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Luminescence Study of Singlet Oxygen Production by Meso-Tetraphenylporphine

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The research in the field of the photodynamic therapy of cancer (PDT) is focused on a development of photosensitizers exhibiting high quantum yield of singlet oxygen production. Direct time-resolved spectroscopic observation of singlet oxygen phosphorescence can provide time constants of its population and depopulation as well as photosensitizer phosphorescence lifetime and relative quantum yields. In our contribution, a study of time and spectral resolved phosphorescence of singlet oxygen photosensitized by meso-tetraphenylporphine in acetone together with the photosensitizer phosphorescence is presented. Time constants of singlet oxygen population and depopulation were determined at wide range of photosensitizer concentrations. The time constant of singlet oxygen generation (0.28 ± 0.01) μs is slightly shorter then the lifetime of photosensitizer's triplet state (0.32 ± 0.01) μs. It is caused by lower ability of TPP aggregates to transfer excitation energy to oxygen. The lifetime of singlet oxygen ($\approx$50 μs) decreases with increasing photosensitizer concentration. Therefore, the photosensitizer acts also as a quencher of oxygen singlet state, similarly to the effects observed in [A. A. Krasnovsky, P. Cheng, R. E. Blankenship, T. A. Moore, and D. Gust (1993). *Photochem. Photobiol.* **57**, 324–330; H. Küpper, R. Dědic, A. Svoboda, J. Hála, and P. M. H. Kroneck (2002). *Biochim. Biophys. Acta Gen. Subj.* **1572**, 107–113]. Moreover, the increasing concentraion of the photosensitizer causes a slight hypsochromic shift of the singlet oxygen luminescence maximum.

KEY WORDS: TPP; singlet oxygen; photodynamic therapy; photosensitizer.

INTRODUCTION

Photodynamic therapy of cancer (PDT) is a progressive method of fighting cancer. Basic principle of PDT is a photogeneration of highly reactive singlet oxygen via excitation energy transfer from so-called photosensitizer. Singlet oxygen is a very strong oxidizer that causes necrosis of the tumour tissue [1]. After photosensitizer administration to the patient's body, it is selectively retained in cancerous tissue. The tumour is then locally irradiated by light absorbed by photosensitizer molecules. Part of photosensitizer molecules undergo intersystem crossing to their triplet state, which can effectively transfer excitation energy to oxygen molecules bringing them into their singlet excited state.

Meso-tetraphenylporphine (TPP) is a hydrophobic acetone soluble photosensitizer. It is sometimes considered to be a common standard for comparing photophysical properties of other photosensitizers. Its quantum yield of singlet oxygen production is 0.65 [2].

In this contribution, a unique experimental set up for measurement of infrared luminescence with simultaneous spectral and time resolution was used for in vitro investigation of concentration dependencies of TPP phosphorescence, and singlet oxygen photogeneration together with phosphorescence of singlet oxygen. Basic spectral properties of TPP were also studied by means of absorption and fluorescence spectroscopy.

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MATERIALS

Meso-tetraphenylporphine was obtained from Frontier Scientific Porphyrine Products. Air saturated acetone was used as a solvent (Lachema, p.a.). Concentration of O_2 in air-saturated acetone is 2 mM [3]. Concentration of TPP in our samples ranged from 1 to 200 μM.

METHODS

TPP absorption, fluorescence, and phosphorescence together with singlet oxygen phosphorescence measurements were carried out. Absorption spectra were measured in standard 1 cm to 0.1 mm spectroscopic cuvettes using Perkin Elmer Lambda 12 spectrometer with 1 nm resolution. Fluorescence spectra were measured in 1 cm fluorescence cuvettes with optically polished bottom. The samples were excited through cuvette's bottom by excimer laser (Lambda Physics LPX 105ICC) pumped dye laser (Lambda Physics FL 3001, Stilbene 3 in methanol). Dye laser beam was focused to a spot of 0.25 mm diameter. The excitation energy ranged from 20 to 30 μJ per puls, with repetition frequency 40 Hz at 420 nm. Duration of the laser pulses was approximately 20 ns. Fluorescence was collected perpendicularly to excitation beam using an optic fibre to multichannel spectrophotometer Avantes S2000 (spectral resolution 1 nm).

Measurements of TPP and singlet oxygen phosphorescence were performed using the same excitation as in fluorescence experiments. IR phosphorescence was spectrally resolved by monochromator Jobin Yvon H20 IR together with two long-pass filters Schott RG 7. Spectral width of monochromator's slit was 16 nm. High IR sensitivity and time resolution of 5 ns was achieved by cooled IR sensitive Hamamatsu R5509 photomultiplier together with Becker-Hickl HF AC-26 dB preamplifier and Becker-Hickl MSA 200 photoncounter. Time and spectral resolved emission of samples was measured between 750 and 1342 nm with 16 nm step to observe TPP and singlet oxygen phosphorescence together (2000 accumulations at each wavelength). The detail of singlet oxygen phosphorescence (Fig. 2) was measured in the region from 1242 to 1306 nm with 4 nm step (4000 accumulations at each wavelength). All fluorescence and phosphorescence data were corrected with respect to spectral sensitivity.

RESULTS AND DISCUSSION

Typical absorption, fluorescence and phosphorescence spectrum of TPP together with singlet oxygen phosphorescence spectrum is displayed at Fig. 1. Extinction coefficients at 420 nm exhibit linear dependence on TPP concentration with a slope of 118000 cm^{-1} $mol^{-1}l$ in the whole concentration region (from 1 to 200 μM). This linear dependence excludes any changes in aggregate to

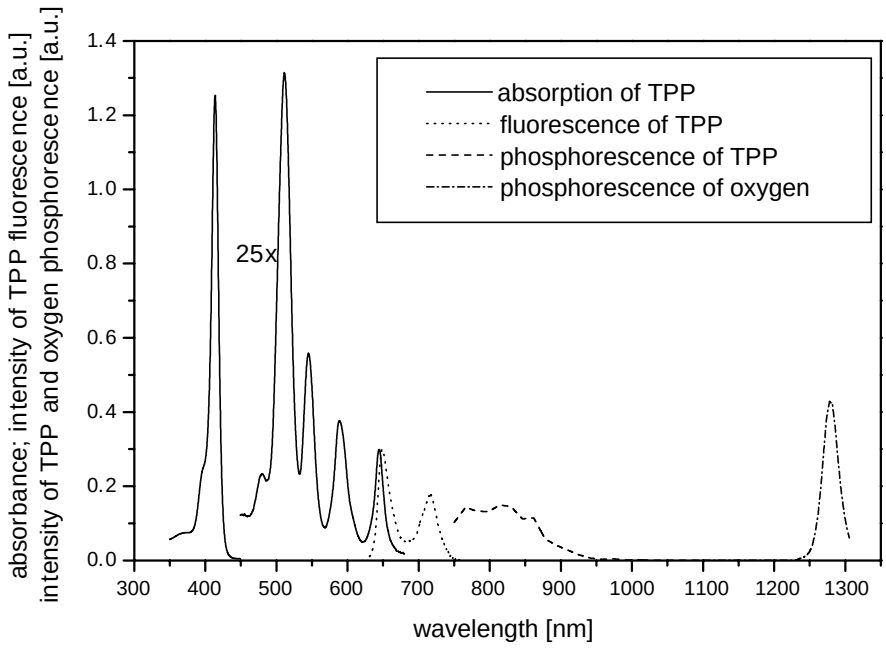

Fig. 1. Typical spectra of 5 μM TPP in air saturated acetone.

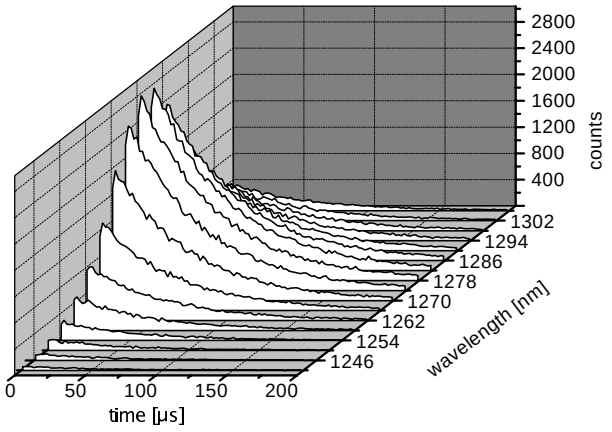

Fig. 2. Time and spectral resolved phosphorescence of singlet oxygen. Its maximum lies at 1280 nm. (5 μM concentration of TPP, time resolution 2 μs).

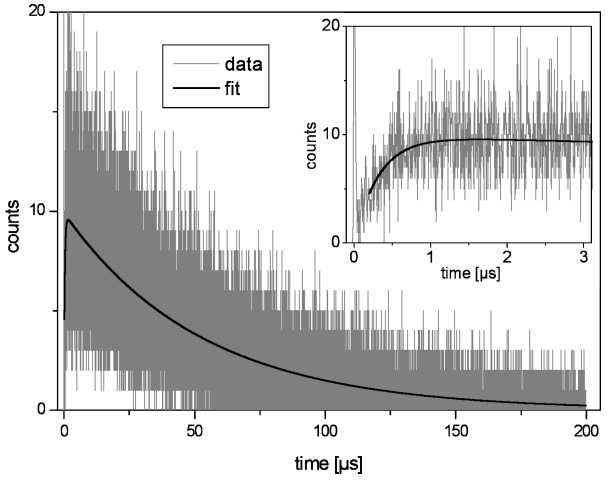

Fig. 3. A typical kinetics of singlet oxygen phosphorescence: the whole measured dependence and a detail of initial rise after excitation. (30 μM concentration of TPP, wavelength: 1278 nm, 5 ns time resolution).

monomer concentration ratio in the studied concentration region. This fact is further confirmed by fluorescence. Reabsorption in optically dense samples was observed at fluorescence spectra but no other changes in spectral positions and shapes of two main fluorescence bands (649 and 717 nm) were found.

Concerning TPP phosphorescence, no concentration induced shifts were observed. Time dependence of phosphorescence intensity $I_{\mathrm{TPP}}(t)$ was fitted by monoexponential decay curve. It provided the lifetime of TPP triplet state, which exhibits neither emission wavelength nor TPP concentration dependence. Averaging of obtained values leads to triplet state lifetime (0.32 ± 0.01) μs.

Figure 2 contains time and spectral resolved singlet oxygen phosphorescence. Wavelength of singlet oxygen emission maximum was reported to occur at 1270 nm [2,3]. Nevertheless, in this study all emission maxima were found to lie at slightly longer wavelengths (about 1280 nm). Moreover, hypsochromic shift of singlet oxygen emission bands with increasing TPP concentration was observed (from 1286 to 1274 nm). Our value is more accurate thanks to substantially better experimental equipment (in sense of spectral resolution and detection sensitivity).

After excitation pulse singlet oxygen emission intensity $I_{\mathrm{SO}}(t)$ follows time evolution described by Eq. (1) [4]

$$I_{\mathrm{SO}}(t) = I_0(-e^{-t/t_1} + e^{-t/t_2}), \qquad (1)$$

where t_2 is the lifetime of singlet oxygen in acetone, t_1 is singlet oxygen generation time constant. Equation (1) embodies singlet oxygen emission rise and decay after the excitation pulse (See Fig. 3).

Fitting of Eq. (1) to measured singlet oxygen kinetics provided t_1 and t_2 values for each TPP concentration. As in TPP phosphorescence measurements, t_1 does not depend on photosensitizer concentration with average value of (0.28 ± 0.01) μs. This value is slightly lower than TPP triplet lifetime. The explanation of this difference assumes that aggregates have lower ability to transfer energy to oxygen and thus the effective TPP triplet lifetime is longer. Singlet oxygen lifetime t_2 exhibits decrease with increasing concentration of TPP. Corresponding rate constant $k_2 = 1/t_2$ depends linearly on TPP concentration (Fig. 4) which fits well with bimolecular quenching of singlet oxygen by TPP described by Stern Volmer equation [5]:

$$k_2(c_{\mathrm{TPP}}) = k_2(0) + k_{\mathrm{q}} \cdot c_{\mathrm{TPP}}, \qquad (2)$$

where $k_2(0)$ is the rate constant of singlet oxygen deexcitation in pure acetone (without TPP), k_{q} is a quenching

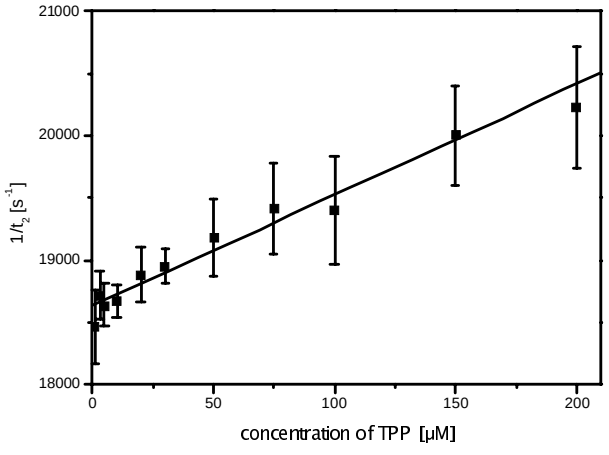

Fig. 4. Linear dependence of rate constant $1/t_2$ indicates quenching of singlet oxygen by TPP.

constant and c_{TPP} is the concentration of TPP. Linear fit provides value of $k_2(0) = (18630 \pm 70)$ s^{-1}. It corresponds to the lifetime of singlet oxygen in pure acetone $t_2(0) = (53.7 \pm 0.2)$ μs. The quenching constant $k_q = (8.9 \pm 1.5) \times 10^6$ M^{-1} s^{-1}. The quenching is attributed to charge transfer complex formation between the photosensitizer and oxygen molecule leading to a fast nonradiative deexcitation [3,4,6].

CONCLUSIONS

Absorption and fluorescence measurements proved that for TPP dissolved in acetone aggregate to monomer concentration ratio remains constant for concentrations up to 200 μM. Phosphorescence spectra of TPP at concentration range from 1 to 200 μM exhibit phosphorescence maxima at 815 nm. Lifetime of TPP triplet state in air-saturated acetone is (0.32 ± 0.01) μs. Singlet oxygen phosphorescence rise time is (0.28 ± 0.01) μs. Both these values are independent of TPP concentration. Singlet oxygen phosphorescence maximum is situated around 1280 nm. The lifetime of singlet oxygen is decreasing with rising TPP concentration due to its quenching caused by charge transfer complex formation between TPP and singlet oxygen. The quenching constant value is $(8.9 \pm 1.5) \times 10^6$ M^{-1} s^{-1} and singlet oxygen lifetime in acetone extrapolated to zero TPP concentration is (53.7 ± 0.2) μs.

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