ISSN 0036-0244, Russian Journal of Physical Chemistry A, 2018, Vol. 92, No. 8, pp. 1621–1626. © Pleiades Publishing, Ltd., 2018.
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PHOTOCHEMISTRY AND MAGNETOCHEMISTRY

Photogeneration of Singlet Oxygen by Tetra(*p*-Hydroxyphenyl)porphyrins Modified with Oligo- and Polyalkylene Oxides

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Received November 16, 2017

Abstract—Mono- and diethylene oxide derivatives of tetra(*p*-hydroxyphenyl)porphyrin (THPP) with different conformation states are synthesized. These compounds exhibit high photosensitization activity in the generation of singlet oxygen in organic and aqueous (in the solubilized state) phases. It is shown that introducing ethylene oxide moieties into the hydroxyphenyl substituents of THPP increases its solubility in chloroform. In addition, the activity of singlet oxygen $^1\Delta_g$ generation in the reaction of anthracene photooxidation by THPP tetra derivatives in chloroform, where the ethylene oxide fragments are introduced into two phenyl rings and hexadecyl fragments are introduced into another two, is higher than the activity of mono- and di-modified THPP molecules with enthylene oxide molecules at one hydroxyphenyl cycle. The activity of tetra-substituted porphyrin in chloroform, however, is comparable to that of nonsubstituted tetraphenylporphyrin, considered to be one of the most active photosensitizers. The produced ethylene oxide derivatives of THPP are solubilized with pluronic F-127 (triblock copolymer of ethylene- and propylene-oxides)—one of the least toxic and effective polymeric detergents—forming water-soluble forms of the respective porphyrins. It is established that the pluronic solubilization capability (the lowest molar concentration of pluronic required for the complete transfer of porphyrin of a particular molar concentration dissolved in organic phase to the water-soluble form) is higher for asymmetrical mono- and di-derivatives of the THPP than for symmetric tetra-substituted THPP. It is shown that the activity of the solubilized water-soluble form of mono- and tetra-derivatives in tryptophan photoxidation is higher than that of unsubstituted THPP and is comparable to the activity of tetraphenylporphyrin.

Keywords: pluronic F127, tetraphenylporphyrins, solubilization, photosensitizer, photooxidation
DOI: 10.1134/S0036024418080277

INTRODUCTION

Photodynamic therapy (PDT) based on the processes of light-activated cell degradation in the presence of reactive oxygen species (mainly singlet oxygen $^1\Delta_g$ generated by photosensitizers (PSes) under the action of light) is mostly used for treating tumors [1]. It was shown recently that PDT is also effective for treating superficial infected wounds, burns, and trophic ulcers [2, 3]. Water-soluble nitrogen-containing macrocycles (either synthetic or natural) have been used as PSes, including phorphyrins (photohem) and their analogs phthalocyanines (photosens), chlorophyll derivatives (pheophorbide *a*), chlorin (fotoditazin, photolon), and 5-aminolevulinic acid (alasens), the precursor of photoporphyrin IX in organisms [4–6].

The PSes with absorption bands in the red region of the spectrum ($\lambda \sim 650$–800 nm) are commonly used in photoexcitation as the region of maximum transparency of tissues [7]. These bands are usually of moderate intensity, so the efficiency of singlet oxygen generation with excitation in the red region of the PS spectrum is not very high, as in the case of water-soluble porphyrin-type PSes. At the same time, the efficiency of singlet oxygen generation on excitation in the Soret band region ($\lambda \sim 400$ nm) of the porphyrin absorption spectrum is several times higher than the one observed with excitation in the long-wave prophyrin absorption band [8]. The synthetic tertaphenylporphyrin and its derivatives are among the most efficient PSes for the generation of singlet $^1\Delta_g$ oxygen, which exhibits one of the highest photogeneration quantum

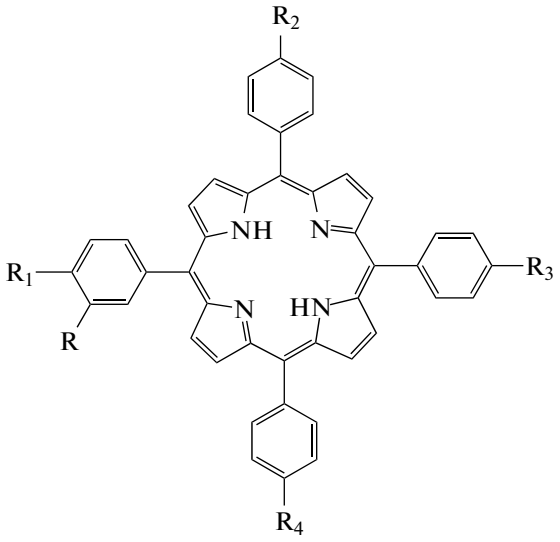

Fig. 1. Structural formula of THPP, mETHPP, dETHPP, and tETHPP. See denotation in Table 1.

yields (Φ_Δ ~ 0.6–0.8) when excited with light in the Soret band region [9]. This offers the possibility of using the Soret band for the excitation of the PSes; it is the main band in the visible range of the porphyrin spectrum with an extinction coefficient exceeding that of red bands by one order of magnitude. Note that the means of synthesizing TPP and its derivatives are quite simple [6].

Hydrophobic TPP derivatives have potential as PSes and in cancer PDT [10–12]. It has also been shown that substituted TPPs display activity toward atherosclerotic plaques [13], along with antiviral (against human immunodeficiency virus) [14] and antimicrobial activity [15, 16]. Amphiphilic derivatives of TPP are of particular interest, since they can bond to blood plasma lipoproteins and be incorporated into tumor cells via the receptor-mediated endocytosis of low-density lipoproteins [17]. Amphiphilic porphyrins are actively investigated as model compounds for determining the total efficiency of photosensitization processes, particularly in the processes of PS aggregation [18, 19] when determining their photosensitization activity in microheterogeneous environments [20].

In this work, we synthesized oligo-ethylene oxide derivatives of tetra- and (*p*-hydroxyphenyl)porphyrine were and considered their photocatalytic activity in reactions of singlet oxygen generation in organic and aqueous phases (in the solubilized state), and in the photooxidation of tryptophan and anthracene. Such ethylene oxide derivatives of tetraphenylporphyrins could have enhanced compatibility with cell lipid bilayers and can be used as efficient photosensitizers in the photodynamic therapy of superficial tumors, wounds, and ulcers. For example, it was shown in [21, 22] that poly(ethylene oxide) and its co-polymers with poly(propylene oxide) could interact with cell membranes and even affected their structural organization in such a way that the cells became more susceptible to the action of some antibiotics (e.g., doxorubicin).

EXPERIMENTAL

Several metal-free porphyrin compounds were prepared and used as PSes: 5,10,15,20-tetraphenylprophyrin (TPP), 5,10,15,20-tetra(4-hydroxyphenyl)porphyrin (THPP), 5-{4-2-[2-(2-methoxyethoxy)ethoxy]ethoxyphenyl}-10,15,20-tris(4-hydroxyphenyl)porphyrin (mETHPP), 5-{3,4-2-[2-(2-methoxyethoxy)ethoxy]ethoxyphenyl}-10,15,20-tris(4-hydroxyphenyl)porphyrin (dETHPP), and 5,10-di{4-2-[2-(2-methoxyethoxy)ethoxy]ethoxyphenyl}-15,20-di(4-hexadecyloxyphenyl)porphyrin (tETHPP) (Fig. 1, Table 1).

TPP and THPP were synthesized according to the procedure in [23]. Substituted tetra(4-hydroxyphenyl)prophyrins were synthesized by means of mixed aldehyde monopyrrole condensation using functionalized benzaldehydes [23, 24]. Substituted benzaldehydes with triethylene glycol residues were produced via the alkylation of 4-hydroxybenzaldehyde and 3,4-dihydroxybenzaldehyde with triethylene glycol mesylate in boiling DMF using cesium carbonate as a base. Pyrrole and substituted benzaldehydes were condensed in stoichiometric ratios in a boiling nitrobenzene/propionic acid/acetic acid mixture (1 : 2 : 1). The target porphyrins was purified via chromatography on a column packed with a silica gel G 60, followed by recrystallization from organic solvents. The yields of asymmetrically substituted porphyrins mETHPP and dETHPP were 10–12%; that of the

Table 1. Structure of THPP, mETHPP, dETHPP, and tETHPP

Porphyrin compounds	R	R_1	R_2	R_3	R_4
THPP	H	H	H	H	H
mETHPP	H	$(CH_2CH_2O)_3CH_3$	OH	OH	OH
dETHPP	$(CH_2CH_2O)_3CH_3$	$(CH_2CH_2O)_3CH_3$	OH	OH	OH
tETHPP	H	$(CH_2CH_2O)_3CH_3$	$(CH_2CH_2O)_3CH_3$	$C_{16}H_{33}$	$C_{16}H_{33}$

tEHTPP compound, 5.5%. TLC, ^{1}H, ^{13}C-NMR spectroscopy, and MALDI-TOF mass spectrometry were used to identify compounds and determine their structures.

Anthracene (chemically pure grade) and D,L-tryprophan (pure grade) manufactured by AO Reakhim (Russia) were used in photooxidation reactions. Pluronic F-127 (Sigma-Aldrich, United States) with a molecular mass of 12 600 was used for the solubilization of PPS. UV spectra were recorded on a Varian Cary 50 spectrophotometer.

Solubilization of tetraphenylporphyrins with pluronic was conducted by preparing a combined solution of polymer and porphyrin in chloroform with a porphyrin concentration of 5.0×10^{-6} M (in experiments on evaluating functional activity) and 3×10^{-5} M (in experiments on evaluating solubilizing activity), and the concentration of pluronic was varied in the range 3.0×10^{-6}–3×10^{-2} M. The solution was evaporated to dryness on a rotary evaporator, forming a 50–100-μm thick pluronic film with porphyrin absorbed in it. Since the aim of this work was to prepare water-soluble porphyrin photosensitizers, the resulting film was dissolved in water, where the degree of solubilization was determined as the fraction of PPS molecules transferred to the aqueous phase as the films dissolved. Degree γ of PPS solubilization was calculated from the electronic absorption spectra (EAS) of porphyrins in water according to the equation $\gamma = C_{Pw}/C_{Ps}$, where C_{Ps} is the concentration of porphyrin photosensitizer in the initial solvent (chloroform), and C_{Pw} is the concentration of PPS in water. With THPP (which is poorly soluble in chloroform), solubilization was conducted by preparing combined solutions of PPS and pluronic in a chloroform–ethanol mixture (25 : 1). The concentration of solubilized PPS was calculated from the intensity of the IV band in the EAS that was affected least during solubilization (Fig. 2, *1*–*3*).

The photooxidation reactions of substrates (thryptophan in water and anthracene in chloroform) were conducted a quartz cuvette (thickness $l = 1$ cm) with mixing using an AFS phototherapeutic apparatus manufactured by OOO Polironik (Russia) for illumination. The operating wavelength was $\lambda = 400$ nm, and the power was 210 mW. The substrate concentration was $C_s = 1 \times 10^{-4}$ M, the PPS concentration was $C_P = 5.0 \times 10^{-6}$ M, and pluronic F-127 concentration C_{Pl} was varied from 5×10^{-6} to 5×10^{-4} M. The kinetics of tryptophan and anthracene photooxidation was monitored using the drop in optical density at the UV absorption bands of tryptophan in water ($\lambda = 280$ nm) and anthracene in chloroform ($\lambda = 360$ nm). With THPP (which is poorly soluble in chloroform), the photooxidation reaction was conducted in a chloroform–ethanol mixture (25 : 1).

The effective rate constants of tryptophan and anthracene photooxidation were determined from the initial parts of the kinetic curves, where the drop in substrate concentration ΔC_s over period of observation Δt was ~20–30%, and calculated according to the equation $k_{\text{eff}} = \Delta C_s / C_s C_P \Delta t$. The values of rate constants were calculated from three experimental replicates. The measuring error was ~10%.

RESULTS AND DISCUSSION

Photosensitization Activity of Substituted TPP in the Oxidation of Anthracene in Chloroform in with and without Pluronic F127

The dependences of k_{eff} for anthracene photooxidation in chloroform with TPP (curve *1*) and substituted TPP (curves *2*–*4*) on the pluronic F127 concentration are presented in Fig. 3. Because THPP is insoluble in chloroform, the photocatalytic activity of the ethylene oxide derivatives of THPP in organic and aqueous phases was compared to the activity of unsubstituted tetraphenylporphyrin, one of most effective photosensitizers of singlet oxygen generation.

It can be seen that with no pluronic F127, the mono- and di-substitutions in TPP reduced PPS activity (the curves in Fig. 3), while tetra-substitution did not affect the porphyrin activity. The effective constant of photooxidation of anthracene for the mono-substituted TPP in particular is 150% lower than the k_{eff} value for unsubstituted TPP, and 300% lower for di-substituted TPP. The asymmetric substitution (in mono- and di-substituted TPP molecules) that shifts the electron density and polarization of the porphyrin molecule obviously increases the PPS's propensity for aggregation.

It is interesting that the pluronic has virtually no effect on the photosensitization activity of TPP (curve *1*) and tetra-substituted THPP (curve *4*); the levels of their activity are approximately the same. At the same time, a 150% increase in activity upon a rise in pluronic concentration is observed for the mono- and di-substituted THPP (curves *2*, *3*). These porphyrins are obviously less soluble in chloroform than TPP and tETHPP and were in an aggregated state. Deaggregation occurs when the pluronic is present, and the photocatalytic activity of the mono- and di-substituted TPP increases as a result. This agrees with the increase in the intensity of the Soret band in the EAS of dETHPP in chloroform with the pluronic (Fig. 2b, curve *2*).

Determining the Degree of Solubilization

The electronic absorption spectra of TPP in chloroform with and without pluronic F127 and of TPPs solubilized with pluronic in water are presented in Fig. 2a. We can see there is no change in the EAS of the unsubstituted TPP in chloroform with the

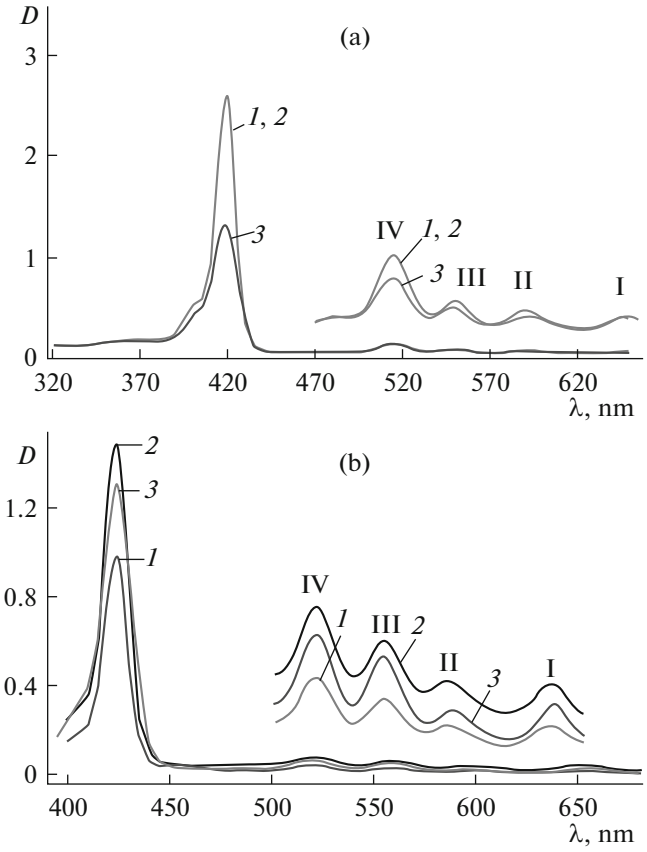

Fig. 2. EAS of (a) TPP and (b) dETHPP solutions: (*1*) in chloroform, (*2*) in chloroform in the presence of pluronic F127, (*3*) PPS solubilized with pluronic F127 in water. Concentrations: PPS, 5×10^{-6} M; pluronic, 5×10^{-4} M.

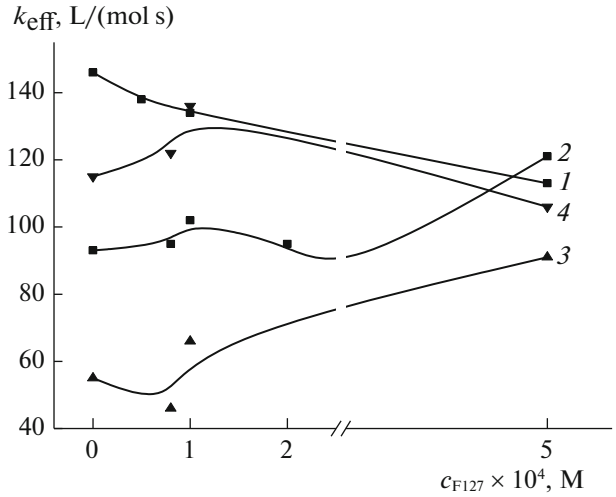

Fig. 3. Dependences of effective constants k_{eff} of photooxidation of anthracene in chloroform in the presence of substituted TPP on pluronic F127 concentration: (*1*) TPP, (*2*) mETHPP, (*3*) dETHPP, (*4*) tETHPP. Concentrations: PPS, 5×10^{-6} M; anthracene, 1×10^{-4} M.

pluronic, while the intensity of all bands increases in the EAS of dETHPP in chloroform with the pluronic (Fig. 2b). This indicates interaction between the dETHPP and pluronic molecules, increasing its solubility in chloroform and the degree of dETHPP de-aggregation.

The EASes of all porphyrins in the aqueous phase show a slight drop in the intensity of all absorption bands, compared to that of these bands in the absorption spectra of porphyrins in chloroform with the pluronic. This agrees with the dependences presented in Fig. 3 that display an increase in the intensities of bands as the pluronic concentration rises, indicating the complete solubilization of porphyrin molecules and their transfer to the aqueous phase in de-aggregated state [25, 26].

The degree of solubilization γ of PPS, which is defined as the fraction of PPS molecules transferred to the aqueous phase as the formed films dissolve, depends only on initial ratio q of the molar concentrations of prophyrin and pluronic. We analyzed dependences $\gamma = \gamma(q)$ obtained during the solubilization of water-insoluble porphyrins of different types (Fig. 4).

It follows from Fig. 4 that the maximum degree of solubilization ($\gamma \sim 1$) is realized in different systems at different values of $q \le q_{max}$, while solubilization is reduced when $q \ge q_{max}$ [27, 28]. The different patterns of solubilization are related to features of the PPS structure. The pluronic displays de-aggregating activity during the solubilization of PPS, and the degree of solubilization depends mainly on the structure of peripheral substituents in the prophyrin molecules that determine the affinity of porphyrin to the pluronic (its ability to bind to polymer).

A sharp drop in the degree of solubilization at $q_{max} = 0.7$ is observed for TPP (Fig. 4, curve *1*), and the degree of solubilization is close to zero at $q_{max} = 1.4$, testifying to the high hydrophobicity of the porphyrin. A sharp drop in the degree of solubilization is observed for tETHPP at $q_{max} \approx 0.2$ (Fig. 4, curve *4*). At the same time, the degree of solubilization of mETHPP and dETHPP does not fall at values of $q \le 3.5$ (Fig. 4, curves *2* and *3*), due to the increased hydrophilicity of these porphyrins and their enhanced ability to bond to the polymer. Similar increased ability to bond to the pluronic was observed in [9] for THPP.

Photooxidation of Tryptophan in Water in the Presence of PPS Solubilized with Pluronic F127

We investigated the effect pluronic has on the catalytic activity of the solubilized PPS in the photooxidation of tryptophan in water. The resulting data are presented in Fig. 5. As was noted above, the increase in the activity of solubilized porphyrins upon an increase in pluronic concentration is related to the de-aggrega-

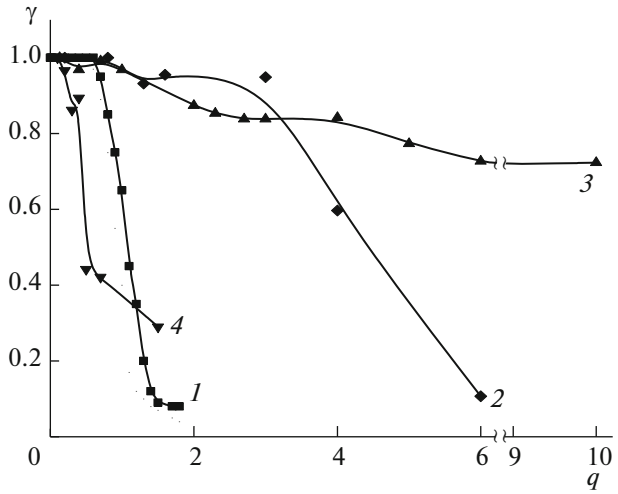

Fig. 4. Dependences of degree of solubilization γ of porphyrins with pluronic F127 on ratio q of molar concentrations of PPS and pluronic: (*1*) TPP, (*2*) mETHPP, (*3*) dETHPP, (4) tETHPP. PPS concentration, 5×10^{-6} M; pluronic concentration varied in the range 3.0×10^{-6}–3×10^{-2} M.

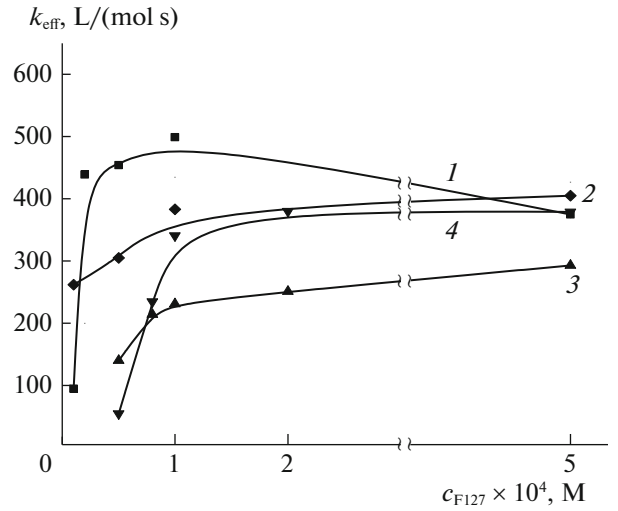

Fig. 5. Dependences of effective constant k_{eff} of photooxidation of tryptophan in the presence of PPS solubilized with pluronic F127 on concentration of pluronic F127: (*1*) TPP, (*2*) mETHPP, (*3*) dETHPP, (*4*) tETHPP. Concentrations: PPS, 5×10^{-6} M; tryptophan, 1×10^{-4} M.

tion of the initially associated porphyrins in aqueous and nonaqueous solutions [25]. The presented data demonstrate first of all that the photocatalytic activity of the solubilized mono- and tetra-derivatives of TPP can be compared to the activity of unsubstituted TPP. Since the affinity of each of the considered TPPs toward cell membranes can be determined from the number of ethylene oxide substituents in the PPS molecules, further investigation of their photosensibilization activity in cell cultures or during the PDT of laboratory animals should allow us to establish whether these PPSes offer any promise for clinical application.

ACKNOWLEDGEMENTS

This work was supported by the Russian Science Foundation, project 16-13-10295, "Investigating the Photosensibilization Activity of Modified Tetraphenylporphyrins"; by the Russian Foundation for Basic Research, project 16-33-00459, "Synthesis of Substituted Tetraphenylprophyrins"; by the Presidential Foundation for Leading Scientific Schools, project no. 7946.2016.11; and by a grant from First Moscow State Medical University.

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Translated by L. Brovko