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Effects of NaCl upon $TPPS_4$ triplet state characteristics and singlet oxygen formation

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Abstract

The quantum yield (φ_T) and lifetime (τ_T) of the triplet state (T) of water-soluble *meso*-tetrasulphonatophenyl porphyrin ($TPPS_4$) and the kinetic profile and quantum yield ($\varphi_{^1O_2}$) of the singlet oxygen $^1O_2$, produced due to the energy transfer from T to molecular oxygen, were investigated for biprotonated (pH 4.0) and non-protonated (pH 7.0) $TPPS_4$ forms as a function of the NaCl concentration. The study was performed with the help of flash-photolysis technique compared with the data obtained by optical absorption spectroscopy. The $^1O_2$ characteristics were monitored by kinetics and spectrum of its infrared phosphorescence.

As shown, for biprotonated $TPPS_4$ the addition of NaCl reduces both φ_T and τ_T due to two processes: "quenching" by Na^+ and/or Cl^- ions and successive formation of H and J $TPPS_4$ aggregates. The triplet state formation for the porphyrin J aggregates was detected. For non-protonated $TPPS_4$, no aggregation in the presence of salt occurs and the observed reduction of φ_T and τ_T was due to the interaction with Na^+ and/or Cl^- ions. The reduction of φ_T was accompanied by the simultaneous reduction of ($\varphi_{^1O_2}$). No NaCl effects on the quenching constant of T by O_2 and on the $^1O_2$ lifetime were observed.

Keywords: $TPPS_4$; Ionic strength; Aggregation; Triplet state; Singlet oxygen

1. Introduction

Meso-tetrasulphonatophenyl porphyrin ($TPPS_4$) (Fig. 1) is considered one of the promising water-soluble synthetic compounds for application in cancer photodynamic therapy (PDT) [1,2]. Cancer treatment by PDT is based on the production of the excited molecular oxygen singlet state (singlet oxygen, $^1O_2$) responsible for neoplasic cell inactivation. The singlet oxygen is formed by the energy transfer from triplet state of a photosensitizer to oxygen molecule [3]. $TPPS_4$ in its non-protonated form is characterized by high quantum yields of the triplet state and the singlet oxygen production, $\varphi_T = 0.78$ [4] and $\varphi_{^1O_2} = 0.62$ [5], respectively, and possesses high affinity to tumor tissues [6]. However, in the presence of salts in acidic media, where $TPPS_4$ exists in biprotonated state, it aggregates [7–9 and therein]. It is known that the aggregation reduces the quantum yield and the lifetime of the excited triplet state of porphyrins [10,11 and therein] and consequently should reduce the $^1O_2$ generation yield.

In this work, we report on the study of the NaCl effects upon $TPPS_4$ triplet state and $^1O_2$ characteristics using the flash-photolysis technique and time resolved detection of $^1O_2$ phosphorescence. The data obtained were analyzed compared with those for optical absorption spectroscopy.

2. Materials and methods

Meso-tetrasulphonatophenyl porphyrin ($TPPS_4$) was obtained from Porphyrin Products, Inc. and not additionally purified.

The experiments were performed at pH 4.0 and 7.0. The pH value was adjusted by adding HCl or NaOH concentrated stock solutions into the experimental cell. pH was controlled by Corn-

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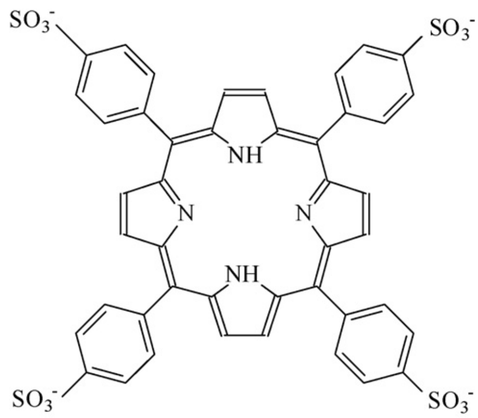

Fig. 1. Structure of $TPPS_4$.

ing 430 pH-meter. The NaCl concentration varied from 0 to 0.4 M. All solutions were prepared in the Milli-Q quality water.

The porphyrin triplet state was produced by the second harmonic short light pulses (532 nm) from Nd:YAG SL400 Spectrum Laser System (with 10 ns pulse duration). $TPPS_4$ concentration was controlled spectrophotometrically with the molar absorption coefficient $\varepsilon^{644\,nm} = 3.26 \times 10^4\,M^{-1}\,cm^{-1}$ at pH 4.0 or $\varepsilon^{515\,nm} = 1.30 \times 10^4\,M^{-1}\,cm^{-1}$ at pH 7.0 [12]. In all experiments, this concentration approached 30 μM resulting in the solution absorbance ≈0.2 at 532 nm.

The decay curves of the $TPPS_4$ triplet state were registered by the triplet–triplet absorption (T_1–T_n) at λ = 470 nm. To study the oxygen effect, the samples were deaerated by bubbling nitrogen through the solution for 20 min.

Optical absorption spectra were monitored in the range from 350 to 750 nm by a Beckman Coulter DU640 spectrophotometer.

The singlet oxygen formation and decay dynamics were monitored directly by its phosphorescence profile at 1270 nm using modified "Edinburgh F900" system.

All experiments were carried out in 1 cm quartz cuvettes at 24(±1) °C.

3. Results and discussion

3.1. *$TPPS_4$ spectral and kinetic characteristics: the effect of pH and NaCl*

In homogeneous aqueous solutions, $TPPS_4$ is characterized by two pK_a points near pH 5.0 [4,13]. Thus, at pH 7.0 $TPPS_4$ is non-protonated ($H_2TPPS_4^{4-}$) with the net charge equal 4−. The absorption is characterized by maxima centered at 413 (the Soret band) and 515, 552, 578, and 632 nm (Q bands) (Fig. 2, curve a). For this $TPPS_4$ state, the excited singlet state S_1 lifetime is $\tau_{S_1} = 9.0$ ns and fluorescence and triplet state quantum yields are $\varphi_{fl} = 0.16$ and $\varphi_T = 0.78$, respectively [4].

In acidic media (pH < 4.8) $TPPS_4$ exists in a biprotonated form ($H_4^{2+}TPPS_4^{4-}$) with the net charge equal 2−. This $TPPS_4$ form is characterized by the Soret band centered at λ = 433 nm and three Q bands at 550, 594 and 644 nm, respectively (Fig. 2, curve b). Protonation reduces τ_{S1} down to 3.6 ns and φ_T to 0.36; at the same time, φ_{fl} increases up to 0.37 [23].

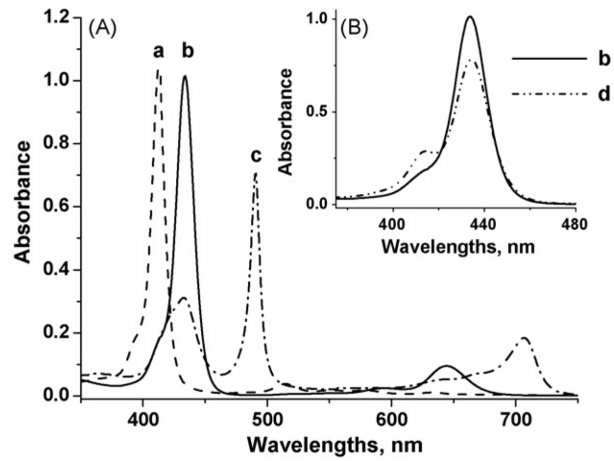

Fig. 2. Absorption spectra of: $TPPS_4$ at pH 7.0 (curve a); $TPPS_4$ at pH 4.0 (curves b in A and B); $TPPS_4$ J aggregates in the presence of NaCl at pH 4.0 (curve c); $TPPS_4$ H aggregates in the presence of NaCl at pH 4.0 (curve d).

Addition of NaCl shifts pK_a points of $TPPS_4$ to the acidic pH region [14]. As shown, at 0.4 M NaCl they approach pH 4.5. Therefore, in the range of NaCl concentrations used at pH 4.0 $TPPS_4$ remains biprotonated and the absorption spectrum is identical to that in the absence of NaCl.

Formerly, it has been demonstrated that the biprotonated $TPPS_4$ aggregates in homogeneous aqueous solutions [8,9,15] and the addition of salt stimulated its aggregation. The aggregation kinetics is characterized by the induction period decreasing from hours to seconds with increasing NaCl and/or porphyrin concentrations. Recently, we have demonstrated that at any NaCl and $TPPS_4$ concentration H aggregates are formed prior to J aggregates and the induction period represent the H aggregates' conversion into the J form [12].

These structural changes are manifested in the absorption spectrum and φ_{fl} alterations of $TPPS_4$ solutions. H aggregates mentioned are characterized by an absorption band centered at 413 nm, whereas J aggregates are characterized by absorption bands centered at 491 and 707 nm (Fig. 2, curves c and d "insertion").

For non-protonated $TPPS_4$ (pH 7.0), NaCl addition in concentrations up to 0.4 M just slightly changes the absorption and fluorescence spectra, φ_{fl} and τ_{S1}. No aggregation of non-protonated $TPPS_4$ in the presence of salt was observed.

3.2. *$TPPS_4$ triplet state characteristics in aqueous solutions: interaction with oxygen*

The 532 nm laser pulse induces the porphyrin excited triplet state formation. The observed variation of optical absorption (ΔA) of the solution is due to a difference between molar absorption coefficients related to the singlet–singlet (ε_{S-S}) and the triplet–triplet (ε_{T-T}) transitions.

$$\Delta A = (\varepsilon_{T-T} - \varepsilon_{S-S})[T] \qquad (1)$$

where [T] is the concentration of the triplet state porphyrin.

The parameter ε_{S-S} of both biprotonated and non-protonated $TPPS_4$ forms is negligibly small ($\varepsilon_{S-S} \cong 0$) at 470 nm, for

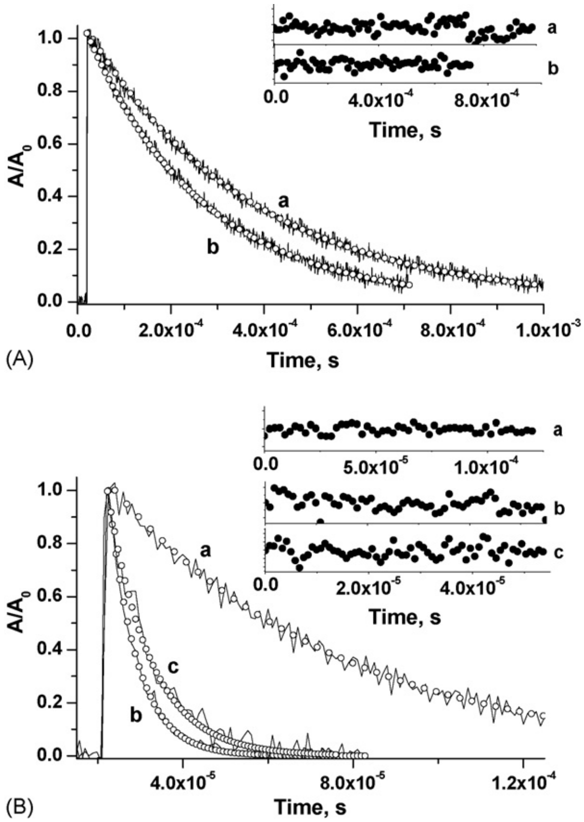

Fig. 3. Normalized decay profiles of the triplet state for [$TPPS_4$] = 30 μM monitored at 470 nm in deoxygenated solutions: at pH 7.0 in the absence (a) and in the presence of [NaCl] = 0.1 M (b); at pH 4.0 in the absence (a) and in the presence of [NaCl] = 0.1 M (b); J aggregates (c) (◯) fitting of these profiles using single-exponential fits. Insertions: residuals of these decay profiles for single-exponential fits.

which ΔA was monitored. In this case, ΔA depends directly on $T_1 \rightarrow T_n$ absorption:

$$\Delta A = \varepsilon_{T-T}[T] \tag{2}$$

At both pH values and all porphyrin concentrations used either in the presence or in the absence of NaCl, the decay profile of the $TPPS_4$ triplet state is mono-exponential (Fig. 3A and B):

$$\Delta A = \Delta A_0 \exp\left(\frac{-t}{\tau}\right) \tag{3}$$

This demonstrates that the contribution of bimolecular quenching between triplets, such as T–T annihilation, was negligibly small under current conditions.

In the absence of oxygen, the $TPPS_4$ triplet state lifetime (τ_T) depends on its protonation state. At pH 4.0 (biprotonated porphyrin), it was $\tau_{T0} = (50 \pm 4)$ μs, whereas at pH 7.0 (non-protonated) $\tau_{T0} = (350 \pm 20)$ μs. The $TPPS_4$ triplet state is effectively quenched by molecular oxygen:

$$k_1 = k_0 + k_q[O_2] \tag{4}$$

For both porphyrin forms, the characteristic quenching constant is $k_q \approx 1.8 \times 10^9\ M^{-1}\ s^{-1}$. This value agrees with that observed elsewhere [10]. In the air-saturated solutions at atmospheric

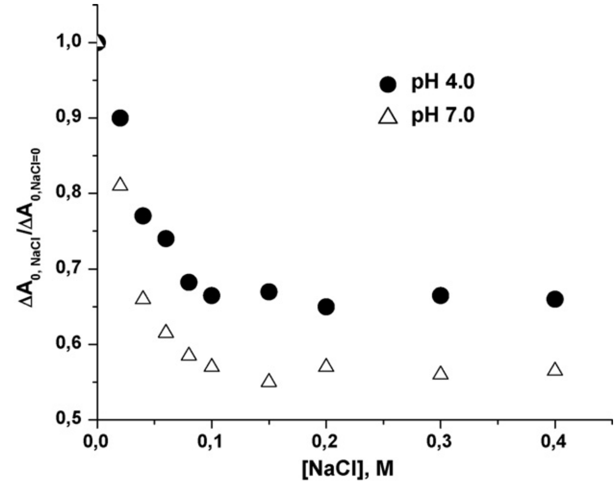

Fig. 4. Normalized values of the maximum $TPPS_4$ T–T, absorbance ($\Delta A_{0NaCl}/\Delta A_0$) at 470 nm as a function of NaCl concentration: (●) pH 4.0; (△) pH 7.0.

pressure ([O_2] = 0.26–0.29 mM [16]) porphyrin triplet lifetime is $\tau_{T1} = 1/k_1 \cong (2.0 \pm 0.1)$ μs for both pH values.

3.2.1. Effects of NaCl

It is reasonable to expect that NaCl, which produces essential modifications in the spectral and kinetic characteristics of $TPPS_4$ in solutions, should also affect the $TPPS_4$ triplet state characteristics.

3.2.1.1. Non-protonated $TPPS_4$ form. Obviously, already for non-protonated $TPPS_4$ form NaCl addition induces a drop of ΔA_0, which depends on the NaCl concentration. This drop reaches ≈40% at [NaCl] > 0.1 M (Fig. 4, curve ●).

In the absence of oxygen, the triplet lifetime reduces to (250 ± 10) μs (Fig. 3A, curve b).

This effect cannot be explained by decreased S–S absorption at 532 nm, since ΔA_0 was corrected using the following equation:

$$\Delta A_0 = \Delta A_{0\,\exp} \frac{D_0}{D_{NaCl}}$$

where $\Delta A_{0\,\exp}$ is the ΔA_0 value obtained in the experiment; D_0 and D_{NaCl} are the S–S absorbances at 532 nm in the absence and in the presence of NaCl, respectively.

Therefore, NaCl does really reduce ΔA_0. This effect may be induced by decreasing ε_{T-T} at the registration wavelength 470 nm and/or decreasing triplet concentration $[T]_0$ immediately after the excited light pulse end. This means a decrease of the porphyrin triplet state quantum yield, φ_T.

The nature of this NaCl effect has not been yet completely clarified. Based on the fact that the effect saturates at [NaCl] > 0.1 M we put forward an idea of Na^+ counter ion "cloud" formation around the $H_2TPPS_4^{4-}$ molecule. However, we cannot exclude completely the possibility of the porphyrin excited state quenching by Cl^- ions, and we pretend to clarify this aspect in our future studies.

The presence of NaCl has no effect on the $TPPS_4$ triplet quenching by oxygen and k_q remains unchanged ($k_q \approx 1.8 \times 10^9\ M^{-1}\ s^{-1}$). The triplet lifetime in the air-saturated solutions is (2.0 ± 0.1) μs.

3.2.1.2. Biprotonated $TPPS_4$ form. The effect of NaCl on the $TPPS_4$ triplet state characteristics is still more pronounced for biprotonated $TPPS_4$. In this case, the addition of NaCl reduces the T–T absorption curve amplitude (ΔA_0) consisting of two phases.

Firstly, similar to non-protonated $TPPS_4$, NaCl addition produces a rapid ($t < 60$ s) ΔA_0 decrease, which reaches 30% in the presence of [NaCl] > 0.1 M (Fig. 4, curve △). At the same time, in the absence of oxygen NaCl (>0.1 M) reduces the triplet lifetime from (50 ± 4) to (7.0 ± 0.1) μs (Fig. 3B, curves a and b).

Similar effect was observed for $TPPS_4$ fluorescence, where addition of 0.3 M NaCl reduced $TPPS_4$ fluorescence quantum yield from 0.37 to 0.23 and the lifetime—from 4.0 to 3.0 ns, respectively [12,17].

Secondly, after the initial ΔA_0 drop, further ΔA_0 reduction is observed (Fig. 5B), with characteristic time τ_1 depending on the NaCl and $TPPS_4$ concentrations (Table 1). This reduction is simultaneous with the increase of $TPPS_4$ H and J aggregate concentrations, which was detected by the S–S absorption (Fig. 5A, Table 1). Based on this observation, we associate this ΔA_0 reduction with $TPPS_4$ aggregation. However, finally, ΔA_{0fin} does not go down to 0, but reaches a stable value at $(\Delta A_0)/2$ after the $TPPS_4$ aggregation to the J form finished (Fig. 5B, curve ●). Since now only J aggregates of $TPPS_4$ are present in the solution, we associate this signal with the J aggregate triplet form, which lifetime in the absence of oxygen is (10.0 ± 0.4) μs (Fig. 3B, curve c).

At the same time, neither NaCl, nor the porphyrin aggregation affect the quenching of the $TPPS_4$ triplet by oxygen and k_q remains $\approx 1.8 \times 10^9\ M^{-1}\ s^{-1}$ for porphyrin monomers and corresponding J aggregates.

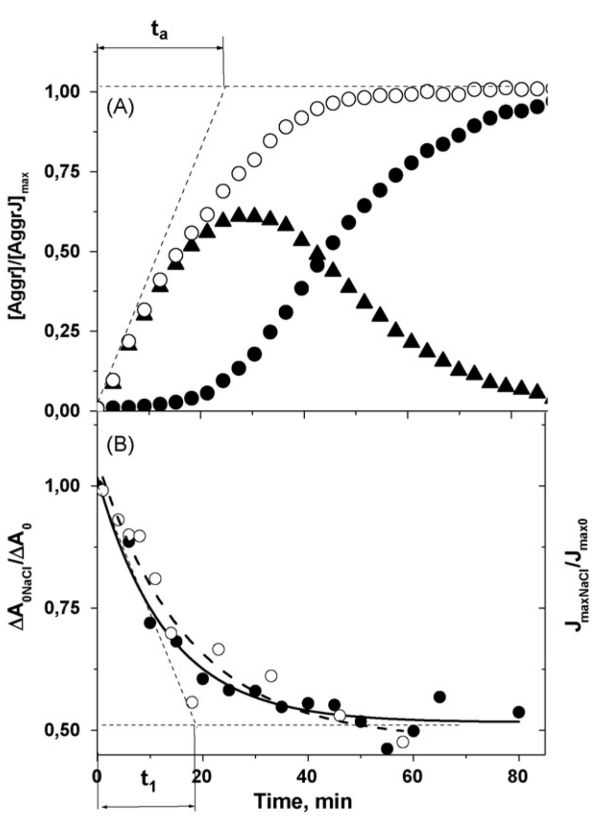

Fig. 5. (A) Relative contents of $TPPS_4$ aggregates as a function of time in the presence of NaCl: (▲) H aggregates; (●) J aggregates; (◯) H + J aggregates; normalized values of the $TPPS_4$ T–T: absorbance at 470 nm ($\Delta A_{0NaCl}/\Delta A_0$) (●) and $^1O_2$ phosphorescence maximum at 1270 nm ($J_{maxNaCl}/J_{max0}$) (◯) as a function of time in the presence of NaCl. Experimental conditions: pH 4.0; $[TPPS_4] = 30$ μM; [NaCl] = 0.1 M.

Table 1
Characteristic times of $TPPS_4$ total aggregation (t_a) and ΔA_0 saturation (t_1) as functions of NaCl and the porphyrin concentrations at pH 4.0 (24 °C)

[NaCl] (M)	$[TPPS_4] = 15$ μM		$[TPPS_4] = 30$ μM	
	t_a (min)	t_1 (min)	t_a (min)	t_1 (min)
0.4	1.7 ± 0.2	>2	0.7 ± 0.1	>2
0.3	3.7 ± 0.4	4.1 ± 0.4	1.5 ± 0.2	>2
0.2	5.5 ± 0.6	6.0 ± 0.6	2.8 ± 0.3	3.0 ± 0.3
0.15	10 ± 1	11 ± 1	4.5 ± 0.5	5.0 ± 0.5
0.1	>60	>60	18 ± 2	20 ± 2

3.3. Singlet oxygen $^1O_2$ formation

Excitation of the $TPPS_4$ solutions, both biprotonated and non-protonated, at 532 nm induces the light emission in the 1220–1340 nm range with a maximum centered at $\lambda \approx 1270$ nm. The emission profile contains two stages: accumulation and decay. These characteristics are typical of $^1O_2$ phosphorescence formed by the energy transfer from the sensitizer triplet state to molecular oxygen [18,19].

In reality the porphyrin triplet state quenching by molecular oxygen can include other mechanisms besides energy transfer, such as electron transfer, which results in the superoxide anion $O_2^{\bullet -}$ formation [20 and therein]. Therefore, we need to separate the quenching constant k_q in two parts:

$$k_q = k_{q\Delta} + k_{q1}$$

where $k_{q\Delta}$ is the $TPPS_4$ triplet state quenching constant due to energy transfer with formation of singlet oxygen and k_{q1} is the total quenching constant describing other oxygen included processes.

In this case, the triplet state decay rate is expressed by the following equation:

$$\frac{d[T]}{dt} = -k_0[T] - (k_{q\Delta} + k_{q1})[T][O_2] = -(k_0 + k_q[O_2])[T]$$

and the $^1O_2$ generation rate is:

$$\frac{d[^1O_2]}{dt} = k_{q\Delta}[T]\,[O_2] - k_{^1O_2}[^1O_2] \quad (5)$$

where $k_{^1O_2}$ is the $^1O_2$ decay constant.

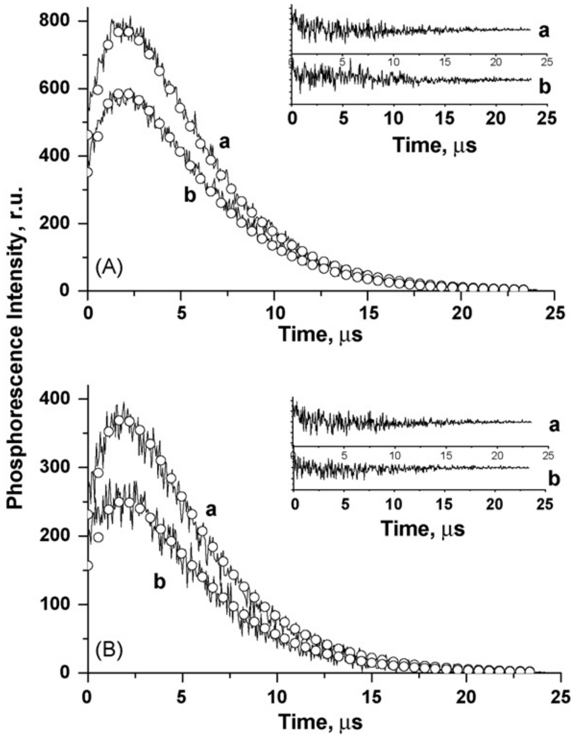

Fig. 6. Singlet oxygen ($^1O_2$) phosphorescence kinetic profiles at 1270 nm: pH 7.0 in the absence (curve a) and in the presence (curve b) of [NaCl] = 0.1 M pH 4.0 in the absence (curve a) and immediately after adding [NaCl] = 0.1 M (curve b) (◯) fitting by the equation $y = A[\exp(-t/\tau_1) - \exp(-t/\tau_2)]$, Insertions: residuals of the profiles for fits. Singlet oxygen was produced by [TPPS$_4$] = 30 μM excitation at 532 nm in aqueous solutions.

The photosensitizer triplet state concentration as a function of time is

$$[\mathrm{T}] = [\mathrm{T}]_0 \exp[-(k_0 + k_q[\mathrm{O_2}])t]$$

Since molecular oxygen concentration [O_2] is approximately 10-fold higher than that of TPPS$_4$ [O_2] should be much higher than [T]. Therefore, we may suppose [O_2] in Eq. (5) to be constant.

In this approximation, the concentration of the singlet oxygen should be expressed by

$$[^1\mathrm{O_2}] = \frac{k_{q\Delta}[\mathrm{O_2}][\mathrm{T_0}]}{k_{^1\mathrm{O_2}} - (k_0 + k_q[\mathrm{O_2}])}\{\exp[-(k_0 + k_q[\mathrm{O_2}])t] - \exp(-k_{^1\mathrm{O_2}}t)\} \quad (6)$$

where the first exponent gives $^1O_2$ accumulation and the second one relates to $^1O_2$ decay.

The best fitting of the phosphorescence curves obtained with the help of Eq. (6) (Fig. 6) demonstrates that for both pH values in the presence and in the absence of NaCl the $^1O_2$ decay constant equals $k_{^1\mathrm{O_2}} = (2.9 \pm 0.1) \times 10^5\,\mathrm{s^{-1}}$ (that gives the lifetime equal $\tau_{^1\mathrm{O_2}} = (3.5 \pm 0.1)\,\mu\mathrm{s}$). This value correlates well with the formerly observed one [21,22].

The concentration of $^1O_2$ reaches a maximum at

$$t_{max} = \frac{1}{k_{^1\mathrm{O_2}} - (k_0 + k_q[\mathrm{O_2}])} \ln\left(\frac{k_{^1\mathrm{O_2}}}{k_0 + k_q[\mathrm{O_2}]}\right) \quad (7)$$

and

$$[^1\mathrm{O_2}]_{max} = \frac{k_{q\Delta}/k_q[\mathrm{T_0}]}{(k_0 - k_{^1\mathrm{O_2}})/k_q[\mathrm{O_2}] + 1}(B^{k_0+k_q[\mathrm{O_2}]/k_{^1\mathrm{O_2}} - (k_0+k_q[\mathrm{O_2}])} - B^{k_{^1\mathrm{O_2}}/k_{^1\mathrm{O_2}} - (k_0+k_q[\mathrm{O_2}])}) \quad (8)$$

where $B = k_{^1\mathrm{O_2}}/(k_0 + k_q[\mathrm{O_2}])$.

In accordance with Eq. (8) $[^1\mathrm{O_2}]_{max}$ depends on both [T_0] and $k_0 = 1/\tau_0$. However, under all current experimental conditions the triplet decay constant determined by triplet quenching by oxygen, which presents in the air ($k_q[\mathrm{O_2}]$) is much higher than the triplet decay constant in the absence of oxygen (k_0) and the sum $k_0 + k_q[\mathrm{O_2}] \cong k_q[\mathrm{O_2}]$ is practically unchanged under these conditions. This means that under atmospheric air pressure due to effective quenching of the TPPS$_4$ triplet state by oxygen practically all TPPS$_4$ "tripletely" excited molecules are quenched by oxygen. Hence, in this case, $^1O_2$ production depends on φ_T and is independent of τ_0, which take part in $^1O_2$ production only when k_0 is comparable to $k_q[\mathrm{O_2}]$.

In our experiments, the decay constant of singlet oxygen $k_{^1\mathrm{O_2}}$ is practically constant. Thus $[^1\mathrm{O_2}]_{max}$ is directly proportional to $[\mathrm{T}]_0$.

The $^1O_2$ quantum yield ($\varphi_{^1\mathrm{O_2}}$) is proportional to $\int_{t_{in}}^{t_{fin}} J(t)\,dt$, where $J(t)$ is $^1O_2$ phosphorescence intensity as a function of time. However, since $J(t)$ profiles are similar under all experimental conditions, it can be affirmed that $\varphi_{^1\mathrm{O_2}}$ is proportional to the phosphorescence kinetics amplitude J_{max}, which in turn is proportional to $[^1\mathrm{O_2}]_{max}$.

On the other hand, φ_T is proportional to $[\mathrm{T}]_0$. Thus, based on Eq. (8), it is possible to deduce that

$$\varphi_{^1\mathrm{O_2}} = K_{\varphi\mathrm{T}}$$

where K is the factor of proportionality.

On the other hand, if every "tripletely" excited TPPS$_4$ molecule transfers its energy to molecular oxygen, φ_T and $\varphi_{^1\mathrm{O_2}}$ should be equal ($K = 1$). However, in reality just a part of these molecules is capable to realize the energy transfer and form $^1O_2$. This part is determined by the ratio $k_{q\Delta}/k_q$.

Indeed, since $k_0 \gg k_{^1\mathrm{O_2}}$ and $k_q[\mathrm{O_2}] \gg k_0$ we can transform Eq. (8) into the form:

$$[^1\mathrm{O_2}]_{max} = \frac{k_{q\Delta}}{k_q}[\mathrm{T_0}](B^{k_0+k_q[\mathrm{O_2}]/k_{^1\mathrm{O_2}} - (k_0+k_q[\mathrm{O_2}])} - B^{k_{^1\mathrm{O_2}}/k_{^1\mathrm{O_2}} - (k_0+k_q[\mathrm{O_2}])}) \quad (9)$$

which demonstrates that $[^1\mathrm{O_2}]_{max}$ is proportional to $k_{q\Delta}/k_q$.

Thus, under our experimental conditions K should be equal $k_{q\Delta}/k_q$ and finally

$$\varphi_{^1\mathrm{O_2}} = \frac{k_{q\Delta}}{k_q \varphi_\mathrm{T}}$$

or,

$$\frac{k_{q\Delta}}{k_q} = \frac{\varphi_{^1\mathrm{O_2}}}{\varphi_\mathrm{T}}$$

For non-protonated TPPS$_4$ $\varphi_T = 0.78$ [4] and $\varphi_{^1\mathrm{O_2}} = 0.62$ [5], and we can conclude that $k_{q\Delta}/k_q = 0.62/0.78 \cong 0.79$. It means that about 79% of "tripletely" excited $TPPS_4$ molecules were quenched by energy transfer, forming singlet oxygen, and just 21% were quenched by other mechanisms.

3.3.1. Non-protonated $TPPS_4$ form

At pH 7.0, NaCl addition induces the immediate reduction of $^1O_2$ phosphorescence intensity maximum (J_{max}) reaching 27% for [NaCl] > 0.1 M (Fig. 6A) and being independent of further NaCl concentration increase. The decrease value was adjusted with the help of the following equation:

$$J_{max} = J_{max\,exp} \frac{D_0}{D_{NaCl}}$$

where $J_{max\,exp}$ is experimentally obtained J_{max} value; D_0 and D_{NaCl} are S–S absorbances at 532 nm in the absence and in the presence of NaCl, respectively.

Since J_{max} is proportional to $[^1O_2]_{max}$, it may be concluded that addition of NaCl in the concentrations above 0.1 M reduces $\varphi_{^1O_2}$ from 0.62 to 0.45.

This effect is similar to the one observed for ΔA_0 under the same conditions. Thus, since $[^1O_2]_{max}$ is directly proportional to $[T]_0$, it may be concluded about direct relation of this drop to a decrease in $[T]_0$. Hence, this indicates φ_T reduction from 0.78 to 0.57, induced by NaCl.

3.3.2. Biprotonated $TPPS_4$ form

At pH 4.0 in the absence of NaCl the corrected J_{max} value is found ≈2-fold lower than that at pH 7.0, indicating $\varphi_{^1O_2}$ reduction from 0.62 for non-protonated to 0.31 for biprotonated $TPPS_4$ form. This reduction agrees with φ_T decrease from 0.78 to 0.36, observed for $TPPS_4$ protonation [23]. Hence, $TPPS_4$ protonation increases the ratio $k_{q\Delta}/k_q$ in favor of $k_{q\Delta}$, thus increasing the yield of the triplet energy transfer from 0.79 to $\varphi_{^1O_2}/\varphi_T = 0.31/0.36 \cong 0.86$.

At pH 4.0, NaCl addition induces rapid J_{max} drop reaching ≈30% at [NaCl] > 0.1 M (Fig. 6B) and being independent of further NaCl concentration increase. Therefore, it may be concluded that the presence of NaCl in concentrations above 0.1 M decreases φ_T of biprotonated $TPPS_4$ down to 0.25, thus reducing $\varphi_{^1O_2}$ from 0.30 to 0.21.

After the initial drop, J_{max} is still decreasing (Fig. 5B, curve ○) with the kinetics symbate to ΔA_0 decrease (Fig. 5B, curve ●), induced by $TPPS_4$ aggregation (Fig. 5A, curve ○), and, finally, reaching the saturation level at 50% of the initial J_{max} value. Since only J aggregates of $TPPS_4$ are present in the solution in this case, it may be assumed that $\varphi_{^1O_2}$ equals ≈0.10 due to excitation of these aggregates, and φ_T is ≈0.12.

4. Conclusion

Based on the above data, we may conclude that NaCl addition reduces the quantum yield and the lifetime of triplet state for biprotonated and non-protonated $TPPS_4$ forms. The effect originates from Na^+ and/or Cl^- interaction with porphyrin molecules, which directly reduces both triplet lifetime and quantum yield and, on the other hand, induces $TPPS_4$ aggregation, thus reducing these characteristics even more. Along with the fact that NaCl addition decreases $TPPS_4$ singlet excited state lifetime and the fluorescence quantum yield, this result allows to conclude that interaction with ions, as well as aggregation, increases the probability of non-radiative dissipation of $TPPS_4$ electronic excitation. Nevertheless, the quantum yield and the lifetime of $TPPS_4$ J aggregate triplet state are comparable with those of $TPPS_4$ monomers.

Under all currently mentioned experimental conditions, the $TPPS_4$ triplet state quenching by molecular oxygen is so effective that all "tripletely" excited molecules loose their energy due to this process. So, the production of singlet oxygen is directly proportional to the $TPPS_4$ triplet state quantum yield and is independent of its lifetime. On the other hand, the difference between φ_T and $\varphi_{^1O_2}$ demonstrates that just a part of "tripletely" excited $TPPS_4$ molecules transfers its excitation energy to molecular oxygen producing singlet oxygen and the other part of $TPPS_4$ triplets is quenched due to different oxygen dependent mechanisms, probably, the electron transfer forming $O_2^{\bullet-}$ radical.

The presence of NaCl does not affect neither the $TPPS_4$ triplet state quenching constant by oxygen nor the lifetime of singlet oxygen.

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