$(2.9\pm0.5)\times10^8$	$(8.5\pm1.5)\times10^8$	$(7\pm3)\times10^9$	$(5.5\pm2)\times10^8$

[a] C, dye concentration.
[b] τ_{rad}, radiative lifetime determined from absorption and fluorescence spectra by use of the Strickler Berg formula [23,24].
[c] $\tau_F = \phi_F \tau_{rad}$.

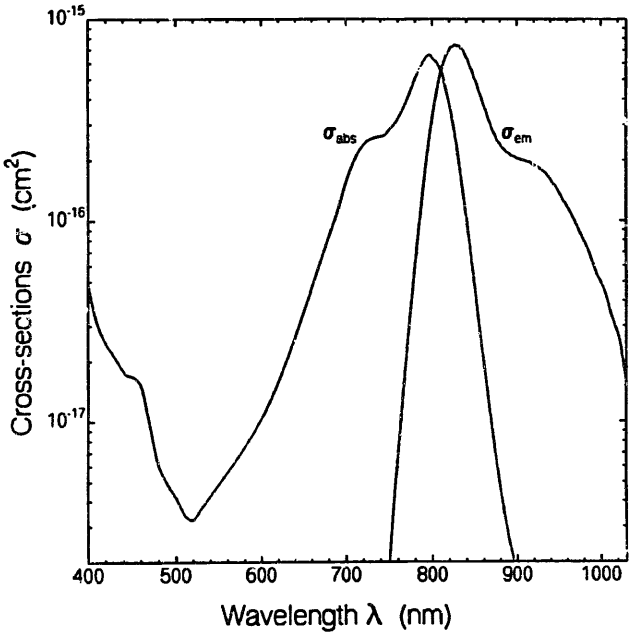

Fig. 3. Absorption cross-section and stimulated emission cross-section spectra of IR 125 in DMSO.

of $[S_{F,c}(2,w_{20},\phi_{S_1T_1})/(S_{F,c}(1,w_{10})]/[S_{F,c}(2,w_{20},\phi_{S_1T_1}=0)/(S_{F,c}(1,w_{10})] = S_{F,c}(2,w_{20},\phi_{S_1T_1})/S_{F,c}(2,w_{20},\phi_{S_1T_1}=0)$ are included in Fig. 2.

The $\phi_{S_1T_1}$ parameters extracted by comparison of the experimental points and the theoretical curves of Fig. 2 are listed in Table 1 together with relevant dye parameters. The absorption cross-section spectrum (from [25]) and the stimulated emission cross-section spectrum (this work, procedure described in [26]) of IR 125 in DMSO are displayed in Fig. 3. The absorption and emission spectra of ICG–NaI in DMSO are identical to the spectra shown in Fig. 3 within our experimental accuracy. The absorption and emission spectra of the other ICG–NaI solutions studied were reported in [11].

4. Discussion

The determined quantum yields of triplet formation are in the range from 10% to 20% for the studied solutions. The strength of intersystem-crossing may be better characterised by the rate of intersystem-crossing, $k_{S_1T_1}$, since $\phi_{S_1T_1}$ depends on the fluorescence lifetime, τ_F. The intersystem-crossing rate is given by

$$k_{S_1T_1} = \frac{\phi_{S_1T_1}}{\tau_F} \quad (1)$$

The obtained $k_{S_1T_1}$-values are included in Table 1. They vary from $k_{S_1T_1} \approx 3\times10^8$ s^{-1} for IR 125 in DMSO via $k_{S_1T_1} \approx 6\times10^8$ s^{-1} for aqueous albumin solution and $k_{S_1T_1} \approx 8\times10^8$ s^{-1} for ICG–NaI in methanol to $k_{S_1T_1}$ 7×10^9 s^{-1} for ICG–NaI in water. The intersystem-crossing rate of ICG–NaI adsorbed to albumin [11] in aqueous solution is approximately a factor of 13 smaller than in pure water. An enhanced intersystem-crossing rate in water compared to other solvents was also reported for hematoporphyrin [27] and eosin Y [18].

In the determination of $\phi_{S_1T_1}$ no back intersystem-crossing from higher excited triplet states to the singlet system is included [28]. Efficient back intersystem-crossing would increase the second pulse fluorescence, and the observed fluorescence reduction would imply larger S_1–T_1 singlet–triplet quantum yields. Efficient back intersystem-crossing requires a depletion of the T_1-state level population by the second excitation pulse. This is not very likely since the saturation energy density of triplet T_1-state depopulation [18], $w_{sat}^{T_1} = h\nu_L \Delta t_L/\sigma_T\tau_T$, is expected to be larger than the second pulse energy density, w_{20}. For a typical triplet absorption cross-section of $\sigma_T = 5\times10^{-17}$ cm^2 [28,29], and a typical triplet state absorption recovery time of $\tau_T = 1$ ps, we estimate $w_{sat}^{T_1} = 0.2$ J cm^{-2} [28,29] while the second pulse energy density was of the order of 0.002 to 0.02 J cm^{-2}.

5. Conclusion

The intersystem-crossing of indocyanine green in some solvents has been investigated. The measured quantum yields of triplet formation in the range from 10% to 20% are suffi-

ciently high for efficient singlet oxygen generation needed for cell destruction in photodynamical cancer therapy [5].

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