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Singlet oxygen produced by quasi-continuous photo-excitation of hypericin in dimethyl-sulfoxide



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ABSTRACT

Singlet oxygen ($O_2(^1\Delta_g)$) production by photo-excited hypericin (Hyp) dissolved in dimethyl-sulfoxide (DMSO) was studied by means of time-resolved phosphorescence measurements. In order to minimize photo-bleaching, the samples were excited in quasi-continuous mode using long-pulse (35 μ s) laser excitation. The measured lifetime of singlet oxygen is $\tau_\Delta = 5.5 \pm 0.3 \mu$ s. This result helps to resolve the discrepancy existing in the literature concerning singlet oxygen lifetime in DMSO. The obtained quantum yield of singlet oxygen photosensitized by Hyp in air-saturated DMSO is $\Phi_\Delta = 0.4 \pm 0.03$. The rate constant for Hyp triplet state depopulation in reaction with ground state molecular oxygen is measured to be $k_q = 1.6 \pm 0.3 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$.

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1. Introduction

Singlet oxygen is a highly reactive molecule which causes oxidation of many biologically important compounds [1]. From medicinal point of view, singlet oxygen has been studied intensively because of its essential role in photodynamic therapy (PDT) of cancer. During PDT, the oxidative stress induced by reactive oxygen species (in most cases by singlet oxygen in the $O_2(^1\Delta_g)$ state) initiates processes leading to cell death [2,3]. Singlet oxygen is mostly produced by energy transfer between light-activated (triplet state) photosensitizer molecules and ground state (triplet, $O_2(^3\Sigma_g^-)$) oxygen molecules [1]. The rate of singlet oxygen deactivation shows significant variation depending on the environment [4]. Singlet oxygen lifetime values ranging from a few microseconds (e.g. 3–4 μ s in water) to milliseconds (e.g. 59 ms in air-saturated CCl_4) were reported for different pure solvents [4,5].

Dimethyl-sulfoxide (DMSO) is clinically used as a penetration enhancer to carry drugs into tissues [6,7] and is known as an efficient solvent for many hydrophobic compounds. Due to these reasons, the information on singlet oxygen properties in DMSO is highly important. In spite of the large number of singlet oxygen lifetime data published during the last four decades, relatively few papers deal with the deactivation rate of singlet oxygen in DMSO.

Moreover, the available data scatter significantly. Lifetime values of 1.8 μ s [8], 5.6 μ s [9] and 30 μ s [10] were published by other authors, while a lifetime value of 19 μ s can be calculated from the quenching rate reported in [11]. One of the objectives of the present work is to provide additional experimental data that help to clarify the contradiction between the previous reports.

Singlet oxygen in the $O_2(^1\Delta_g)$ state can be detected through its phosphorescence around 1270 nm. The lifetime of singlet oxygen is readily determined by time-resolved phosphorescence decay measurements following pulsed photo-activation of photosensitizer molecules [12]. Most of the previous experiments were carried out with short-pulse laser excitation using laser pulses in the nanosecond or sub-nanosecond range (see e.g. [1,12–17]). An alternative excitation scheme using longer (microsecond) laser pulses was applied by Lee and co-workers [18,19]. In our previous work, the duration of the laser pulses was set long enough so that steady-state concentrations of the photosensitizer's triplet state and $O_2(^1\Delta_g)$ were reached at the end of the laser pulse [20]. The major advantage of this so-called quasi-continuous excitation scheme was that the peak laser power was reduced significantly when compared to laser pulses of nanosecond duration, which resulted in a lower bleaching rate of the used photosensitizers. Moreover, steady-state conditions at the end of the laser pulse allowed for analytical solution of the differential equations describing the time-evolution of $O_2(^1\Delta_g)$ concentration after the excitation pulse [20].

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Photo-excited hypericin (Hyp) was used as a source of the singlet oxygen in this work. Hyp is a naturally occurring potent photosensitizer that is being studied extensively for its possible application in PDT [21–25]. Under light illumination Hyp causes anti-proliferative and cytotoxic effects in many tumor cell lines. Singlet oxygen is supposed to play the major role in the photo-activity of Hyp at aerobic conditions. $O_2(^1\Delta_g)$ generation via energy transfer from Hyp triplet state (^3Hyp) to ground state oxygen molecules was theoretically predicted [26] as well as experimentally confirmed in several organic solvents and lipid environments [27–29]. The corresponding quantum yields for singlet oxygen formation by Hyp vary between 0.25 and 0.45 [27–29]. Stock solutions of Hyp used in PDT-related experiments are often prepared in DMSO [30–33]. On the other hand, Hyp forms non-fluorescence aggregates in aqueous environment [21,34]. These aggregates are not able to produce singlet oxygen [15,35].

In the present work, besides the determination of singlet oxygen lifetime in DMSO, we report also on the rate of ^3Hyp deactivation by oxygen in DMSO and the quantum yield of singlet oxygen production by Hyp in this solvent.

2. Experimental

Hypericin, Rose Bengal, dimethyl-sulfoxide (> 99.9%), RuPhen (dichlorotrakis(1,10-phenanthroline) ruthenium(II) hydrate) and protoporphyrin IX disodium salt (PpIX) were purchased from Sigma-Aldrich.

The purity of DMSO was checked by measuring its freezing point before the experiments. The results (17.5 ± 0.5 °C) indicated water content of about 0.5 wt%. According to the work of Staicu et al. [9], the effect of this water content on singlet oxygen lifetime in DMSO is below the error range of the present experiments.

The experimental apparatus used for time-resolved phosphorescence measurements has been described in details previously [20]. Briefly, the excitation laser beam (532 nm, Cobolt Samba) passed vertically through a cuvette ($1 \times 1 \times 4$ cm) filled with 2 ml of the sample solution. The cuvette holder was thermostated at 22 °C. The sample was continuously flushed with a nitrogen/oxygen mixture (at 25 standard cubic centimeter per minute, sccm) and was stirred by a magnetic stirrer. An acousto-optic modulator (AOM, Isomet 1205C) was used to switch the excitation laser beam (on/off) creating 35 μs long laser pulses with a repetition rate of 8 kHz. The average laser power on the sample was 1.4 mW. The switching time of the AOM was approx. 400 ns. The emitted phosphorescence light was passing through a 1250–1350 nm bandpass filter and was detected with a Hamamatsu H10330A-75 photomultiplier tube (PMT) operated in photon counting mode. The time evolution of the photon rate was acquired with a multichannel scaler (Becker & Hickl, MSA-300). Typically, a signal of $3 \cdot 10^6$ pulses was collected at each experimental condition. The obtained time-dependences were corrected for the phosphorescence background of the optical components as measured in pure DMSO.

In case of RuPhen measurements the sample was excited by a 476 nm (Coherent 90 C FreD) laser. The repetition rate of 20 μs long laser pulses was set to 2 kHz and the average laser power at the sample was 0.7 mW.

The concentration of oxygen in the sample was calculated from the gas-phase oxygen pressure [36]. Rose Bengal (RB) was used to calibrate the experimental apparatus for quantum yield measurements of singlet oxygen production (see below). The calibration was carried out with a RB solution (in DMSO) having the same absorbance (0.022 per cm at 532 nm) as the used sample of Hyp. The quantum yield of singlet oxygen production by RB in DMSO

was estimated to be 0.76 (see also [37]) for air-saturated conditions.

The lifetime data obtained with quasi-continuous excitation were compared with those measured by conventional short-pulse experiments. The apparatus was equipped with a Q-switched Nd:YAG laser (Spectra-Physics Quanta-Ray INDI-40-10) pumping an OPO (optical parametric oscillator, Spectra-Physics basiScan) that was tuned to 532 nm. The repetition rate of the 5–7 ns long laser pulses was 10 Hz producing an average beam power of 1.2 mW at the sample. The phosphorescence signal of 3000 pulses was collected at each experimental condition.

3. Data analysis

The decay of the $O_2(^1\Delta_g)$ concentration $[\Delta]$ after long pulse (quasi-continuous) excitation is described by [20]:

$$[\Delta] = \frac{S_0 \tau_\Delta}{\tau_\Delta - \tau_T} \left(\tau_\Delta e^{-\frac{t}{\tau_\Delta}} - \tau_T e^{-\frac{t}{\tau_T}} \right), \quad (1)$$

where S_0 is the rate of $O_2(^1\Delta_g)$ production at steady-state conditions, and τ_Δ and τ_T are the lifetimes of $O_2(^1\Delta_g)$ and the photosensitizer's triplet state, respectively. The $O_2(^1\Delta_g)$ phosphorescence intensity detected at 1270 nm is proportional to $[\Delta]$ through an equipment specific factor c and the $O_2(^1\Delta_g)$ phosphorescence emission rate constant k_Δ^e :

$$I_{\text{meas}}^{1270} = ck_\Delta^e [\Delta] = F \frac{\tau_\Delta}{\tau_\Delta - \tau_T} \left(\tau_\Delta e^{-\frac{t}{\tau_\Delta}} - \tau_T e^{-\frac{t}{\tau_T}} \right), \quad (2)$$

where $F = S_0 k_\Delta^e c$. The given time dependence differs from the kinetic curves used to analyze short-pulse (nanosecond) experiments [12], where (following the short excitation pulse) the phosphorescence signal first increases, then reaches its maximum and finally decays. By contrast, dependence (2) gives a monotonous decrease of the phosphorescence intensity with its time derivative being zero at $t=0$ (right after the excitation pulse). Fig. 1 shows a typical experimental $O_2(^1\Delta_g)$ phosphorescence decay curve obtained with quasi-continuous excitation of Rose Bengal dissolved in DMSO using 35 μs excitation pulses. Rose Bengal was chosen as a photosensitizer here because (unlike Hyp) it has a low level of fluorescence at 1270 nm. Right after turning the laser on (at $t = -35$ μs in Fig. 1) the fluorescence of RB appears instantaneously. The onset of the fluorescence is mostly limited by the switching time of the AOM. During the laser pulse the signal increases due to a gradually increasing contribution from singlet oxygen phosphorescence, which reaches steady-state intensity at the end of the pulse. When the laser is switched off (at $t=0$) the

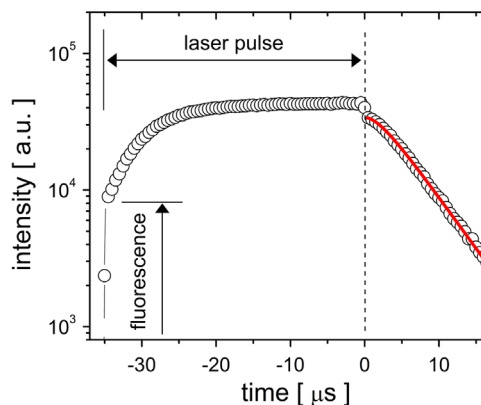


Fig. 1. Typical time-dependence of the emission signal detected around 1270 nm during long-pulse excitation of Rose Bengal (not all points are shown). The red curve represents data fit according to (2). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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