Laser induced singlet-oxygen-sensitised delayed fluorescence of dyes in aqueous solutions

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***Abstract*.** **It is shown that water-soluble derivatives of phthalocyanines – poly(diethoxyphosphinylmethyl)substituted aluminium phthalocyanines – emit intense singlet-oxygen-sensitised delayed fluorescence upon laser-induced formation of singlet oxygen in air-saturated aqueous (D_2O) solutions. The delayed fluorescence is emitted by the dye molecules which accepted energy from two molecules of singlet oxygen. The quantum efficiency of delayed fluorescence in aerated D_2O of the chloroaluminium complex of octa(diethoxyphosphinylmethyl) phthalocyanine corresponds to the rate constant of population of excited dye molecules which is equal to $(5.5 \pm 3) \times 10^{12}$ $mole^{-2}$ L^2 s^{-1}. This value is only an order of magnitude smaller than that for tetra(4-*tert*.-butyl)phthalocyanine earlier studied in aerated organic solvents. It is shown that these phthalocyanine derivatives can be used as highly sensitive luminescence indicators of singlet oxygen produced in aqueous solutions of different compounds upon laser excitation.**

Keywords: *singlet molecular oxygen, delayed fluorescence, phosphorescence, phthalocyanine, aqueous solutions of photosensitisers.*

1. Introduction

We reported earlier that photoexcitation of dye molecules in air-saturated organic solvents causes formation of singlet $^1\Delta_g$ molecular oxygen ($^1O_2$), whose deactivation is accompanied by IR phosphorescence at 1270 nm and the short-wavelength emission in the region from 600 to 800 nm [1 – 4]. The decay kinetics of the short-wavelength emission and dependence of its intensity on the excitation intensity suggest that two $^1O_2$ molecules are responsible for emission of one photon [3 – 5].

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Spectral studies showed that the nature of the short-wavelength emission depends substantially on the photophysical properties of dyes. In solutions of the dyes whose main fluorescence band was located at $\lambda \leqslant 600$ nm, this emission was mainly related to dimers (dimols) $(^1O_2)_2$ [3, 4, 6]. In dye solutions with the major fluorescence maximum located at $\lambda \geqslant 685$ nm, the emission spectrum coincided with the fluorescence spectrum of the dye, i.e., singlet-oxygen-sensitised delayed fluorescence of dyes (SOSDF) was observed [3 – 10]. The most intense SOSDF was found in solutions of phthalocyanines and naphthalocyanines. The SOSDF quantum yield reached 0.2 – 0.4 under pulsed laser excitation of solutions of tetra (4-*tert*.-butyl)phthalocyanine, which exceeds the quantum yield of IR phosphorescence of $^1O_2$ by 2 – 4 orders of magnitude [10,11]. Therefore, the use of laser induced delayed fluorescence of phthalocyanines for detecting $^1O_2$ in chemical systems is of great interest. So far, SOSDF was observed only in organic media. In this paper, we observed this emission in aqueous solutions of poly(diethoxyphosphinylmethyl)substituted aluminium phthalocyanines.

2. Materials and methods

Time-resolved measurements of delayed fluorescence were performed using a laser spectrophosphorimeter [12], which was modified as described in papers [7, 8]. The excitation source was a 337-nm LGI-505 nitrogen laser (pulse duration, pulse energy and pulse repetition rate were 10 ns, 7 mJ and 700 Hz, respectively). The energy distribution of laser radiation over the laser-beam cross-section was almost uniform. The diameter of the illuminated spot was 2 mm, i.e., the illuminated area was ~ 0.03 cm^2 and the corresponding excitation energy density was ~ 0.23 mJ cm^{-2}.

The solutions were studied in a rectangular spectrophotometric cell of size 2×10 mm. The laser beam was directed horizontally to a wide (10 mm) cell face. To reduce reabsorption of delayed fluorescence in dye solutions, the laser beam was displaced so that the distance from its centre to the edge of the cell was 2.5 mm. Luminescence was measured from the narrow 2 mm face of the cell at an angle of 90° to the laser beam.

The luminescence was focused by a quartz lens on the entrance slit of an MS-80 monochromator (relative aperture 1:3) through a red KS-11 filter during the measurement of delayed fluorescence, and through an infrared IKS-7 filter during the measurement of the $^1O_2$ phosphorescence. During spectral and kinetic measurements, the width of

the monochromator slits was 4 mm, corresponding to the spectral width of 16 nm. Monochromatic radiation was focused on the photocathode of a FEU-83 photomultiplier cooled to $-60\,°C$.

Single-photon photomultiplier pulses passed through a preamplifier, discriminator and a delay generator and then were fed to a NTA-1024 multichannel (256 or 512 channels) pulse counter. The channel dwell time was 5 μs. The counter was activated by the scattered light of the laser pulse with the aid of a second photomultiplier. The counter summed up the photomultiplier pulses in each channel after a large number of laser pulses, thus forming the luminescence kinetic curve. The luminescence intensity was calculated as the ratio of the number of the pulses in the channels to the number of laser pulses.

The luminescence spectra were determined by recording the kinetic curves at different wavelengths. It was sufficient to use about 10^4 laser pulses to determine the kinetic curves and the spectra required 500 shots for one wavelength. The data were processed by a PC coupled to the counter using the Grafit and Excel programs. The spectral sensitivity of the recording system was determined using a standard incandescent lamp from the accessories of the an MPF-44B Perkin-Elmer spectrofluorimeter (USA).

The absorption spectra were measured with a Hitachi U-3400 spectrophotometer (Japan). The fluorescence spectra were recorded with an MPF-44B Perkin-Elmer spec-trofluorimeter or with a spectrofluorimeter of a laboratory design with an increased sensitivity in the near infrared region.

Water-soluble aluminium complexes of poly(diethoxy-phosphinylmethyl)substituted phthalocyanines containing from 4 to 8 phosphite groups were synthesised from poly(chloromethyl)phthalocyanine and triethylphosphite using the Arbuzov reaction [13, 14]. The structure of the complexes was determined from the data of elemental analysis and the spectra of the proton magnetic resonance. A solvent was deuterium oxide D_2O ('Isotope', St. Peters-burg, Russia).

3. Results and discussion

We used phenalenone as a photosensitiser of the $^1O_2$ formation, which generates singlet oxygen with the quantum yield of 0.95 [15]. The phthalocyanine derivatives served as acceptors of singlet oxygen energy. We found that octa(diethoxyphosphinylmethyl)phthalocyanine (Pc^{P}AlCl) (Fig. 1) exhibited the most intense SOSDF upon laser excitation and we used it for detailed studies.

The absorption spectrum of Pc^{P}AlCl in D_2O (pD 7.4) is shown in Fig. 2. The most intense absorption bands are located at 693 nm and 353 nm. Judging from the absorption spectra, Pc^{P}AlCl remained monomeric in the pD interval 7–10 and the concentration range 0.18–1.8 μmole L^{-1}. The molar extinction coefficient of Pc^{P}AlCl at the maximum of the long-wavelength band was 1.3×10^5 mole^{-1} L cm^{-1}. At the maximal Pc^{P}AlCl concentration used in our experiments (1.8 μmole L^{-1}), the optical density of this compound at the wavelength of laser radiation (337 nm) was 0.02. At other Pc^{P}AlCl concentrations, the optical density was proporti-onally less. The optical density of phenalenone at 337 nm was 0.12–0.24 in all experiments. Therefore, the laser radiation was absorbed mostly by phenalenone. Upon exci-tation of the solutions containing phenalenone and Pc^{P}AlCl

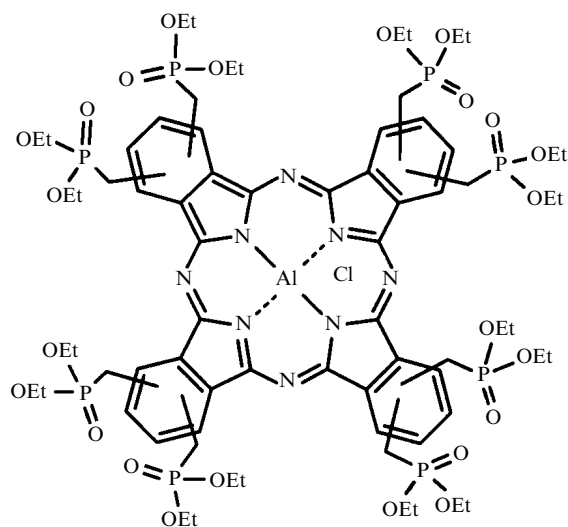**Figure 1.** Chemical structure of the aluminium-chloride complex of octa(diethoxyphosphinylmethyl)substituted (Pc^{P}AlCl).

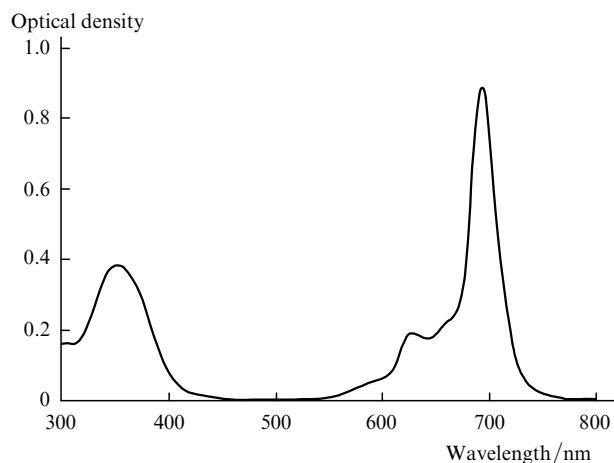**Figure 2.** The absorption spectrum of Pc^{P}AlCl in D_2O at room tem-perature.

by laser pulses, we observed phenalenone-photosensitised phosphorescence of singlet oxygen at 1270 nm, whose lifetime was 50 ± 5 μs (Fig. 3). In Pc^{P}AlCl solutions without phenalenone, this emission was not detected.

Along with the phosphorescence of $^1O_2$, singlet-oxygen sensitised delayed emission was observed at the shorter wavelengths. As shown in Fig. 4, the spectrum of this emission coincided with the Pc^{P}AlCl fluorescence spectrum in the same solution. The maximum of the spectrum is located at 701 nm. In the long-wavelength region, another maximum is observed at 770 nm. The kinetics of the delayed fluorescence upon pulsed laser excitation is shown in Fig. 3. If delayed fluorescence was measured with the 10-μs delay after a laser pulse, its decay kinetics was exponential with the lifetime being approximately two times shorter than that of the phosphorescence. The intensity of the delayed fluorescence increased linearly with the Pc^{P}AlCl concen-tration within the concentration range indicated above. As shown earlier, these data indicate that the delayed fluo-rescence appears due to consecutive processes, as a result of which one dye molecule accumulate energy of two singlet oxygen molecules [3–10].
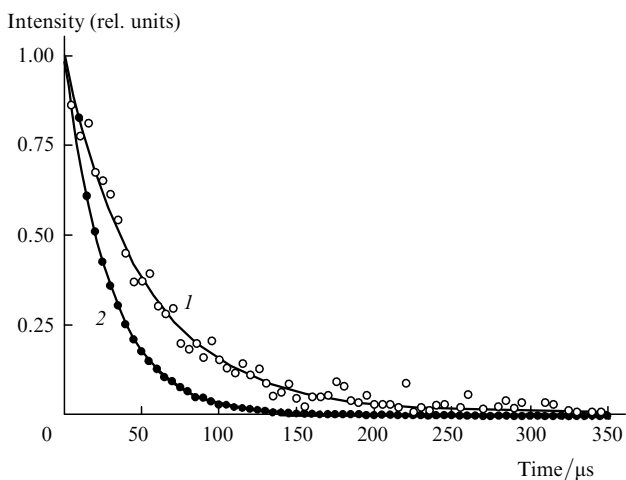
Figure 3. Decay kinetics of phenalenone-photosensitised phosphorescence of singlet oxygen (*1*) and singlet-oxygen sensitised delayed fluorescence of Pc^{P}AlCl (*2*) in D$_2$O after pulsed laser excitation. The decays are normalised to the initial luminescence intensity and obtained by signal averaging over 200 000 (*1*) and 20 000 (*2*) laser pulses.
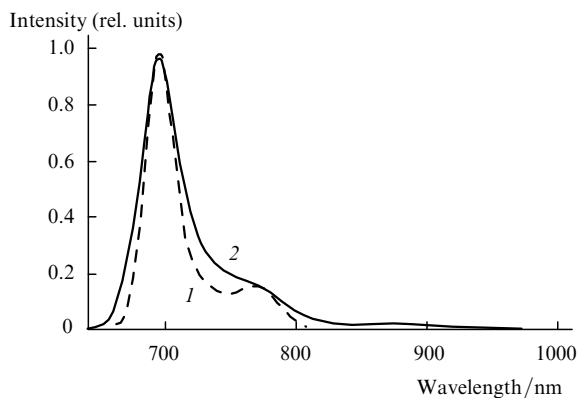

Figure 4. Fluorescence (*1*) and SOSDF (*2*) spectra of Pc^{P}AlCl in D$_2$O at room temperature. The optical densities of the solutions at the long-wavelength absorption maximum of Pc^{P}AlCl did not exceed 0.05 (*1*) and 0.1 (*2*). The spectral widths of the monochromator slits were 2 (*1*) and 16 nm (*2*).

As in the case of SOSDF of dyes in organic solvents [16–18], delayed fluorescence of Pc^{P}AlCl in D$_2$O decreased approximately five times after oxygen purging, i.e., proportionally to the partial oxygen pressure, which can be explained by the involvement of the triplet state of Pc^{P}AlCl or a Pc^{P}AlCl complex with oxygen in SOSDF.

We determined the quantum efficiency of ^{1}O$_2$-sensitised formation of excited Pc^{P}AlCl molecules from the coefficient [18]:

$$\alpha = \frac{(I_{df}^0/I_{ph}^0)k_r}{\gamma_f[^1O_2]_0[D]}, \tag{1}$$

where I_{df}^0 и I_{ph}^0 are the initial intensities of the SOSDF and the ^{1}O$_2$ phosphorescence, respectively, obtained by the extrapolation of the luminescence decay curves to zero time; k_r is the rate constant of the radiative deactivation of ^{1}O$_2$; γ_f is the quantum yield of the dye fluorescence; $[^1$O$_2]_0$ is the concentration of singlet oxygen immediately after a laser pulse; and $[D]$ is the dye concentration. The coefficient α corresponds to the effective rate constant of formation of excited Pc^{P}AlCl molecules resulting from interaction of two ^{1}O$_2$ and one Pc^{P}AlCl molecules.

The parameters needed for calculation of the coefficient α are indicated below. According calculations performed in paper [19] using the data [12, 20, 21], $k_r \approx 0.12$ s^{-1} in D$_2$O. The molar concentration $[^1$O$_2]_0$ was calculated from the expression

$$[^1O_2]_0 = \frac{1000W_{las}(1-T)\gamma_\Delta}{h\nu Nl} \tag{2}$$

where W_{las} is the energy density of the laser radiation in mJ cm^2; $h\nu$ is the energy of 337-nm photons, equal to 5.8×10^{-16} mJ; $N = 6.023 \times 10^{23}$ mole^{-1} is the Avogadro number; $(1-T)$ is the absorption coefficient of phenalenone at the laser wavelength; γ_Δ is the quantum yield of the ^{1}O$_2$ production by phenalenone upon laser excitation ($\gamma_\Delta = 0.95$); l is the cell thickness (0.2 cm). In our experiments, $[^1$O$_2]_0 = 0.7 - 1.3$ μmole L^{-1}.

The intensities I_{df}^0 and I_{ph}^0 were measured at the maxima of the SOSDF and phosphorescence spectra (at 700 and 1270 nm, respectively) using the monochromator with the spectral slit width of 16 nm. Then, we corrected these data with the aid of computer simulation taking into account the areas under the SOSDF and phosphorescence spectra and the spectral sensitivity of the photomultiplier.

The fluorescence quantum yield γ_f of Pc^{P}AlCl, which is needed for calculation of the coefficient α, was not known. It was measured by comparing the fluorescence intensity of solutions of Pc^{P}AlCl and tetra(*p*-sulfophenyl)porphyrin (TPPS) in a 10×10 mm cell upon excitation at 370 nm. The optical density of dye solutions at this wavelength was 0.08. The fluorescence intensity was calculated using the Excel software as the area under the fluorescence spectrum taking into account the spectral sensitivity of the spectrofluorimeter. The data indicate that the quantum yield of Pc^{P}AlCl fluorescence was 3.9 times higher, than that of TPPS. It is known that $\gamma_f = 0.1$ in TPPS solutions ([22] and references therein). Hence, $\gamma_f \approx 0.4$ for Pc^{P}AlCl.

The value of α calculated from the experimental data was $\alpha = (5.5 \pm 3) \times 10^{12}$ mole^{-2} L^2 s^{-1}. One can see that the error of measuring α is rather high, which is caused by the necessity of quantitative account for a large number of technical parameters.

A comparison of this value of α with its value obtained earlier [18] for air-saturated solutions of tetra(4-*tert.*-butyl)-phthalocyanine ($\alpha = (4 \pm 2) \times 10^{13}$ mole^{-2} L^2 s^{-1}) shows that the quantum efficiency of Pc^{P}AlCl as the SOSDF emitter in aqueous solutions is rather high.

4. Conclusions

The data obtained in this paper show that Pc^{P}AlCl is an efficient emitter of SOSDF and therefore this molecule can be used as a luminescence indicator of laser induced singlet oxygen formation in aqueous solutions. Because the SOSDF signal is stronger by several orders of magnitude than that of phosphorescence of ^{1}O$_2$, the dose of laser radiation needed for reliable ^{1}O$_2$ detection from the SOSDF measurement is less by several orders of magnitude than that for ^{1}O$_2$ detection from the phosphorescence measurement. It is especially important for the analysis of aqueous solutions of biologically important compounds which are usually rapidly destroyed upon exposure to light.

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