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Investigation of photo-physical properties of selected diaminoacid protoporphyrin derivatives ($PP(AA)_2Arg_2$). II. Determination of quantum yield of singlet oxygen FD

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A new generation of photosensitizers for photodynamic therapy (PDT) has been investigated. Different amino acids were bound to protoporphyrin (PP). Two methods of determination the quantum yield of generated singlet oxygen in porphyrin solutions were investigated. In the first method, tryptophan was used as a singlet oxygen acceptor. In the second one, the amount of singlet oxygen was determined by phosphorescence at 1272 nm. The purpose of this work was to evaluate the quantum yield of singlet oxygen of different $PP(AA)_2Arg_2$ derivatives, since it is crucial for choosing proper ingredients of photosensitizer for diagnosis and therapy in PDT.

Keywords: photosensitizers, photodynamic therapy, quantum yield of singlet oxygen.

1. Introduction

In earlier work [1], we have studied the basic optical properties of diaminoacid protoporphyrin derivatives ($PP(AA)_2Arg_2$) used for photodynamic diagnosis. In photodynamic therapy, one of the parameters for determination of efficiency of photosensitizer is a quantum yield of generation of singlet oxygen.

An oxygen particle in a ground state is commonly known because it is chemically very active and indispensable for aerobic cells in a breathing process. Investigations of oxygen properties have been carried out for 200 years but its properties in the excited state have been investigated over the last three decades.

The investigation results show that oxygen in the excited state, *i.e.*, singlet oxygen takes part in photo-oxidation reaction as a strong oxidiser. This effect is used for destruction of bacteria, viruses, and tumor cells. In a photodynamic method of tumor treatment, singlet oxygen is used as photo-chemical therapeutic factor. Singlet oxygen affecting healthy cells, depending on its concentration at the time of influence, can

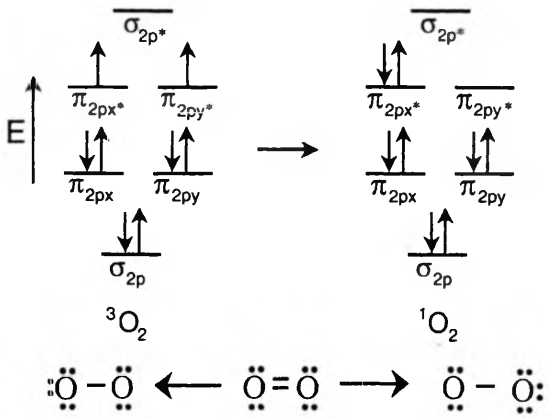

Fig. 1. Electron state of oxygen in various excited states (after [2]).

cause mutation and carcinogenesis as well as damage of bio-polymers important for a cell life [3]. Electron ground state of oxygen particle is a triplet state of zero angular momentum having two unpaired p electrons at the anti-bounding orbits π_{2px} and π_{2py}, as shown in Fig. 1.

In a ground state, the external electrons are arranged according to the Hund rule, *i.e.*, at the anti-bounding orbits p_x and p_y. When these orbitals are degenerated and electrons have the same spines and electrons have the same spins, the quantum numbers describing these two electrons are the same and according to the Paulie exclusion principle they have to occupy separate orbitals.

In a reaction with excited photosensitizer, a spin of one of the electrons is inverted which causes a change in the value of the spin number. This allows formation of electron pair at one anti-bounding orbital. Such a change in electron structure of oxygen particle is energy-dependent one and it can occur only after absorption of energy

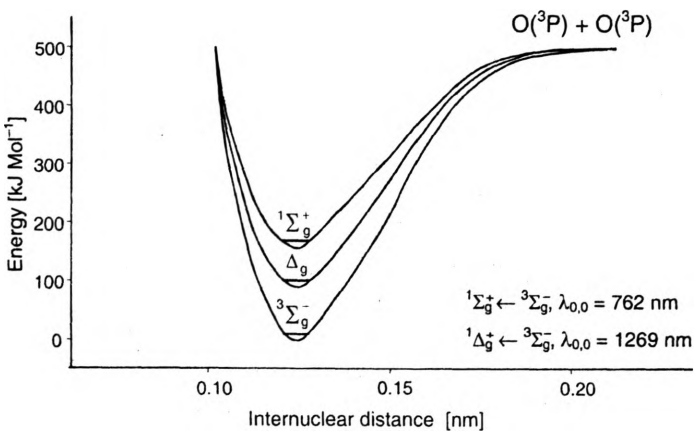

Fig. 2. Energy curves for oxygen particle in ground and excited states (after [4]).

σ_{2p^*}					
π_{2p^*}	↑ ↑	↑↓	↑ ↓	↑↓ ↑	↑↓ ↑↓
π_{2p}	↑↓ ↑↓	↑↓ ↑↓	↑↓ ↑↓	↑↓ ↑↓	↑↓ ↑↓
σ_{2p}	↑↓	↑↓	↑↓	↑↓	↑↓
σ_{2s^*}	↑↓	↑↓	↑↓	↑↓	↑↓
σ_{2s}	↑↓	↑↓	↑↓	↑↓	↑↓
σ_{1s^*}	↑↓	↑↓	↑↓	↑↓	↑↓
σ_{1s}	↑↓	↑↓	↑↓	↑↓	↑↓
Name	triplet oxygen	singlet oxygen	singlet oxygen	anion radical superoxide	superoxide ion
Symbol	$^3\Sigma_g O_2$	$^1\Delta_g O_2$	$^1\Sigma_g^+ O_2$	$O_2^{-\cdot}$	O_2^{2-}
Relative energy	0	94 kJ/mol	157 kJ/mol		
Lifetime in water	—	2 μs	< 10 ps		

Fig. 3. Distribution of electrons on molecular orbits of ground state oxygen and various reactive oxygen forms.

quantum, which next destabilizes $^1O_2$ particle making it very reactive. Figure 1 shows that a singlet oxygen particle can be presented as highly polarized two bipolar ion. Thus, particle excitation from the ground triplet state to the excited singlet state occurs after absorption of light quantum. Electron structure of oxygen particle under $h\nu$ influence can be changed in two ways, as can be seen from Figs. 2 and 3.

These two forms of singlet oxygen differ in energy and half-lifetime $T_{1/2}$. Two electron excited singlet states, that are from the same electron configuration, have various paired energy of spins of these electrons $^1\Delta_g$ and $^1\Sigma_g^+$, 94 and 157 KJmol^{-1} above the ground state, respectively. Although the transitions $^1\Delta_g \leftarrow {}^3\Sigma_g^-$ and $^1\Sigma_g \leftarrow {}^3\Sigma_g^-$ are strongly forbidden, their absorption and emission can be observed in the upper layers of atmosphere due to zero–zero transitions for λ_{max} 1270 and 762 nm and estimated lifetimes are 64 min and 10 s, respectively [4]. The lifetime of both forms of singlet oxygen depends on medium in which it was generated. Especially short lifetimes are in polar solutions, also in water.

When quantum yield of singlet oxygen is determined, one should consider a medium in which it was generated and the factors influencing its quenching. These factors can be both chemical and physical ones. Also, self-quenching effect can occur. It is observed when photosensitizer quenches the singlet oxygen.

Quantum yield of the singlet oxygen Φ_Δ is a result of separating the populations between the excited singlet state S_1 and the excited triplet state T_1 minus the sum of quenching effects. Thus, Φ_Δ will be dependent on oxygen concentration, and concentration of "quenchers", both in S_1 and T_1 states. This situation can be expressed as

$$\Phi_\Delta = P_S^{O_2} f_\Delta^S + \Phi_T^{O_2} P_T^{O_2} f_\Delta^T \tag{1}$$

where $P_S^{O_2}$ is a portion of the state S_1 quenched with oxygen, f_Δ^S – the fraction of S_1 from which the singlet oxygen is formed, $\Phi_T^{O_2}$ – the quantum yield of a triplet state in the presence of oxygen, $P_T^{O_2}$ – the portion of T_1 quenched with oxygen, f_Δ^T – the fraction of T_1 quenched with oxygen generating singlet oxygen. Because the value of $P_S^{O_2} f_\Delta^S$ is very low, it can be neglected in calculations and then quantum yield of singlet oxygen can be calculated from the simplified equation

$$\Phi_\Delta = \Phi_T^{O_2} P_T^{O_2} f_\Delta^T. \tag{2}$$

2. Experimental results

The subject of our investigation was quantum yield of singlet oxygen of the selected diaminoacid of protoporphyrin (PP) derivatives and their complexes with arginine. Their general chemical structure is shown in Fig. 4.

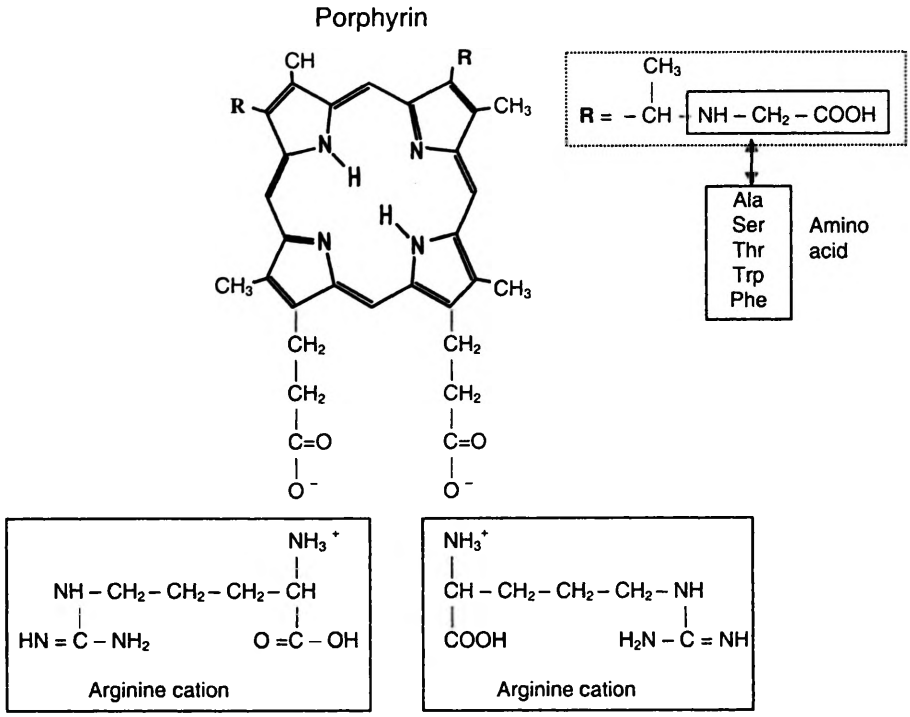

Fig. 4. Diaminoacid derivatives of protoporphyrins $PP(AA)_2Arg_2$.

The quantum yield of singlet oxygen Φ_Δ can be estimated by:

– determining the loss of a substance reacting with singlet oxygen and forming a photo-product,

– determining a phosphorescence decay of singlet oxygen for which the emission band is near infrared λ_{max} = 1270 nm.

All the $PP(AA)_2Arg_2$ under investigation, shown in Fig. 4, have been synthesized at the Biochemistry and Spectroscopy Laboratory, Military University of Technology (MUT), Warszawa, Poland, using the method described in [5] and [6]. Purification was carried out using column chromatography method and for purity determination, the HPLC was used. For investigation purposes, the preparations were used with 65–70% diaminoacid derivatives and 30–35% of hydroxy derivatives of PP formed during reaction and were difficult to separate. The efficiency of preparations of the same composition was checked in volunteers suffering from advanced tumors. Satisfactory diagnostic and therapeutic results have been obtained. The quantum yield of singlet oxygen had to be determined for particular derivatives as a value characterizing their usefulness for therapy.

2.1. Determination of the quantum yield Φ_Δ of $^1O_2^*$ from tryptophan degradation by singlet oxygen

In indirect measuring method, tryptophan was used as a substrate in photooxidation with singlet oxygen. The mechanism being the base of measurement of quantum yield $^1O_2$ can be described using the following equations:

$$^1O_2^* + A \xrightarrow{k_A} AO_2,$$

$$^1O_2^* + A \xrightarrow{k_q} {}^3O_2 + A,$$

$$^1O_2^* \xrightarrow{k_d} {}^3O_2,$$

$$^1O_2^* \xrightarrow{k_p} {}^3O_2 + h\nu$$

where A is the tryptophan as singlet oxygen acceptor, k_A (6.6×107 $M^{-1}s^{-1}$ [7]) – the reaction rate constant of singlet oxygen reaction with tryptophan, k_q – the rate constant of physical quenching of tryptophan, k_d (2.5×10^5 s^{-1}) – the decay rate constant of the excited state of singlet oxygen (quenching with a solvent) [8], k_p – the constant of radiant quenching rate.

Finally, the quantum yield $^1O_2$ has been determined from the equation

$$-\Phi_\Delta I_{abs} t + [A]_0 - [A] = \frac{k_d}{k_A} \ln \frac{[A]}{[A]_0} \tag{3}$$

where Φ_Δ is the quantum yield of singlet oxygen generation, I_{abs} – the intensity of absorbed light, [A] – the tryptophan concentration [M], and t – the exposure time [s].

The amount of the reacted tryptophan with $^1O_2$ generated by excitation of a photosensitizer has been determined from the change in intensity of tryptophan emission band. Four solutions were used for investigation in which tryptophan concentration was constant and equal to 1.6×10^{-4} M. The concentration of $PP(AA)_2Arg_2$ varied from 1.6×10^{-5} M to 2.85×10^{-5} M. A mixture of $PP(AA)_2Arg_2$ and tryptophan

was buffered to pH = 7.2 in phosphate buffer solution (PBS) and measured in a cuvette of the optical path equal to 1 cm. Before the first illumination, the solutions have been oxidized for 20 min and next illuminated for 10 min. After each cycle of illumination, the absorption spectra of tryptophan and $PP(AA)_2Arg_2$ were registered with Cary 50 Bio UV-VIS spectrophotometer. Before the consecutive measurement the solution was again illuminated for 5 min in order to supplement the oxygen content in the solution. The system used for illumination is shown in Fig. 5.

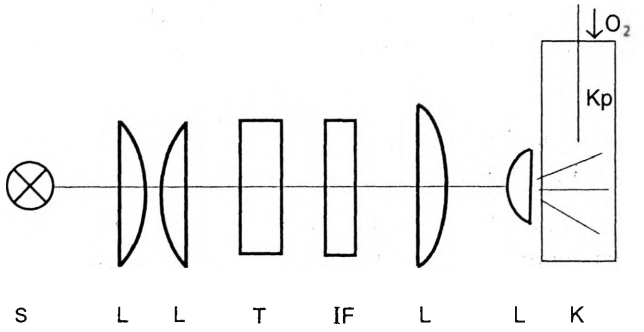

Fig. 5. System used for determination of quantum yield of singlet oxygen. S – the radiation source (incandescent lamp P = 150 W made by Osram firm), L – the lens, T – the thermal IR filter, IF – monochromatic filter (UV SIF 383 nm), K – the cuvette (optical path 1 cm), and Kp – the set of capillaries.

After each illumination cycle, 50 μl of the investigated solution was taken. This sample was diluted 50 times with a buffer and its emission spectra were registered for excitation λ = 280 nm and excitation spectra of tryptophan for λ = 360 nm using LS-5B Perkin–Elmer spectrophotometer. The solutions of concentration 3×10^{-6} M were used in spectral investigations. For this concentration, the dependence of fluorescence intensity on tryptophan concentration is nearly linear, as can be seen from Fig. 6.

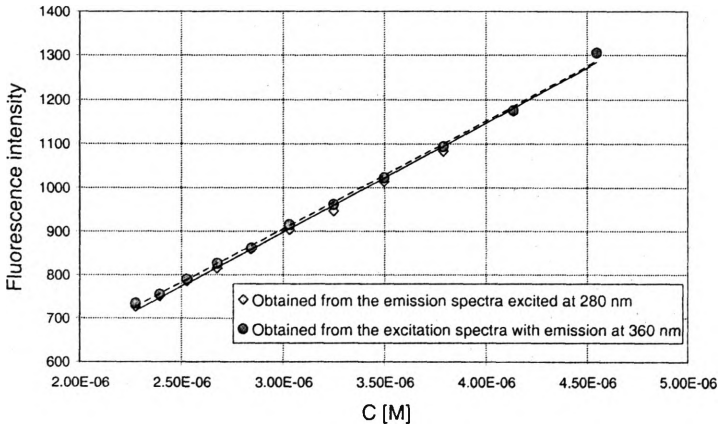

Fig. 6. Intensity of tryptophan fluorescence *vs.* its concentration.

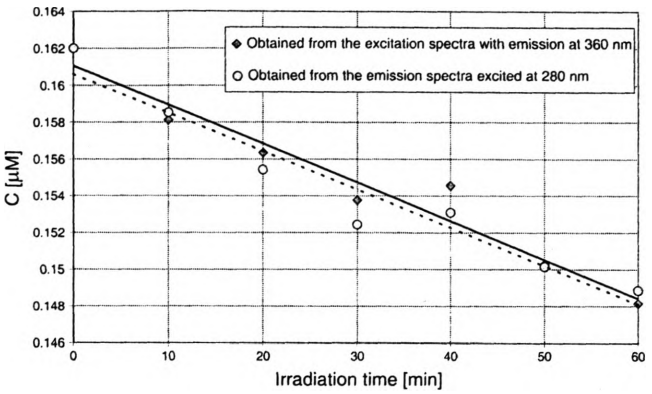

Fig. 7. Kinetics of tryptophan degradation for mixture solution $PP(Ser)_2Arg_2$ ($C_0 = 2.81\times10^{-5}$ M) and tryptophan ($C_0 = 1.62\times10^{-4}$ M) in PBS at pH 7.2 as a function of irradiation time.

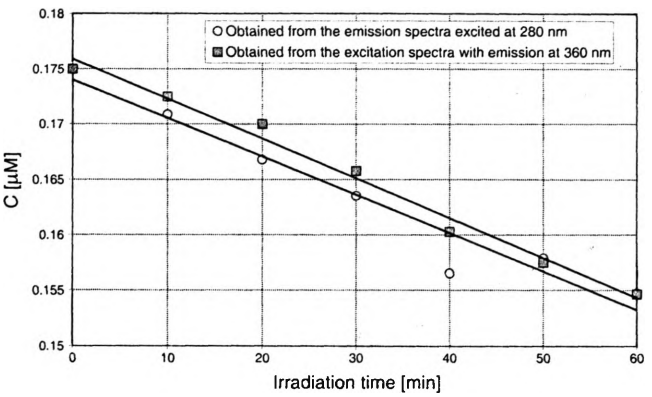

Fig. 8. Kinetics of tryptophan degradation for mixture solution $PP(Ser)_2Arg_2$ ($C_0 = 2.85\times10^{-5}$ M) and tryptophan ($C_0 = 1.60\times10^{-4}$ M) in PBS at pH 7.2 as a function of irradiation time.

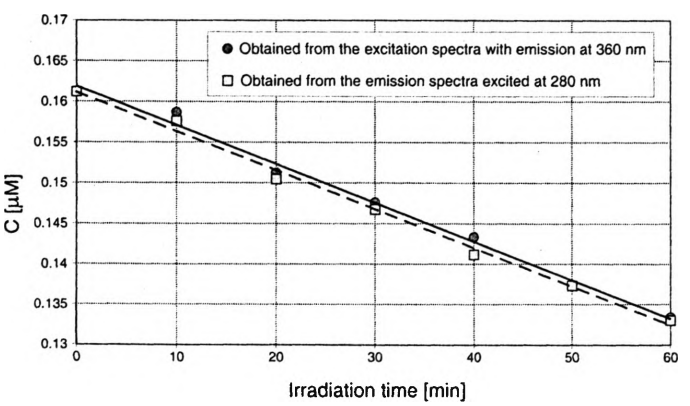

Fig. 9. Kinetics of tryptophan degradation for mixture solution $PP(Thr)_2Arg_2$ ($C_0 = 1.66\times10^{-5}$ M) and tryptophan ($C_0 = 1.61\times10^{-4}$ M) in PBS at pH 7.2 as a function of irradiation time.

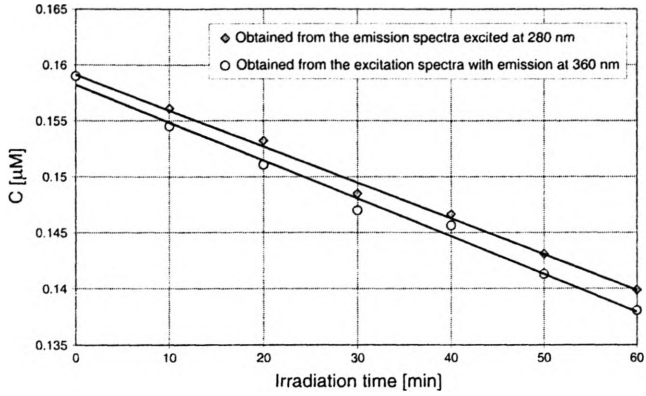

Fig. 10. Kinetics of tryptophan degradation for mixture solution $PP(Phe)_2Arg_2$ ($C_0 = 1.70\times10^{-5}$ M) and tryptophan ($C_0 = 1.59\times10^{-4}$ M) in PBS at pH 7.2 as a function of irradiation time.

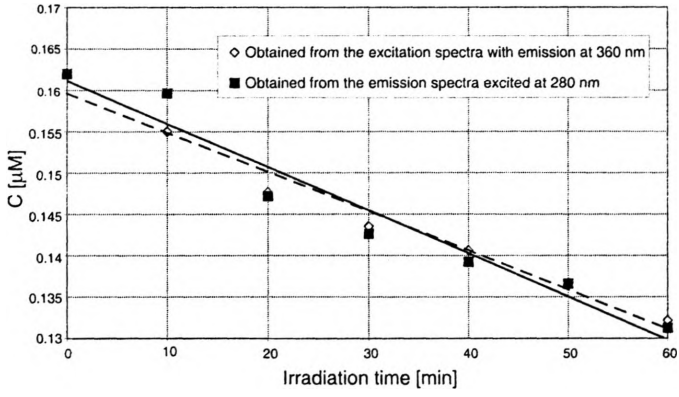

Fig. 11. Kinetics of tryptophan degradation for mixture solution $PP(Trp)_2Arg_2$ ($C_0 = 2.66\times10^{-5}$ M) and tryptophan ($C_0 = 1.62\times10^{-4}$ M) in PBS at pH 7.2 as a function of irradiation time.

T a b l e 1. Quantum yields of singlet oxygen Φ_Δ for five derivatives of $PP(AA)_2Arg_2$ group calculated from tryptophan degradation with consideration of emission and excitation spectra.

Compound	Concentration [μM]	Emission spectrum	Excitation spectrum	Average values
$PP(Ala)_2Arg_2$	28.1	0.093	0.098	0.096
$PP(Ser)_2Arg_2$	28.5	0.132	0.133	0.133
$PP(Phe)_2Arg_2$	17.0	0.156	0.171	0.164
$PP(Thr)_2Arg_2$	16.6	0.225	0.213	0.219
$PP(Trp)_2Arg_2$	26.6	0.228	0.244	0.236

The amount of tryptophan (Trp) reacted during photo-oxidation was determined from dependence of fluorescence intensity on Trp concentration. It should be pointed out that for $\lambda = 360$ nm, $PP(AA)_2Arg_2$ does not show its own fluorescence. The kinetics

of tryptophan degradation as a function of illumination time for particular derivatives is presented in Fig. 7 for $PP(Ala)_2Arg_2$, Fig. 8 for $PP(Ser)_2Arg_2$, Fig. 9 for $PP(Thr)_2Arg_2$, Fig. 10 for $PP(Phe)_2Arg_2$, and Fig. 11 for $PP(Trp)_2Arg_2$. Table 1 illustrates the calculated quantum yields of singlet oxygen Φ_Δ for these five derivatives.

2.2. Determination of the quantum yield Φ_Δ of $^1O_2^*$ versus dihydrochloride of hematoporphyrin from luminescence decay of singlet oxygen in NIR region

A chromatographically purified dihydrochloride of hematoporphyrin (HP) of the Porphyrin Products has been used as a standard. The solutions of $PP(AA)_2$ were prepared in chloroform. The solutions of $PP(AA)_2Arg_2$ were prepared in D_2O and obtained from the Nuclear Chemistry Institute in Świerk, Poland. The pH value of

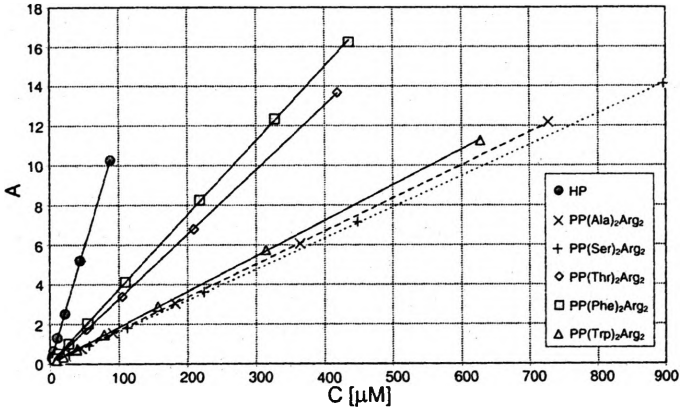

Fig. 12. Dependence of absorption of $PP(AA)_2Arg_2$ solution in D_2O with 2% TX-100 *vs.* concentration of porphyrin; absorbance was normalized for l = 1 cm.

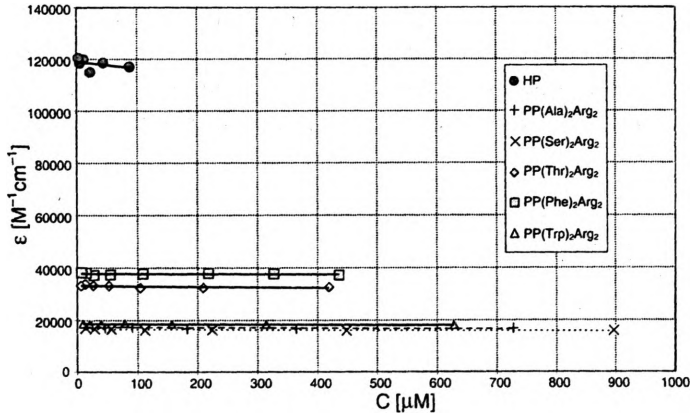

Fig. 13. Dependence of molar absorption coefficients of $PP(AA)_2Arg_2$ in D_2O with 2% TX-100 *vs.* concentration of porphyrin.

solutions was equal to 7.2. Pure chloroform was used and after dehydration it was oxidized with dry oxygen. Because of high tendency to $PP(AA)_2Arg_2$ aggregation, 2% (mass %) detergent TX-100 of Merc firm was added to their solution in D_2O. In water solution with TX-100, the porphyrins are in the form of monomer. Figure 12 illustrates linear changes of absorbance of these solutions as a function of porphyrin concentration. Molar absorption coefficients for the Soret band are significantly higher in 2% TX-100 solution than in pure water at μM concentration of porphyrin. The molar absorption coefficients determined for $PP(AA)_2Arg_2$ and HP in D_2O with 2% TX-100 are shown in Fig. 13.

Solutions were in the cuvette of the optical path 1 cm and volume 4 ml. Porphyrins were excited with a xenon lamp of 450 W with a monochromator adjusted at 400 nm. Excitation radiation was introduced into the middle part of the cuvette at an adequate angle in order to maintain linear dependence of absorption and emission as a function of concentration. Luminescence of singlet oxygen was measured using L-900 Edinburgh Analytical Instrument fluorometer.

The luminescence intensity of $_1O^2$ at 1272 nm for protoporphyrin derivatives *vs.* their concentration was measured. The concentration of HP as a standard was 83 μM. Light intensity absorbed by various derivatives was the same as for the standard. Because of various molar absorbance coefficients of $PP(AA)_2Arg_2$, their concentrations were in the range of 1–100 μM and concentrations of arginized derivatives of $PP(AA)_2Arg_2$ within the range 15–650 μM.

The quantum yield of phosphorescence of singlet oxygen was determined as the ratio of the total number of photons emitted by singlet oxygen (luminescence at 1272 nm) to the number of absorbed photons in the whole range of absorption spectrum

$$\Phi_p^{\Delta} = \frac{I_f}{I_a} \tag{4}$$

where Φ_p^{Δ} is the phosphorescence quantum yield of singlet oxygen, I_f – the total fluorescence intensity, I_a – the intensity of the absorbed light. The sample and the standard were irradiated in the same conditions. Next, dependence of luminescence intensity on concentration of the solutions of compounds being examined was investigated. The following equations were used for calculation of quantum yield of singlet oxygen.

$$\frac{\Phi_{\Delta}(S)}{\Phi_{\Delta}(W)} = \frac{\Phi_p^{\Delta}(S)}{\Phi_p^{\Delta}(W)} = \frac{I_f(S)I_a(W)}{I_f(W)I_a(S)}, \tag{5}$$

$$\frac{I_a^S}{I_a^W} = \frac{1 - 10^{-A^S}}{1 - 10^{-A^W}} \tag{6}$$

where S is the compound examined and W is the standard.

Figures 14 and 15 show phosphorescence intensities of singlet oxygen emitted by the solutions of $PP(AA)_2$ and $PP(AA)_2Arg_2$ derivatives *vs.* their concentrations.

According to literature data, quantum yield of singlet oxygen for hematoporphyrin excited at $\lambda = 532$ nm in D_2O with TX-100 is 0.53, [9]. Because diaminoacid derivatives of protoporphyrin have maximum absorption for $\lambda = 400$ nm, comparative investigation was carried out for standard solution HP of the concentration 83 μM in D_2O with 2% TX-100. The system was excited at $\lambda = 400$ nm and 532 nm, respectively. Intensities of singlet oxygen luminescence and absorbance were written. The quantum yields of singlet oxygen were calculated from Eqs. (4) and (5). The yield of solution excited at $\lambda = 400$ nm is by 10% higher than that of excited solution at $\lambda = 532$ nm. This difference was used for correction of the experimental results obtained. Quantum

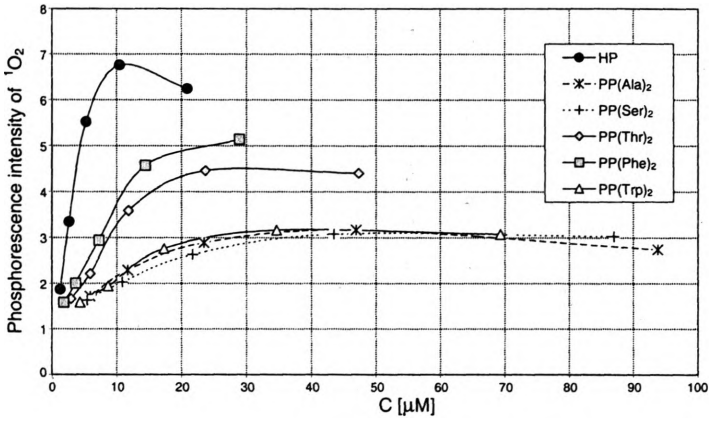

Fig. 14. Phosphorescence intensities of singlet oxygen for $PP(AA)_2$ in $CHCl_3$ *vs.* photosensitizer concentration at 1272 nm.

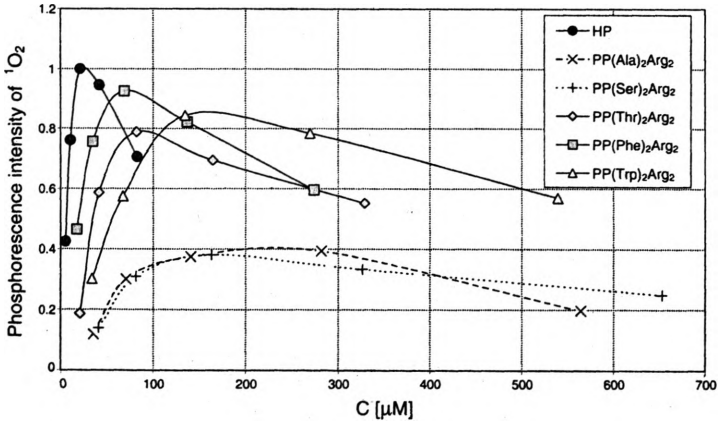

Fig. 15. Phosphorescence intensities of singlet oxygen for $PP(AA)_2Arg_2$ in D_2O *vs.* photosensitizer concentration at 1272 nm.

Table 2. Quantum yields of singlet oxygen determined from phosphorescence decay of $^1O_2^*$ for $PP(AA)_2$ in $CHCl_3$.

HP		$PP(Ala)_2$		$PP(Ser)_2$		$PP(Thr)_2$		$PP(Phe)_2$		$PP(Trp)_2$	
C [μM]	Φ_Δ	C [μM]	Φ_Δ	C [μM]	Φ_Δ	C [μM]	Φ_Δ	C [μM]	Φ_Δ	C [μM]	Φ_Δ
20.93	0.79	93.84	0.35	86.95	0.38	47.35	0.55	28.93	0.65	69.32	0.39
10.47	0.85	46.92	0.40	43.47	0.39	23.67	0.56	14.47	0.58	34.66	0.40
5.23	0.70	23.46	0.36	21.74	0.33	11.84	0.45	7.23	0.37	17.33	0.35
2.62	0.42	11.73	0.29	10.87	0.26	5.92	0.28	3.62	0.25	8.67	0.24
1.31	0.23	5.86	0.22	5.43	0.21	2.96	0.21	1.81	0.20	4.33	0.20

Table 3. Quantum yields of singlet oxygen determined from phosphorescence decay of $^1O_2^*$ for $PP(AA)_2Arg_2$ in D_2O with 2% TX-100.

HP		$PP(Ala)_2Arg_2$		$PP(Ser)_2Arg_2$		$PP(Thr)_2Arg_2$		$PP(Phe)_2Arg_2$		$PP(Trp)_2Arg_2$	
C [μM]	Φ_Δ	C [μM]	Φ_Δ	C [μM]	Φ_Δ	C [μM]	Φ_Δ	C [μM]	Φ_Δ	C [μM]	Φ_Δ
82.95	0.53	564.30	0.15	653.20	0.19	329.15	0.41	274.00	0.45	539.19	0.43
41.48	0.71	282.15	0.30	326.60	0.25	164.57	0.52	137.00	0.62	269.60	0.59
20.74	0.75	141.07	0.28	163.30	0.29	82.29	0.59	68.50	0.70	134.80	0.64
10.37	0.77	70.54	0.24	81.65	0.24	41.14	0.46	34.25	0.60	67.40	0.46
5.18	0.42	35.27	0.12	40.83	0.14	20.57	0.18	17.13	0.45	33.70	0.30

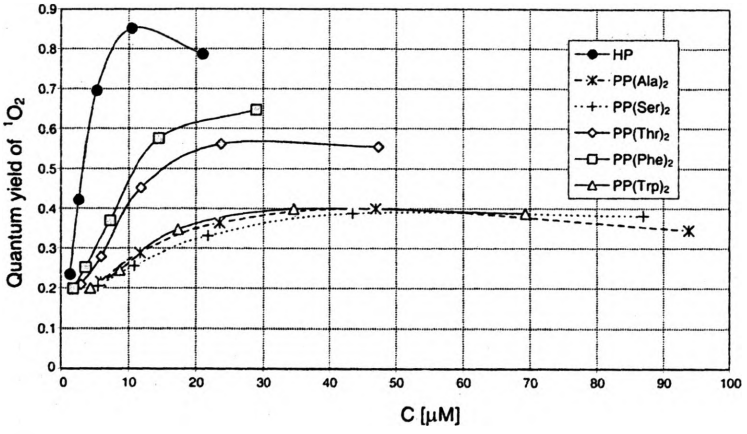

Fig. 16. Quantum yields of singlet oxygen for $PP(AA)_2$ in $CHCl_3$ *vs.* photosensitizer concentration at 1272 nm.

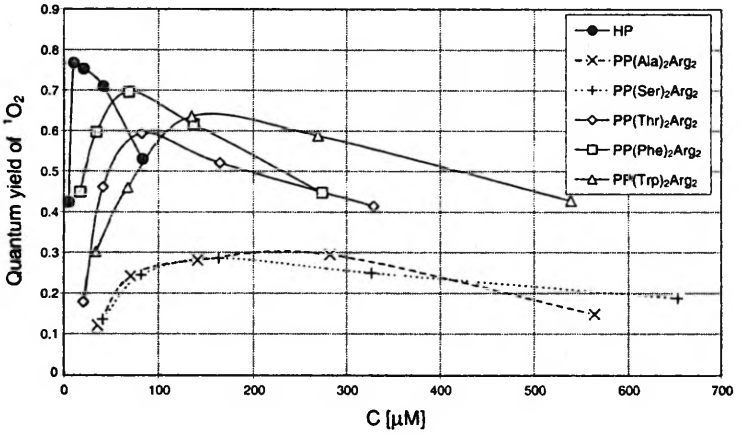

Fig. 17. Quantum yields of singlet oxygen for $PP(AA)_2$ in D_2O *vs.* photosensitizer concentration at 1272 nm.

yields of singlet oxygen, determined with the method of direct measurement of the luminescence decay of $^1O_2$ as a function of concentration are shown in Figs. 16 and 17. The digital values of Φ_Δ are shown in Tab. 2 for $PP(AA)_2$ and in Tab. 3 for $PP(AA)_2Arg_2$.

3. Discussion on results

Quantum yield of singlet oxygen is the other important parameter characterizing usefulness of the given photosensitizer for therapy. Of course, there is no simple dependence between quantum yield determined experimentally in laboratory conditions and production of $^1O_2$ by the given photosensitizer under *in vivo* conditions. To have effective cytotoxic singlet oxygen, produced photodynamically, a photo-

sensitizer must be located in adequate sub-cellular organellas, the best place for it is mitochondrium. However, determination of the ability of a given sensitizer to generate singlet oxygen is the basis for estimation of its usefulness in therapy with photodynamic method.

First, quantum yield of singlet oxygen Φ_Δ was determined with indirect method applying tryptophan as acceptor of singlet oxygen. A decrease in intensity of the emission band and excitation band in tryptophan spectrum was investigated. Investigation of changes in absorption band intensity was difficult because absorption bands of tryptophan photo-product cover the absorption bands of non-oxidized tryptophan. It can be seen from Tab. 1 that quantum yield of singlet oxygen Φ_Δ, determined with indirect method, depends on aminoacids substituted in a porphyrin ring and it increases with the substituent value. This is probably connected with aggregation ability of particles of the given derivative. As is known, the higher stage of aggregation reduces the ability of a photosensitizer to generate $^1O_2$ and larger (in volume) substituent can counteract it due to steric hindrance. It should be pointed out that yields of singlet oxygen determined from excitation and emission spectra are almost of the same value.

Also, the quantum yield of singlet oxygen was determined from the measurement of phosphorescence decay for λ_{max} = 1272 nm. Investigation was made for the standard HP as well as for $PP(AA)_2$ in $CHCl_3$ and $PP(AA)_2Arg_2$ in D_2O with addition of TX-100. In these conditions, lifetimes of $^1O_2$ are longer and higher intensity of phosphorescence of $^1O_2$ can be registered. Analogous values were obtained for the values of quantum yields of singlet oxygen (shown in Figs. 16 and 17). In a non -polar medium, the lifetime of $^1O_2$ is significantly longer than in water medium. It results from numerical data of quantum yield of $^1O_2$ for $CHCl_3$ and D_2O, presented in Tabs. 2 and 3, that quantum yield of $^1O_2$ for both $PP(AA)_2$ and $PP(AA)_2Arg_2$ depends on concentration, *i.e.*, on aggregation degree.

Comparing the values $\Phi_{^1O_2}$ for similar values of concentrations, one can see that the highest quantum yield of singlet oxygen has been obtained for $PP(Phe)_2Arg_2$. For solutions in a non-polar solvent and concentrations 2–10 μM, the compounds under investigation are in monomeric form, which is demonstrated by the higher $\Phi_{^1O_2}$ values. For water solutions, despite measurement in heavy water with TX-100 addition, the comparable quantum yields $^1O_2$ were obtained at higher concentrations in comparison with the solutions in chloroform. It results from the analysis of the data presented in Tabs. 2 and 3 that quantum yield of singlet oxygen depends on many factors such as medium polarity, chemical structure of the compound and its ability to aggregate. Under *in vivo* conditions, the values $\Phi_{^1O_2}$ can be additionally affected by such factors as dissociation of mers under influence of adequate enzymes, which causes an increase in both fluorescence quantum yield Φ_f and quantum yield of singlet oxygen $^1O_2$, medium pH, and interaction with proteins that are in tissues medium.

References

[1] SHU YE., CZUBA M., ROMISZEWSKA A., KAROLCZAK J., GRACZYK A., Opt. Appl. **33** (2003), 489.
[2] TURRO N.J., *Singlet oxygen and chemiluminescent organic reactions*, Modern Molecular Photochemistry, University Science Books, California 1991, pp. 583–593.
[3] FOOTE C.S., *Mechanisms of photo-oxygenation*, [In] *Porphyrin Localization and Treatment of Tumors*, [Eds.] D.R. Doiron, C.J. Gomer, Alan R. Liss, New York 1984, pp. 3–18.
[4] WILKINSON F., HELMAN W., ROSS A., J. Phys. Chem. Ref. Data **22** (1993) 113.
[5] PADZIK-GRACZYK A., KONARSKI J., SOBCZYŃSKA J., *Method of hemins synthesis*, PL 165248B1/94 (1994), (in Polish).
[6] GRACZYK A., KONARSKI J., *Complex Salts Hematoporphyrin and its derivatives, their synthesis and Therapeutic Agents*, US 005451599A , 1995; EP0539960A2/97, 1997.
[7] LAMBERT C.R., REDDI E., SPIKES J.D., RODGERS M.A.J., JORI G., Photochem. Photobiol. **44** (1986), 595.
[8] REDDI E., RODGERS M.A.J., SPIKES J.D., JORI G., Photochem. Photobiol. **40** (1984), 415.
[9] RODGERS M.A., SNOWDEN P.T., J. Am. Chem. Soc. **104** (1982), 5541.

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