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On the Ability of Bacteriochlorophyll *a* to Generate Singlet Oxygen upon Photoexcitation in Aqueous Environment

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Dedicated to the memory of Academician Oskar Iosifovich Koifman

The quantum yields of singlet oxygen generation (Φ_Δ) photosensitized by bacteriochlorophyll a have been measured in diethyl ether and aqueous (D_2O and H_2O) dispersions, in which hydrophobic bacteriochlorophyll molecules are solubilized by a detergent Triton X-100. According to the absorption spectra, bacteriochlorophyll molecules are monomeric in all samples studied. For measurements, both detection of photosensitized phosphorescence of singlet oxygen at 1270 nm and singlet oxygen trapping by 1,3-diphenylisobenzofuran were applied. In ether, both methods gave the highest quantum yields (Φ_Δ=0.60±0.05) and slightly lower Φ_Δ=0.3–0.4 was obtained in micellar dispersions in deuterium oxide. The lowest, but still reasonable, value of Φ_Δ was found in aqueous (H_2O) dispersions (Φ_Δ=0.12±0.02). In detergent-free water, where aggregated pigment molecules predominate, $^1O_2$ generation was not detected. Generation of $^1O_2$ was always accompanied by active quenching of $^1O_2$ by bacteriochlorophyll with the rate constants $k_q=(1.4–2.2)\cdot10^9$ $M^{-1}s^{-1}$. The data indicate that monomeric bacteriochlorophyll molecules actively photosensitize generation of $^1O_2$ in organic solvents and do not lose this ability in aqueous environment, which most likely determines photodynamic action and PDT by bacteriochlorophyll and its derivatives.

Keywords: Bacteriochlorophyll *a*, singlet oxygen, phenalenone, porphyrins, phosphorescence, trapping, quenching, photodynamic therapy (PDT).

О способности бактериохлорофилла *a* генерировать синглетный кислород при фотовозбуждении в водных системах

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Методами регистрации фосфоресценции синглетного кислорода (1270 нм) и окисления ловушки синглетного кислорода (1,3-дифенилизобензофурана) измерены квантовые выходы (Φ_Δ) фотосенсибилизированной бактериохлорофиллом а генерации синглетного кислорода в диэтиловом эфире и водных (D_2O и H_2O) дисперсиях детергента Тритона X-100. Согласно спектрам поглощения, молекулы пигмента мономерны во всех этих средах. Установлено, что в диэтиловом эфире молекулы бактериохлорофилла генерируют синглетный кислород с наибольшей эффективностью (Φ_Δ=0,60±0,05). Несколько ниже активность пигмента в мицеллярных растворах детергента в оксиде дейтерия (Φ_Δ=0,3–0,4). В водных (H_2O) дисперсиях детергента значение Φ_Δ еще ниже (0,12±0,02), но остается достаточно высоким. В водных средах без детергента, где доминируют агрегированные молекулы пигмента, генерация $^1O_2$ не обнаруживалась. Генерация $^1O_2$ сопровождалась активным тушением $^1O_2$ бактериохлорофиллом с константами скорости $k_q=(1.4–2.2)\cdot10^9$ $M^{-1}c^{-1}$. Данные позволяют сделать вывод, что мономерные молекулы бактериохлорофилла эффективно генерируют $^1O_2$ в органических растворителях и не теряют эту способность в водном окружении, что является наиболее вероятной причиной фотодинамического действия и ФДТ бактериохлорофилла и его производных.

Ключевые слова: Бактериохлорофилл *a*, синглетный кислород, феналенон, порфирины, фосфоресценция, метод химических ловушек, тушение, фотодинамическая терапия (ФДТ).

Introduction

It is known that excited oxygen molecules in the singlet $^1\Delta_g$ state ($^1O_2$) are effectively formed in aerobic systems upon photoexcitation of photosensitizer dyes (Dye) due to the energy transfer from the triplet states of dyes (^{3}Dye*) to molecular oxygen (Figure 1).[1-4]

Singlet ($^1\Delta_g$) oxygen molecules are highly reactive and therefore play a crucial role in photodynamic damage of living cells. This fact is used in medicine for the photodynamic elimination of cancer tumors.[1-4] It is also known that near-infrared laser radiation penetrates deeper into biological tissues than radiation in the visible spectral region.[4,5] Therefore, the synthesis and investigation of the IR photosensitizers[5-7] (so-called second generation photosensitizers) are of great interest for the treatment of subcutaneous tumors. Bacteriochlorophylls (BChls) *a* and *b* and their magnesium-free analogs (bacteriopheophytins, BPh) – the main pigments of purple photosynthetic bacteria are natural IR photosensitizers. The main absorption maxima of the monomeric molecules of these pigments are at 760–780 nm.[8] The generation of $^1O_2$ by BChl and BPh inside bacterial cells is extremely undesirable, since it leads to the destruction of bacterial membranes and interferes with the process of photosynthesis. Nevertheless, Gaffron reported almost a century ago that bacteriopheophytin molecules isolated from bacterial cells photosensitize oxidation of thiourea by oxygen.[9] This fact caused many-year discussion in the scientific community whether BPh and BChl are capable of photosensitized $^1O_2$ formation.

In nineteen seventies, our group observed for the first time that BChls and BPhs photosensitize $^1O_2$ luminescence at 1270 nm in aerated carbon tetrachloride.[10,11] Further studies revealed similar effects in solutions of bacteriochlorophyll and bacteriopheophytin *b*.[12] The quantum yields of the $^1O_2$ generation were shown to be 0.6–0.75.[10-12] It was also found that unexcited molecules of these pigments in organic solvents quench singlet oxygen via a physical deactivation mechanism:

$$^1O_2 + \text{BChl} \rightarrow {}^3O_2 + \text{BChl} + \text{heat}$$

The rate constants for $^1O_2$ quenching by bacteriochlorophylls were reported to be equal to $(1–1.5)\cdot10^9$ $M^{-1}s^{-1}$, what is only an order of magnitude smaller than the rate constant for the diffusion-limited reactions. The quenching rate constants for BPhs were about ten-fold lower.[10-12] Contribution of chemical oxidation of BChl by $^1O_2$ to the overall quenching process was found to be about 3%.[12] The same ability to physically $^1O_2$ was found also in chlorophyll and pheophytin *a*.[10-12] Thus, it was established that pigments of this group are powerful photodynamic sensitizers. On the other hand, they can serve as protectors of photosynthetic cells from destruction by singlet oxygen generated by bacteriochlorophylls themselves ([2] and refs. therein).

Subsequent time-resolved measurements of $^1O_2$ phosphorescence (1270 nm) under pulsed laser excitation confirmed the main conclusions of the previous studies. Borland and Truscott observed that BChl and BPh efficiently generate $^1O_2$ in methanol, MeOD and benzene.[13] They also confirmed that BChl is a strong $^1O_2$ quencher (Table 1). Further kinetic measurements of our group revealed $^1O_2$ luminescence in diethyl ether, pyridine and in a mixture of D_2O with nonionic detergent Triton X-100 (Table 1). The data indicate that in organic solvents and detergent micelles, in which the pigments are monomeric, the $^1O_2$ quantum yield was rather high varying in the range of 0.1–0.6. The generation of singlet oxygen could not be reliably measured in deuterium oxide without detergents, in which strong aggregation of the pigments occurs.[14,15]

It should be noted here that BChl is rapidly oxidized by air oxygen under irradiation. The quantum yield of BChl oxygenation changes within two orders of magnitude depending on solvents. The highest photooxygenation rate was observed in micellar aqueous dispersions.[17-19] In diethyl ether, the oxygenation rate is about 100-fold smaller. It is possible that the significant scattering in the data listed in Table 1 is due to photobleaching of the pigments during the measurement time. From this point of view, the most accurate results were obtained in ref.[12], in which the degree of the pigment destruction during the measurement procedure was less than 2%.

It is most difficult to detect BChl-sensitized singlet oxygen in aqueous (H_2O) environment, since the phosphorescence yield and lifetime of singlet oxygen in water are manifold smaller than in other solvents. Besides, due to strong photobleaching of BChl, one cannot increase the power of the exciting light in order to build up the luminescence intensity. Probably, for these reasons, in the papers cited in Table 1 no one reported the detection of singlet oxygen in BChl solutions in H_2O. Only in D_2O, preliminary data were obtained.[15] No singlet oxygen phosphoresce was observed in aqueous solutions of the PDT photosensitizer WST11 (a derivative of water-soluble Pd-bacteriopheophorbide).[20] Similar result was obtained with a synthetic photodynamic dye tetrahydroporphyrin derivative (THPTS).[21] Therefore, it was proposed that BChl and its derivatives lose an ability to generate singlet oxygen under excitation in aqueous environment, and photodynamic action proceeds without $^1O_2$ due to activities of superoxide and hydroxyl radicals.[20,21] Currently, we have much more sensitive methods for $^1O_2$ detection,[22-25] than those that were used in the works cited above. The objective of present work is to apply these methods to measurement the quantum yields of $^1O_2$ formation by BChl in aqueous environment (D_2O and H_2O) and compare the results with those obtained in organic solvents.

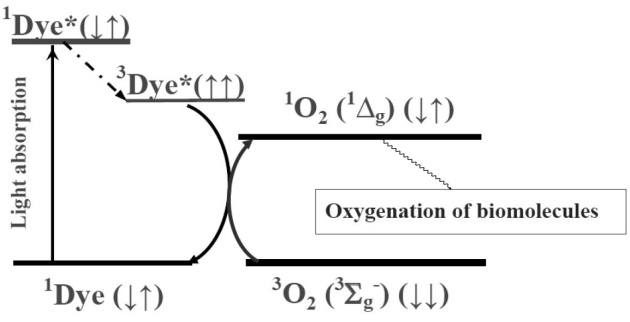

Figure 1. Simplified energy diagram describing the mechanism of photosensitized formation of singlet oxygen as the primary stage of the type II photodynamic reactions.

Table 1. The quantum yields (Φ_Δ) and rate constants (k_q) of $^1O_2$ quenching by BChl *a* reported in the preceding papers.

Solvent	Φ_Δ	$k_q \times 10^{-9}$ $M^{-1}s^{-1}$
CCl_4	0.6 [12]	0.9 ± 0.2 [12]
Benzene	0.35 [13]	1.7 [13]
Monodeuterated benzene	0.34 [13]	-
Monodeuterated methanol	0.22 [13]	-
Diethyl ether	0.42 [14,15]	0.22±0.03 [15]
Pyridine	0.43 [14,15]	0.59±0.08 [15]
D_2O + 2% Triton X-100	0.10 [14,15]	-
D_2O (no detergent)	< 0.02 [14,15]	-

Experimental

Two methods were used for studies of singlet oxygen generation in BChl solutions. One is based on detection of photosensitized IR phosphorescence of singlet oxygen, which appear due to energy transfer to oxygen from triplet molecules of the photosensitizer followed by formation of singlet ($^1\Delta_g$) oxygen molecules and emission of luminescence at 1270 nm. The measurements were performed using the phosphorescent spectrometer recently assembled at the A.N. Bach Institute of Biochemistry (FRC Biotechnology RAS).[22] The phosphorescence was excited by a LED with the emission band maximum at 395 nm and a half-width of 30 nm (Polironic, Moscow, Russia). Pulse repetition rate was 1–5 kHz, flash duration was 1–2 μs. LED radiation was focused in a spot of 5 mm on the surface of a quartz spectrophotometric cell with a testing solution. Phosphorescence was recorded by an S-1 photomultiplier FEU-112 (Ekran Optical Systems, Novosibirsk, Russia) cooled by liquid nitrogen vapour to –30° C. The phosphorescence spectrum was analyzed using a set of three interference light filters with the transmission maxima at 1230, 1270, and 1310 nm. The photomultiplier signal was passed through the preamplifier and then processed by a computer photon counter synchronized with the LED pulse. The spectrometer allowed estimation of the emission spectrum and recording the phosphorescence decay traces by averaging a signal after an unlimited number of exciting pulses using the method of multi-channel photon counting. Typically, the signal accumulation time did not exceed 30 min. The average power of the exciting light was typically 1–9 mW.

Another method for singlet oxygen detection was based on measuring the rate of chemical trapping of singlet oxygen by 1,3-diphenylisobenzofuran (DPIBF). The modification of this method applied at present work was described elsewhere.[23-25] The maximum of the DPIBF absorption spectrum is at 414 nm. In the region of 500–900 nm, DPIBF has no absorption bands. The reaction of DPIBF with singlet oxygen causes accumulation of colourless products having no absorption bands in the visible spectral range. The rate of singlet oxygen formation was determined from the rate of reduction of absorbance in the maximum of the DPIBF absorption band. The initial absorbance of DPIBF was 1±0.1 at 414 nm in a 10 mm quartz cell that corresponds to the DPIBF concentration of 42±4 μM. The quantum yields of singlet oxygen generation were determined using as reference compounds phenalenone, which was employed in phosphorescence measurements. *meso*-Tetra(*p*-sulfonatophenyl)porphyrin tetra-sodium salt (TPPS) and *meso*-tetraphenylporphyrin (TPP) were used in chemical trapping experiments in water and organic solvents respectively. According to the literature, the most probable values for the quantum yields of singlet oxygen generation are 0.70±0.05 for TPP and TPPS[23-25] and 0.95±0.05 for phenalenone.[26,27] Fluorescence was recorded using the computerized spectro-fluorimeter Perkin Elmer MPF-44B.

The power of the exciting light was measured using ThorLabs PM-100D with a S120VC sensor head (ThorLabs, USA) and Ophir ORION-TH with a 20C-SH sensor head (Ophir Optronics, Israel). The absorption spectra were measured using the SF-56 spectro-photometer (LOMO-Spektr, St. Petersburg, Russia) and quartz cells with an optical path length of 10 and 5 mm.

Bacteriochlorophyll *a* was extracted from cells of purple bacteria *Rhodospirillum rubrum* and *Ectothiorhodospira haloalkali-phile* and purified by column chromatography or HPLC as described elsewhere ([12,15,16] and refs therein). BChl concentration was calculated using absorbance of solutions in the main IR absorption maximum and the value of 95000 $M^{-1}cm^{-1}$ for the molar absorption coefficient in diethyl ether ([12,15] and references therein). Diethyl ether (analytical grade, Soyuzkhimprom, Novosibirsk, Russia), deuterium oxide (99.8%, Chemical line, St. Petersburg, Russia), and doubly distilled H_2O were used as solvents. A detergent Triton X-100 was obtained from MP Biomedicals (France), 1,3-diphenylisobenzofuran (DPIBF) – from Acros Organics (99%, Belgium), TPP, TPPS and phenalenone – from Merck (97%, USA).

Results and Discussion

Absorption Spectra

Figure 2 shows the absorption spectra of BChl *a* in the solvents employed in the present paper. As a reference, diethyl ether is used, since as mentioned above, BChl is most stable in this solvent. It is seen that in ether and detergent containing aqueous dispersions the main IR absorption maxima are at 771–781 nm. In water without detergent, the main absorption maximum is shifted to 850 nm that indicates that pigment molecules are strongly aggregated ([15] and refs therein).

Phosphorescence Measurements

Decays of $^1O_2$ phosphorescence in solutions of the reference pigment (phenalenone) and BChl in diethyl ether are shown in Figure 3. The excitation power was 9 mW. Irradiation time was 5 min. Under these conditions, no bleaching of phenalenone and 5% bleaching of BChl were observed. The decays are rather noisy since we used relatively low excitation power in order to minimize the BChl photobleaching. The decays were exponential in both phenalenone and BChl solutions (Figure 3).

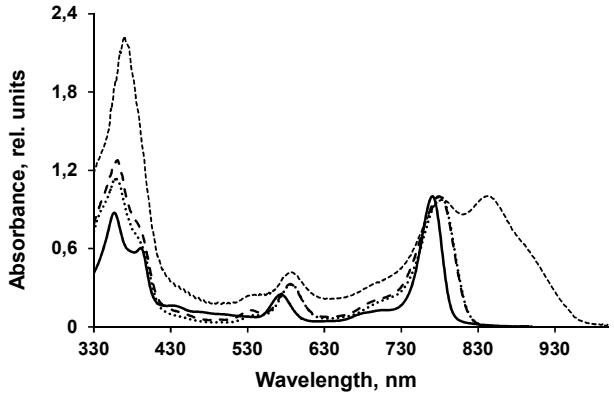

Figure 2. The absorption spectra of BChl *a* in diethyl ether (solid line), in D_2O and H_2O (dashed and dotted lines) in the presence of micelles of Triton X-100 and in H_2O without detergent (upper dotted line). All spectra are normalized at 780 nm.

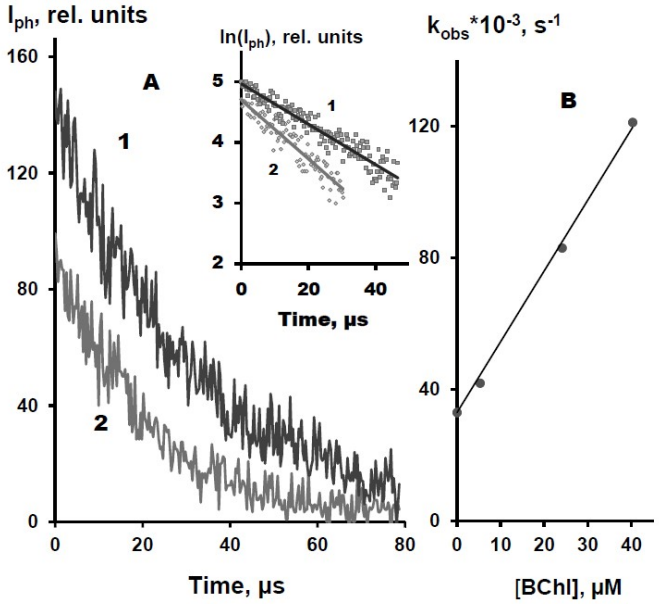

Figure 3. Decays of $^1O_2$ phosphorescence at 1270 nm in aerated solutions of phenalenone (1) and bacteriochlorophyll *a* (2) in diethyl ether after excitation by 1 μs LED pulses at 395 nm with the 5 kHz repetition rate in Cartesian and semilogarithmic coordinates (A). The absorbance of both solutions was about 0.35 at the excitation wavelength in 5 mm quartz cell. Acquisition time was 5 min, average excitation power 9 mW. B: Stern-Volmer plot for the dependence of the decay rate constants (k_{obs}, s^{-1}) for $^1O_2$ phosphorescence in phenalenone solutions on the concentrations of added BChl.

The decay time (τ_Δ) in phenalenone solution was of 31 μs, the decay rate constant ($k_{obs} = \tau_\Delta^{-1}$) was equal to 32000 s^{-1}. Addition of BChl caused the decrease of the decay time which obeyed the Stern-Volmer equation (Figure 3):

$$k_{obs} = k_o + k_q[BChl], \quad (1)$$

where k_o is the decay rate constant in phenalenone solutions without BChl. The bimolecular quenching rate constants (k_q) calculated from the Stern-Volmer plot are indicated in Table 2. The obtained values are higher than those reported previously being however of the same order of magnitude (Table 1). The quantum yield (Φ_Δ) was obtained using the following equations:

$$I_{ph}(t) = I_{ex}(1\text{-}10^{-A})\ \Phi_\Delta k_r(exp\ (-t/\tau_\Delta)) \quad (2)$$

where $I_{ph}(t)$ is the instant phosphorescence radiative rates in Einstein per second, I_{ex} the intensity of short exciting LED pulse in Einsteins per pulse. A is absorbance of the pigments at the excitation wavelength. As phosphorescence decays were exponential, extrapolation of the exponential functions to the zero time after exciting pulse is equal to:

$$I_{ph}(0) = I_{ex}(1\text{-}10^{-A})\ \Phi_\Delta k_r \quad (3)$$

This function makes it possible to compare (Φ_Δ) values for BChl and phenalenone:

$$(\Phi_\Delta)_{BChl}/(\Phi_\Delta)_{phen} = \\ = [I_{ph}(0)/(I_{ex}(1\text{-}10^{-A}))]_{BChl}/[I_{ph}(0)/(I_{ex}(1\text{-}10^{-A}))]_{phen} \quad (4)$$

Knowing that $(\Phi_\Delta)_{phen} = 0.95$, one can obtain $(\Phi_\Delta)_{BChl}$ using eq. 4. The data were corrected for the BChl bleaching by using for calculations the mean between the values of BChl absorbance before and after irradiation. The value of Φ_Δ we obtained in diethyl ether was equal to 0.6 (Table 2).

Phenalenone solutions were prepared by direct dissolution of a phenalenone powder in deuterium oxide. Figure 4 shows the decays of $^1O_2$ phosphorescence in phenalenone solution in D_2O. The decay time obtained 69±1 μs coincides with the previously reported data.[28,29] In the presence of 4% Triton X-100, quenching of $^1O_2$ by the detergent occurs.[30] We obtained that the lifetime decreased to 27 μs after addition of 4% Triton X-100 (Figure 4).

BChl was solubilized in deuterium oxide by addition of 4% Triton X-100. BChl is rapidly bleached under photoexcitation in detergent micelles in deuterium oxide. To reduce the influence of this effect, we applied LED with low average power of 1 mW. Irradiation time was equal to 3 min. Under these conditions, 20% bleaching of the IR absorption band of BChl was observed. After one trial, the phosphorescence signal was too weak to be analysed. Therefore, the procedure was repeated 10 times, each time with a freshly prepared solution. The results were accumulated by a computer and compared with those obtained in the phenalenone solution after 30 min irradiation. Under these irradiation conditions, phenalenone bleaching was not observed. The obtained Φ_Δ is near that in diethyl ether Table 2. The physical quenching of $^1O_2$ by BChl is rather strong also in Triton X-100, being about 1.5-fold smaller than in ether.

Table 2. The values of the quantum yields of singlet oxygen obtained in the present work.

	Diethyl ether	D_2O + 4% Triton X-100	Diethyl ether + DPIBF	D_2O + 4% Triton X-100 + DPIBF	H_2O + 4% Triton X-100 + DPIBF
	Phosphorescence		Trapping		
Φ_Δ	0.60±0.05	0.40±0.05	0.60±0.05	0.30±0.05	0.12±0.02
$k_q \times 10^{-9}$, $M^{-1}s^{-1}$	2.2±0.2	1.4±0.2			
τ_Δ, μs	31.5	27±2	-	-	-
Φ_{fl}	0.18	0.080	0.18	0.080	0.066

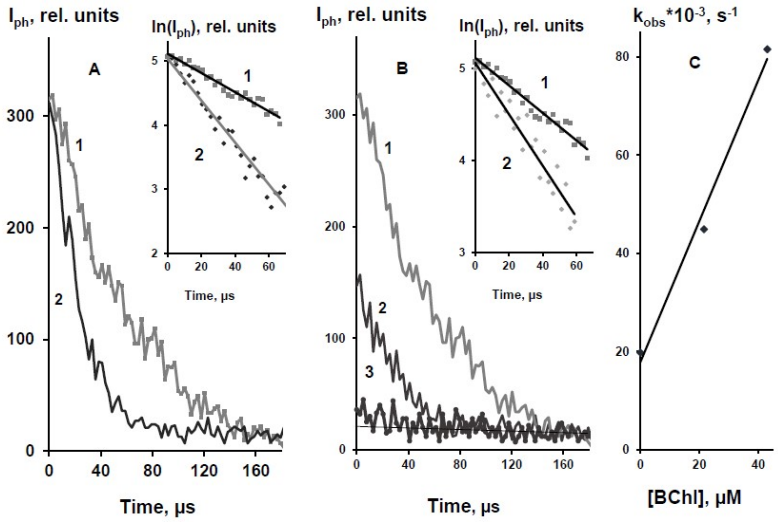

Figure 4. Decays of $^1O_2$ phosphorescence at 1270 nm in aerated solutions of pigments in deuterium oxide. A: phenalenone without Triton X-100 (1), phenalenone in the presence of 4% Triton X-100 (2) in Cartesian and semilogarithmic (inset) coordinates; B: phenalenone in D_2O without triton X-100 (1), bacteriochlorophyll *a* in deuterium oxide with 4% Triton X-100 (2) and without Triton X-100 (3) in Cartesian and semilogarithmic (inset) coordinates. Phosphorescence was excited at 395 nm by 1 μs LED pulses with the 1 kHz repetition rate, average excitation power was 1 mW, the absorbance of solutions was ~ 0.35 at the excitation wavelength in 5 mm quartz cell. Accumulation time was 30 min. C: Stern-Volmer plot of the dependence of the phosphorescence decays in phenalenon solutions on the concentrations of added BChl in D_2O with 4% Triton X-100.

Time-resolved measurement of $^1O_2$ phosphorescence decays in aqueous (H_2O) micellar solutions of BChl cannot be done using our equipment, since to obtain reliable results, one needs to apply LED pulses of much higher power (>20 mW). With this power BChl is completely destroyed during several seconds. To solve this problem, we have applied a steady-state trapping method, which is by several orders more sensitive. This method does not allow measurement of the $^1O_2$ lifetimes and quenching rate constants, but makes it possible to measure the Φ_Δ under very low power of steady-state excitation.

$^1O_2$ Trapping Experiments

For these experiments, mixed solutions were prepared containing BChl, 1,3-diphenylisobenzofuran and a detergent Triton-100 (4%) in water or deuterium oxide. Apparently, lipophilic DPIBF and Bchl molecules were absorbed by detergent micelles. Parameters of micelles of this detergent are known.[31] The molar concentration of 4% detergent was 62 mM (molecular weight of one Triton molecule is 650). The molar weight of one micelle is known to be 90000 Da (138 detergent molecules in one micelle), therefore the molar concentration of micelles was of 445 μM. The average concentration of DPIBF was of 42 μM. The BChl concentration was of about 1 μM. Whence, the concentration of micelles is 10-fold higher than the DPIBF concentration and 445-fold higher than BChl concentration. It follows from this calculation that the probability of the presence in one micelle of two and more BChl molecules or two and more molecules of DPIBF or one BCchl and one DPIBF molecule in one micelle are close to zero. In accord with this conclusion, the absorption spectrum of BChl dispersion indicates that the pigment is monomeric in the aqueous Triton X-100 dispersions.

The solutions were irradiated by monochromatic light of the xenon lamp eliciting the monochromator of the Perkin Elmer MPF-44B fluorimeter. The wavelength of the exciting light (770–780 nm) corresponded to the main absorption maximum of BChl. During irradiation, bleaching of both the IR and blue BChl absorption bands were observed. The rate of DPIBF photobleaching was calculated using absorbance of the mixture at 414 nm which corresponds to the main absorption maximum of DPIBF. Bleaching of BChl at 770 nm was accompanied by simultaneous increase of absorbance in the blue region caused by accumulation of a product of BChl degradation. Fortunately for our experiments, at 414 nm there is an isosbestic point, at which absorbance of BChl was not changed during photodestruction of the pigment. Therefore, change in absorbance at 414 nm corresponded to change of the DPIBF concentration only.

The quantum yield of DPIBF oxygenation was measured using as a reference TPP in diethyl ether and TPPS in aqueous micellar dispersions. For excitation, the 515 nm monochromatic light was applied. As mentioned above, the Φ_Δ for TPP and TPPS is equal to 0.70±0.05. The obtained Φ_Δ values are indicated in Table 2. In diethyl ether, both the trapping and phosphorescence methods yielded similar values of Φ_Δ. In D_2O the two-fold lower value was obtained. In water (H_2O), Φ_Δ was 2.7-fold smaller than in D_2O. Addition of the $^1O_2$ quencher sodium azide (0.1 M) to the water-BChl-Triton-DPIBF mixture caused 4-fold decrease of the rate of DPBF oxygenation. This observation provides clear evidence that DPIBF photobleaching in this mixture is due to $^1O_2$ produced by BChl. In aqueous solutions lacking the detergent, where BChl forms long wavelength aggregates (Figure 2), no generation of singlet oxygen was detected. This fact agrees with the prior measurements of Egorov *et al.*[15] and the data of Gurinovich *et al.*[28] indicating that the quantum yield of triplets in BChl aggregates is of a few percents.

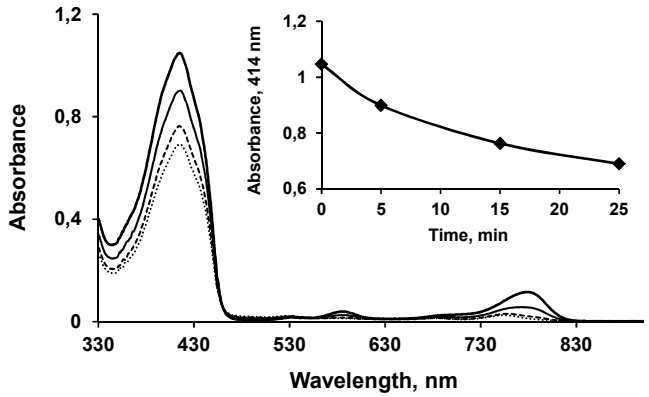

Figure 5. Absorption spectra of a mixture of bacteriochlorophyll *a* and 1,3-diphenylisobenzofuran in aqueous (D_2O) dispersion of Triton X-100 (4%) in 10 cm quartz cell before and after 5, 15 and 25 min irradiation by monochromatic light (780 nm, 99 μW). The initial BChl concentration in this experiment was 1.3 μM. Inset indicates the time course of the change of absorbance at 414 nm under illumination of the mixture at 780 nm.

Conclusion

Thus, the studies of IR phosphorescence and chemical trapping of singlet oxygen provide unambiguous evidence that monomeric molecules of bacteriochlorophyll *a* generate singlet oxygen under photoexcitation in aerated organic solvent (diethyl ether) and in aqueous (D_2O and H_2O) dispersions of a detergent Triton X-100, in which molecules of BChl are adsorbed by detergent micelles. The highest quantum yield (0.60) was obtained in diethyl ether. This value coincides with that obtained previously in carbon tetrachloride[12] (Tables 1 and 2). In papers of other groups, 1.5–1.8-fold smaller values of Φ_Δ were obtained in organic solvents (Table 1). Perhaps, the difference is due to the influence of the BChl photobleaching during the measurement procedure.

The photosensitizing activity of BChl in aqueous systems was found to be lower than in ether. Besides, in D_2O the Φ_Δ is about 3-fold greater than in H_2O. Since we observed parallel change of BChl fluorescence in these samples (Table 2), one can propose that these effects are connected with the state of BChl molecules in detergent dispersions. For instance, as mentioned above, in aqueous solutions lacking the detergent, where BChl forms long wavelength aggregates (Figure 2), no generation of singlet oxygen was observed (see also[15]). This effect might be explained using the mechanism proposed by Gurinovich *et al.*[28] who suggested that aggregation of BChl molecules leads to appearance of a very efficient non-radiative channel of the ^{1}BChl transition to the BChl ground state. In this case, BChl fluorescence and the rate of population of the BChl triplet states (^{3}BChl) are strongly reduced. Since $^1O_2$ is generated by ^{3}BChl molecules (Figure 1), the decrease in the ^{3}BChl yield causes proportional suppression of $^1O_2$ generation. However, this suppression mechanism seems unlikely in our experiments, since we have one BChl molecule for one micelle. It is possible that fluorescence is partly suppressed due to interaction with OH-groups.[33] At the moment, we cannot understand why the trapping and phosphorescence methods, which yield similar values of Φ_Δ in ether, show smaller values of Φ_Δ in heterogeneous dispersions of Triton X-100 in D_2O and especially in water. Perhaps, the kinetics equations which we used for analysis do not reflect the real behavior of $^1O_2$ in heterogeneous systems. It might be that Φ_Δ is suppressed due to efficient deactivation of $^1O_2$ by BChl itself in viscous interior of micelles just after the act of $^1O_2$ generation by the same BChl molecule. Further experiments are needed.

Nevertheless, previous and present data of our group provide clear evidence that $^1O_2$ is efficiently formed by bacteriochlorophyll and its derivatives in aqueous environment. The results of our work agree with the data of other groups. For instance, Sharonov *et al.*[34] using a chemical trapping method (RNO) found that monomeric molecules of cycloimide bacteriochlorin *p* derivatives (CIBs) generate $^1O_2$ very efficiently in aqueous micelles of Cremophor EL. Depending on the chemical structures of CIBs, Φ_Δ varied in the range of 0.07-0.57. Unlike BChl *a*, CIBs derivative are very stable and bleaching is much weaker. Aggregated molecules of CIBs derivatives, which are formed in aqueous suspensions without detergents, did not generate $^1O_2$. Hoebeke and Damoiseau[35] have established using a chemical trapping with anthracene-9,10-dipropionic acid as a trap that bacteriochlorin *a* efficiently generates $^1O_2$ in aqueous dispersion of dimiristoyl-*L*-phosphatidylcholine liposomes with $\Phi_\Delta = 0.33$.

Therefore, an assumption advanced in papers[20,21] that singlet oxygen is not involved in photodynamic action of certain bacteriochlorophyll derivatives and its role is played by superoxide or other radicals, is very unlikely. The impossibility of recording photosensitized phosphorescence at 1270 nm cannot be an argument to deny the formation of singlet oxygen since measuring this luminescence in water is a difficult technical problem. On the other hand, superoxide, hydroxyl and other radicals are always formed in oxidation processes initiated by singlet oxygen.[2-4] We believe that the results of the present work are important in connection with fundamental aspects of PDT and mechanisms of protection of photosynthetic organisms from damage by solar radiation.

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