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SINGLET MOLECULAR OXYGEN PHOTOSENSITIZATION UPON TWO-PHOTON EXCITATION OF PORPHYRIN IN AQUEOUS SOLUTION

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The high photodynamic activity of the water-soluble porphyrins is well documented. However, use of the water-soluble porphyrins as the photosensitizers in photodynamic therapy (PDT) is of limited usefulness because of the insufficient absorbance in the red wavelength region, where the tissues are transparent. The process by which these molecules can overcome these restrictions is the two-photon excitation (TPE). Up until now, this process is considered as being too inefficient and having no practical interest. In this study the two-photon absorptivity and singlet oxygen photosensitization by 5, 10, 15, 20-tetrakis-(4-N-methylpyridyl)-21H, 23H-porphin in aqueous solution have been examined directly. Two-photon absorption cross-section σ_{TPA} shows the value ranging from 60 up to 180 GM (Göppert-Mayer units), when tuning the excitation wavelength from 800 to 730 nm. This absorbance at the blue side of the one-photon Soret band (B-band) is found to be due to two-photon allowed excitation into the state of even parity (i. e. $g \rightarrow g$ transition). TPE into Q-states is parity forbidden ($g \rightarrow u$ transitions), and σ_{TPA} does not exceed 6 GM over 1100–1400 nm excitation wavelength range. TPE of porphyrin at 780 nm in air-saturated aqueous (D_2O) solution results in efficient singlet molecular oxygen ($^1\Delta_g$) photosensitization, which is detected by its $^1\Delta_g \rightarrow {}^3\Sigma_g^-$ luminescence. Our findings prove the applicability of the TPE in photodynamic therapy and allow determining the requirements to the two-photon absorptivity of photosensitizer to be used.

Keywords: water-soluble porphyrins, two-photon absorption, photosensitization, singlet oxygen, photodynamic therapy

PACS: 42.65.-k, 33.W2

1. Introduction

Water-soluble cationic 5, 10, 15, 20-tetrakis-(4-N-methylpyridyl)-21H, 23H-porphine (Fig. 1), hereafter referred as H_2TMPyP^{4+}, and its metallated complexes are extensively studied during the last two decades. Interest in these compounds is called mainly due to their high photodynamic activity and capability to form complexes with proteins and DNA [1–7]. It has been noted that the photocytotoxicity of these compounds exceeds that of anionic and neutral porphyrins. In particular, H_2TMPyP^{4+} is much more efficient in the induction of single- and double-strand breaks in ColE1 plasmids than anionic porphyrins, including hematoporphyrin derivatives [2]. Cationic H_2TMPyP^{4+} photoinduces direct inactivation of Gram-positive (G+) and Gram-negative (G-) bacteria, thereby differing from neutral and anionic porphyrins that can photosensitize only after permeating the outer membrane of G- bacteria [8]. So far, the use of H_2TMPyP^{4+} as a

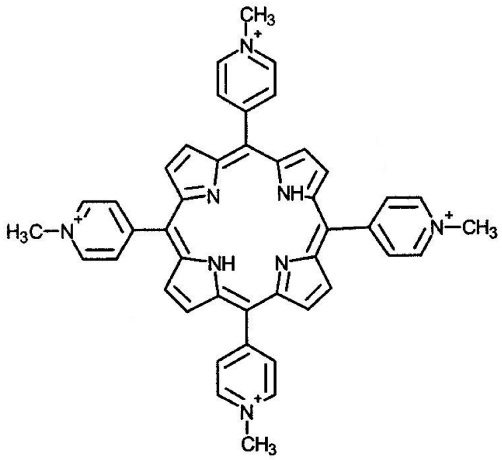

Fig. 1. Structure of H_2TMPyP^{4+} porphyrin.

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photosensitizer in photodynamic therapy has been limited by the fact that its lowest absorption band, centred at 638 nm in aqueous solution, has a small absorbance ($\varepsilon_{638} = 1300\,\mathrm{M}^{-1}\mathrm{cm}^{-1}$) [9]. Also, the above wavelength is out of tissue transparency window (750–1000 nm), which makes one-photon photoexcitation *in vivo* difficult [10–12].

To overcome this common for the porphyrin photosensitizers difficulty, many efforts have been made to synthesize new compounds with strong absorption band shifted to 750–850 nm [11, 12]. Alternatively, this difficulty can be overcome by means of two-photon excitation [13–15], where the excitation photon energy equals one half of the transition energy. Then the excitation light with a wavelength corresponding to the tissue transparency window can be used, thus providing for a longer penetration depth. Since the efficiency of TPA is proportional to the instantaneous radiation intensity squared [16], this effect can be dramatically enhanced by using the laser pulses of short duration. By applying modest average power in the form of one–two hundred femtosecond duration pulses, the efficiency of two-photon excitation can be brought close to that of one-photon excitation. It is evident that the peak power of the ultrashort pulses is much higher as compared to that of longer (hundreds of ps – tens of ns) laser pulses with the same energy. Nevertheless, the tissue damage threshold is higher in this case [15], thus proving the use of ultrashort pulse laser excitation in a variety of biological applications [15, 17, 18].

To be of practical interest, the photosensitizer needs to have a large probability of TPE. Early papers reported rather small σ_{TPA} values of 1–10 Göppert-Meyer (1 GM = $1{\cdot}10^{-50}$ cm^{4}s per photon) for tetrapyrrolic photosensitizers [19, 20]. For this reason, it was traditionally assumed that TPA in porphyrins had a little practical value for PDT applications if any. Recently, for the porphyrin molecules the two-photon absorption cross-section amounting up to 1600 GM has been reported by our group [21–26]. With some of these compounds we demonstrated the possibility of efficient two-photon photosensitized production of singlet molecular oxygen [21, 24–26]. In these papers we have demonstrated that efficient two-photon excitation of tetrapyrrolic compounds could be achieved by using an allowed transition between the states of the same parity, i. e. $g \rightarrow g$ transition. This way of excitation turned out to be possible due to the specific energy level ordering in tetrapyrrolic compounds, possessing excited states of g parity much above the first excited singlet S_1-state [27].

In the experimental studies presented here we deal with water-soluble porphyrin whose photodynamic activity is well known. We report the absolute value of two-photon absorption cross-section for H_2TMPyP^{4+} porphyrin in aqueous solution in different spectral regions. The measurements of the singlet molecular oxygen photosensitization have been done for solutions in heavy water with excitation by the commercial femtosecond mode-locked Ti:sapphire laser at 780 nm. Two-photon excitation of the H_2TMPyP^{4+} porphyrin results in efficient singlet molecular oxygen $^1\Delta_g$ photosensitization, which is detected by means of its $^1\Delta_g \rightarrow {}^3\Sigma_g^-$ luminescence. On the basis of the obtained results we discuss the two-photon excitation scheme for PDT as well as the requirements imposed on the σ_{TPA} value of the porphyrin photosensitizer to meet PDT criteria.

2. Materials and methods

2.1. Sample preparation

The tetratosylate salt of 5, 10, 15, 20-tetrakis-(4-N-methylpyridyl)-21H, 23H-porphin was purchased from Aldrich and has been used as received. We carefully examined its absorbance and fluorescence properties and found that there was no evidence of either chlorin derivative or some other impurities. Deionized H_2O was obtained using standard procedure. Deuterium oxide D_2O (99.96% D) was obtained from Aldrich. All measurements were carried out in rectangular 1×1 cm^2 quartz cells in air-equilibrated solutions at 293 K. Electronic absorption spectra were recorded using Lambda 900 Perkin Elmer spectrophotometer. Sample concentrations were $\sim1{\cdot}10^{-5}$ M (= mol/litre) and have been determined spectrophotometrically using known extinction coefficients [9, 28].

2.2. Instrumentation

The laser system comprising a mode-locked Ti:sapphire laser (Coherent Mira 900) and a Ti:sapphire regenerative amplifier system (Clark MRX CPA-1000) operating at 1 kHz repetition rate was used for excitation. The amplified pulses had duration of 150 fs and energy of 0.8 mJ at 780 nm. Tuning of the excitation wavelength was achieved with an optical parametric amplifier, OPA (TOPAS, Quantronix), which converted 780 nm pulses to near infrared (1100–1600 nm). Glass colour filters were used to cut off any residual light from the OPA. Pulse duration and temporal profile were measured with an autocorrelator. Laser spectrum

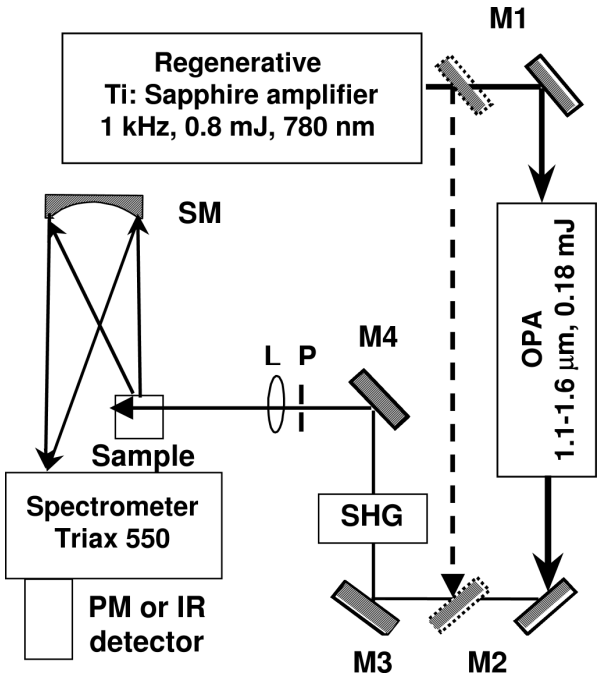

Fig. 2. Sketch of experimental setup: L is lens, P is pinhole, M1–M4 are mirrors, SM is spherical mirror, OPA is optical parametric amplifier, SHG is nonlinear crystal for generation of the second harmonics, PM is photomultiplier.

was recorded with a Lambda 900 Perkin Elmer spectrophotometer coupled with an optical fiber, in which the laser beam was collected.

Samples were irradiated at energy in the range of 5–50 μJ/pulse. The laser beam was slightly focused with an $f = 50$ cm spherical lens. The focal point was out from cell, providing the 1.5 mm diameter of the beam in the sample cell. The pinhole with a radius much smaller than laser beam diameter was placed in front of the sample, that is why a variation in intensity over the beam cross-section could be neglected.

2.3. TPA cross-section measurements

TPA cross-section has been measured by a method consisting in the comparison of the sensitizer fluorescence intensity under two- and one-photon excitation [19, 24, 29]. The fluorescence emitted at a right angle to the excitation was collected by a concave mirror and focused onto entrance slit of TRIAX 550 Jobin–Yvon/Spex monochromator (Fig. 2). The signal was measured with a Hamamatsu photomultiplier coupled with a lock-in amplifier.

The measurements were performed at 1 kHz pulse repetition rate of the laser system. Under these conditions with two-photon excitation the fluorescence intensity was verified to depend quadratically on the illumination power. This fact means that all the possible effects due to population bottleneck and/or excited state absorption of the porphyrin should be ruled out.

The values of the TPA cross-section σ_{TPA} have been calculated according to the following formula [24]:

$$\sigma_{\mathrm{TPA}} = \frac{\sqrt{2}\pi^{3/2}}{\sqrt{\ln 2}} \frac{F_{\mathrm{TPA}} \nu_{\mathrm{TPA}} \tau r_0^2 E_{\mathrm{OPA}}}{F_{\mathrm{OPA}} \nu_{\mathrm{OPA}} E_{\mathrm{TPA}}^2} \sigma_{\mathrm{OPA}} ,$$

where F_{TPA} and F_{OPA} are the fluorescence intensities upon two-photon and one-photon excitation, ν_{TPA} and ν_{OPA} are the frequencies of the two-photon and one-photon excitation light, τ is a pulse duration (FWHM), r_0 is the laser beam (pinhole) radius, E_{OPA} and E_{TPA} are the energies of laser pulse used for one- and two-photon excitation, and σ_{OPA} is the linear one-photon absorption cross-section at the frequency ν_{OPA}.

2.4. Singlet oxygen luminescence measurements

Steady-state spectra of $^1\Delta_g \rightarrow {}^3\Sigma_g^-$ molecular oxygen luminescence were measured with a liquid nitrogen-cooled Ge-detector coupled with a lock-in amplifier and TRIAX 550 Jobin–Yvon/Spex monochromator. The sample concentration, position of the cell, as well as the laser pulse duration and power were exactly the same as in the case of TPA cross-section measurements, thus providing the same way of porphyrin photoexcitation, i. e. we only replaced the photomultiplier tube by IR-detector to detect the $^1\Delta_g \rightarrow {}^3\Sigma_g^-$ luminescence of molecular oxygen. Singlet oxygen formation is due to the energy transfer from photoexcited porphyrin molecule. Thus, if the photosensitizer is excited in a two-photon process, the photosensitization has the same law. To facilitate the singlet oxygen luminescence detection, D_2O was used as a solvent instead of H_2O because in the former case singlet oxygen possesses much longer $^1\Delta_g$ lifetime [30]. The dissolved oxygen concentration in both D_2O and H_2O is the same ($2.8{\cdot}10^{-4}$ M).

A number of control experiments have also been performed. Specifically, singlet oxygen signals were not observed under the same excitation conditions neither upon porphyrin sample bubbling with nitrogen (leading to decrease in dissolved oxygen concentration), nor from free D_2O. We were also unable to reveal $^1\Delta_g \rightarrow {}^3\Sigma_g^-$ luminescence from porphyrin solution of the same concentration prepared in H_2O, because in such a case its intensity was much lower, and sensitivity of our instrument was not sufficient to detect it.

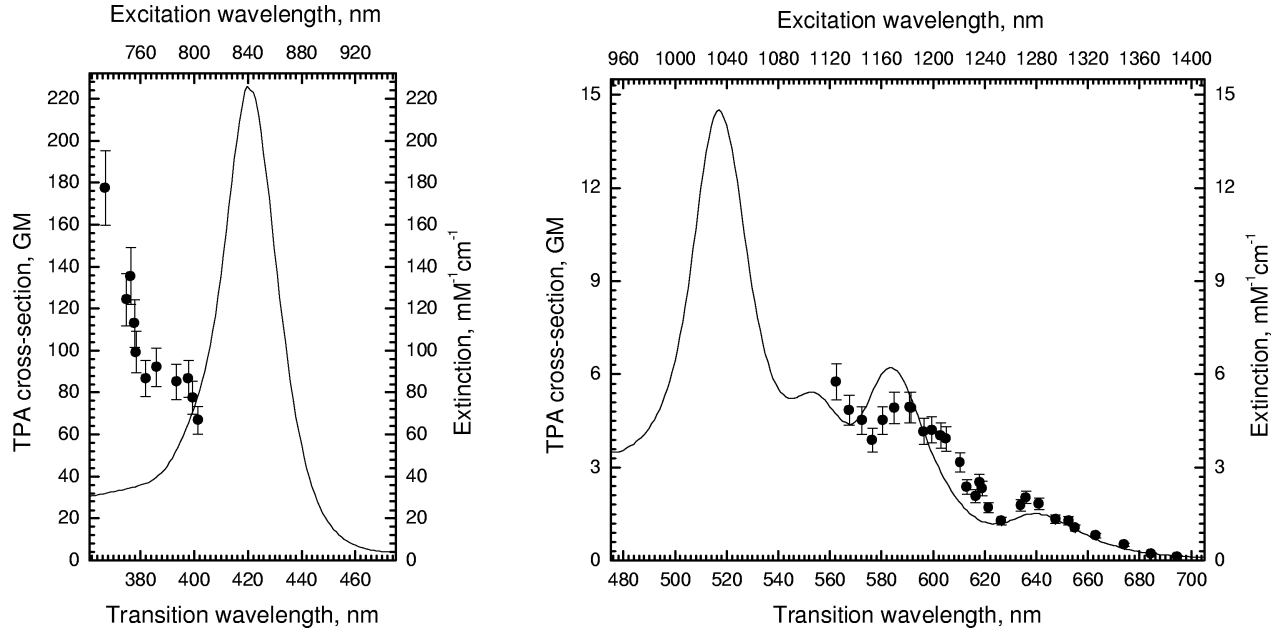

Fig. 3. H_2TMPyP^{4+} porphyrin two-photon absorption spectra (closed circles and left scale) in (a) Soret band and (b) Q-band regions. Solid line is the corresponding linear absorption spectrum (right scale).

3. Results and discussion

3.1. Two-photon absorption spectra and cross-sections

Figure 3 represents one- and two-photon absorption spectra of H_2TMPyP^{4+} in aqueous solution measured in the region of (a) Soret and (b) Q-bands. In the short wavelength region the linear (i. e. one-photon) spectrum of H_2TMPyP^{4+} has strong Soret band with maximum at 422 nm (Fig. 3(a)). In the long wavelength region the linear spectrum is of phyllo-type and consists of four bands centred at 638, 583, 552, and 516 nm (Fig. 3(b)). No changes in the spectrum have been found when changing solvent from H_2O to D_2O. All these features are in good agreement with the published data [9, 28, 31]. We ascertained that for the samples used in two-photon experiments, the absorbance in the wavelength range 730–1400 nm did not exceed 0.001.

In the visible region the measured TPA bands practically coincide in energy with those of linear spectrum (Fig. 3(b)). The TPA cross-section has moderate values and does not exceed 6 GM over the whole Q band range studied (i. e. 560–700 nm and 1120–1400 nm for the transition and excitation wavelengths, respectively). Comparison of the two-photon absorption spectrum pattern with the corresponding one-photon one indicates that two-photon transition into vibronic $Q_x(0,1)$ state is enhanced in contrast to that into the pure electronic $Q_x(0,0)$ state. This behaviour is explained by the symmetry considerations. Assuming H_2TMPyP^{4+} has a centre of inversion, two-photon transition into pure electronic $Q_x(0,0)$ state (it is of u parity) will be parity forbidden since the ground state is of g parity. Prohibition is lifted because of the molecular symmetry distortions originating mainly from interactions between the porphyrin macrocycle and the four methylpyridyl substituents in the *meso* positions. Dihedral angle Θ between the mean porphyrin plane and the plane of pyridyl ring is about 65° [32]. Thus, the observation of the TPA transition into $Q_x(0,0)$ state in H_2TMPyP^{4+} indicates that its symmetry is actually lower than D_{2h}, which is usually attributed to symmetrically substituted free base porphyrins. The increase of TPA in vibronic band is due to the coupling between electronic and vibrational wavefunctions [24]. The parity of the vibronic wavefunction results from parities of electronic and vibrational wavefunctions. Since, for the first excited $Q_x(0,0)$ state, the electronic moiety is of u parity, its combination with u parity vibrational wavefunction leads to overall g parity state: $\phi_u\chi_u = \Phi_g$. Therefore, two-photon transition into the vibronic $Q_x(0,1)$ state should be considered as an allowed one [24].

In UV region (360–400 nm) the σ_{TPA} value has been measured to be as high as 60–180 GM (Fig. 3(a)). We should stress that this band in TPA spectrum does not coincide with the Soret band and has no counterpart at all in one-photon spectrum, which consists of a series of $g \rightarrow u$ transitions. Nearly monotonic increase of σ_{TPA} towards higher frequency as well as rather high

cross-section values can be explained by the presence of allowed two-photon $g \rightarrow g$ transitions, which are predicted to lie near or above the B state [27]. Thus, the measured TPA spectrum can be attributed to several overlapping $g \rightarrow g$ transitions, giving rise to a broad TPA absorption band. For all the wavelengths we attested that the two-photon excited fluorescence does have a quadratic dependence on laser power. At lower excitation wavelength, this dependence gradually transforms into linear one because of the onset of one-photon absorption. This experimental circumstance prevents the extension of spectral range of TPA measurements deeper into UV where the maximum of this new TPA band should lie. The effect of resonance enhancement can also contribute to TPA cross-section growth, since the frequency of the excitation photons is close to the frequency of the one-photon allowed Q-transition [22]. More detailed analysis of the TPA spectra features for 15 porphyrin molecules including several tetra-aryl-substituted derivatives has been reported in our recently published paper [24].

There is some controversy in the values of quantum yield of the singlet oxygen photosensitization Φ_Δ by H_2TMPyP^{4+} porphyrin, and we want to clear up the situation before discussing the experimental results in this field. Several values of Φ_Δ for H_2TMPyP^{4+} porphyrin were obtained so far, varying from 0.58 [33] to 0.9 [6]. The last value measured by Kruk et al. [6] seems to be overestimated because the authors have relied on an overestimated ($\Phi_\Delta = 0.70$) value for H_2TSPP^{4-} [34], which they have used as a reference compound. Recent report by Braslavsky's group clearly indicates that the latter value has to be corrected to 0.62±0.03 [33]. Taking this into account, the value for H_2TMPyP^{4+} reported in Ref. [6] should be replaced by 0.77, which is close to 0.74 reported by Verlhac et al. [3]. The quantum yield of the singlet oxygen photosensitization Φ_Δ by H_2TMPyP^{4+} porphyrin averaged over all the reported values in Refs. [3, 6, 33, 35] is 0.71.

3.2. Singlet oxygen photosensitization upon TPE of porphyrin and its implications for PDT

Figure 4 shows the $^1\Delta_g \rightarrow {}^3\Sigma_g^-$ luminescence spectra of molecular oxygen measured with two- and one-photon excitation of H_2TMPyP^{4+} photosensitizer for the sample prepared in D_2O. For one-photon excitation we used nonlinear crystal for generation of the second harmonic of Ti:sapphire laser at 390 nm. In this experiment the intensity of $^1\Delta_g \rightarrow {}^3\Sigma_g^-$ luminescence

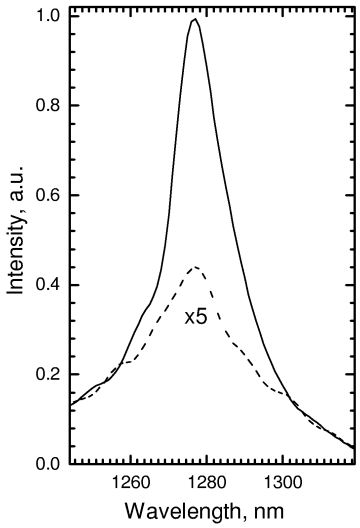

Fig. 4. $^1\Delta_g \rightarrow {}^3\Sigma_g^-$ luminescence spectra of molecular oxygen in air-equilibrated heavy water solution of H_2TMPyP^{4+} porphyrin. Dashed and solid curves represent, respectively, the spectra measured with two- and one-photon excitation.

signal increased linearly with laser power. Two-photon excitation of H_2TMPyP^{4+} porphyrin in these experiments with $^1\Delta_g$ photosensitization has been done at 780 nm. At this wavelength sensitizer has a moderate two-photon absorption cross-section $\sigma_{\mathrm{TPA}} = 85$ GM, whereas σ_{TPA} value growths up to 180 GM when the excitation wavelength is shifted to 735 nm. We used the following arguments to make a wavelength choice in such a way. Firstly, the excitation wavelength should fall in the tissue transparency window. Secondly, it was desirable to measure the $^1\Delta_g$ oxygen photosensitization upon excitation with commercially available laser system. $\lambda = 780$ nm is the operating wavelength of the commercial Ti:sapphire laser and it meets both above requirements. Both $^1\Delta_g \rightarrow {}^3\Sigma_g^-$ luminescence spectra measured with one- and two-photon excitation have the same shape and the maximum position at 1276±1 nm. This finding is in agreement with literature data for one-photon excitation [36]. The luminescence intensities of the same order of magnitude have been found in two cases, when the average laser power was 15 $\mathrm{W/cm^2}$ and 0.5 $\mathrm{W/cm^2}$ for two- and one-photon excitation, respectively.

Fluence rate of 15 $\mathrm{W/cm^2}$ is one order of magnitude higher than those used in PDT protocols [12], where it is varied from 100 $\mathrm{mW/cm^2}$ for Foscan® up to 1 $\mathrm{W/cm^2}$ in case of Photosens®. Therefore, it is important to compare the former figure with that for tissue damage threshold to conclude if such a fluence rate can be used in clinical practice. Low absorption of the tissues in near-IR region greatly reduces potential

for hyperthermal effects, and femtosecond duration of pulses prevents the initiation of collisional ionization and other avalanche damage mechanisms [15]. As a result, the irradiance from unfocused Ti:sapphire beam (as it was in our case) is found to be approximately six orders of magnitude below the tissue damage threshold [15]. This fact means that the photosensitization efficiency comparable with that found with one-photon excitation can by achieved with two-photon excitation of molecules having the σ_{TPA} value of about 100 GM and higher at the fluence rate well below the tissue damage threshold.

The two-photon absorbance by living tissues has been shown to be negligibly small and not interfering with two-photon absorbance of photosensitizer embedded into living tissues [15, 17, 18, 37]. It is known that focused ultrashort pulses suffer from spatial and temporal dispersion [38]. However, it has been shown experimentally that for laser pulses with halfwidth of 100–200 fs these effects are not substantial even in the living tissues for the experiments *in vitro* [37]. That is why it is possible to localize an excitation in the volume of order of few $\mu\mathrm{m}^3$ and to achieve efficient two-photon excitation. It is worthwhile to note that for next generation of TPE photosensitizers, having TPA cross-section of about several hundred GM, the excitation with picosecond lasers can be applied. Thus, the restrictions limiting an application of ultrashort laser pulses for two-photon excitation in biology can be successfully overpassed [37].

As we already have pointed out above, a major shortcoming of the porphyrins as photodynamic agents consists in their weak absorption in the red region of visible spectrum. For this reason, many of the known porphyrin sensitizers, even possessing a high yield of singlet oxygen photosensitization and meeting biological requirements of optimal balance between hydrophobic and hydrophilic properties, have been ruled out from potential candidates for PDT application. Out of the various possible ways to overcome the above limitation, simultaneous two-photon excitation seems to be a promising one (Fig. 5). We have to note once more that for centrosymmetric molecules (and for quasicentrosymmetric molecules, in fact) the selection rules for two-photon or one-photon absorption are alternative. Therefore the initial excited states populated in both cases are different. However, independently of excitation mechanism, the lowest excited triplet state, T_1, is ultimately populated as a result of intramolecular radiationless transitions in porphyrin photosensitizer [13]. Hence, provided that high

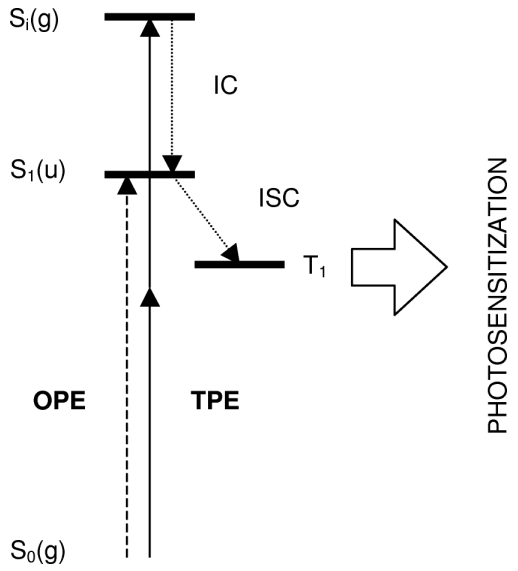

Fig. 5. Sketch of the energy levels for porphyrin photosensitizer. $\mathrm{S}_0(g)$, $\mathrm{S}_1(u)$, $\mathrm{S}_i(g)$, and T_1 represent, respectively, the ground, first singlet, ith excited singlet, and lowest triplet states of the photosensitizer. The symbols in the parentheses denote *gerade* (g) and *ungerade* (u) symmetry of the corresponding states. Upon one-photon excitation (OPE), the lowest singlet state S_1 of photosensitizer is populated. Upon two-photon excitation (TPE), a transition occurs to one of the higher excited states $\mathrm{S}_i(g)$. In this case the energy of the excitation photon is much lower than that used in OPE and falls into the tissue transparency window. Because of the internal conversion (IC) and intersystem crossing (ISC) the T_1 state is ultimately populated. This is followed by energy transfer from photosensitizer to the ground state oxygen, resulting in the singlet oxygen photosensitized formation: $\mathrm{T}_1 + {}^3\Sigma_g^- \rightarrow \mathrm{S}_0 + {}^1\Delta_g$.

efficiency of two-photon excitation is accomplished, the pronounced photodynamic effect can be obtained. This two-photon excitation scheme, which we first proposed in Ref. [21], differs from those suggested earlier [13, 15] in that we have proposed to excite the photosensitizer into one of the higher excited S_i states (actually, for free base porphyrins it is higher than S_4 state), whereas in earlier schemes S_1 state has been proposed. Because of the alternative selection rules the σ_{TPA} value for two-photon excitation of porphyrins into S_1 state is too low to have any practical application (see Fig. 3(b)), whereas two-photon allowed $g \rightarrow g$ transition has high σ_{TPA} value (Fig. 3(a)). Our results prove that efficient two-photon excitation can be achieved for water-soluble $\mathrm{H_2TMPyP}^{4+}$ porphyrin (TPA cross-section $\sigma_{\mathrm{TPA}} = 85$ GM at the excitation wavelength), giving rise to an observable ${}^1\Delta_g$ singlet oxygen photosensitization. Thus, from the point of view of two-photon absorptivity the *meso*-tetrapyridyl-substituted porphyrins seem to have the advantages over their *meso*-tetraphenyl-substituted counterparts ($\mathrm{H_2TSPP}^{4-}$ porphyrin has $\sigma_{\mathrm{TPA}} = 21$ GM at 780 nm).

4. Conclusions

The experimental data reported in this paper prove a new excitation scheme for porphyrin photosensitizers based on simultaneous two-photon absorption in the $g \rightarrow g$ transitions. This approach seems to be of importance in PDT because it does not require strong absorption by porphyrin photosensitizer at the red edge of visible spectrum and allows considering many porphyrin molecules, which reveal photodynamic activity *in vitro*, as potential drugs for PDT. Owing to reduced absorption and scattering of IR light this excitation scheme can provide a greater depth of penetration into biological tissues, high spatial localization of excitation, and can minimize the laser-induced hyperthermia [37]. Our observations open the perspective to increase the potential of well-studied porphyrin photosensitizers in photodynamic therapy.

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SINGULETINIO MOLEKULINIO DEGUONIES FOTOJAUTRINIMAS, DVIFOTONIŠKAI ŽADINANT PORFIRINĄ VANDENS TIRPALE

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Santrauka

Gerai žinomas vandenyje tirpaus porfirino fotodinaminis aktyvumas. Tačiau porfirinų fotojautrinantį panaudojimą fotodinaminėje terapijoje riboja nepakankama sugertis raudonajame spektro ruože, kuriame kūno audiniai nesugeria spinduliuotės. Šių molekulių dvifotonis sužadinimas (DFS) padeda išvengti šio apribojimo. Lig šiol DFS buvo laikomas neefektyviu ir neturinčiu praktinės vertės. Šiame darbe dvifotonė savitoji sugertis ir singuletinio deguonies fotojautrinimas 5,10,15,20-tetrakis-(4-N-metilpiridil)-21H,23H-porfirinu vandens tirpale buvo tiesiogiai patikrinti. Dvifotonės sugerties skerspjūvio σ_{DFS} didumas kinta nuo 60 ligi 180 GM, derinant sužadinimo bangos ilgį nuo 800 iki 730 nm. Atrasta, kad mėlynajame Soret'o juostos (B juosta) krašte ši sugertis pasireiškia dėl dvifotonės leistino šuolio sugerties į lyginio lygiškumo būseną ($g \rightarrow g$ šuolis). DFS į Q būsenas yra uždraustas pagal lygiškumą ($g \rightarrow u$ šuolis) ir σ_{DFS} didumas neviršija 6 GM 1100–1400 nm spektro ruože. Porfirino DFS ties 780 nm oro prisotintame sunkiojo vandens tirpale efektyviai jautrina singuletinį molekulinį deguonį ($^1\Delta_g$), kuris detektuojamas per $^1\Delta_g \rightarrow ^3\Sigma_g$ liuminescenciją. Mūsų išaiškinimais parodytas dvifotonio sužadinimo tinkamumas fotodinaminei terapijai ir galimybė nustatyti reikalavimus panaudojamos fotojautrinančios medžiagos dvifotonei savitajai sugerčiai.