ELSEVIER

Journal of Photochemistry and Photobiology A: Chemistry 156 (2003) 139–144

Journal of Photochemistry and Photobiology A:Chemistry

www.elsevier.com/locate/jphotochem

The triplet excited state changes of amphiphilic porphyrins with different side-chain length in AOT reverse micelles

Jun Hua Yu [a], Yu Xiang Weng [b], Xue Song Wang [a,*], Lei Zhang [b], Bao Wen Zhang [a,*], Yi Cao [a]

[a] *Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing 100101, China*
[b] *Institute of Physics, Chinese Academy of Sciences, Beijing 100080, China*

Received 30 September 2002; received in revised form 18 October 2002; accepted 5 December 2002

Abstract

Two amphiphilic porphyrins terminated with carboxyl were studied in AOT/*iso*-octane/water reverse micelles, intending to mimic the relationship between microenvironments in organism and the amphiphilic properties of porphyrins for photodynamic therapy (PDT) drugs. The quenching of porphyrins by methyl viologen chloride and the laser flash photolysis experiments show that the longer side-chain length would promote the firm embedding of porphyrin molecules in the interfacial region of reverse micelles. Such an embedding may shift porphyrins from aggregated to monomeric form in reverse micelles and, accordingly, the slower excited state decay may increase the efficiency of the photosensitizer in PDT. The effect of amphiphilic properties on the status of porphyrins in microenvironments provides a light on the synthesis of other amphiphilic porphyrins for PDT drugs.

Keywords: Amphiphilic porphyrins; Excited triplet state; AOT reverse micelles; Side-chain length

1. Introduction

Porphyrin derivatives are potentially photodynamic therapy (PDT) drugs known for their high triplet quantum yields and long lifetimes of excited triplet state, which are regarded as the prerequisites to initiate efficient PDT process [1]. The photosensitizers in their excited states may react directly (type I mechanism) or indirectly (type II mechanism) with abnormal cells, resulting in their destruction [2]. In either type, the photoreaction proceeds via the lowest excited triplet state of the sensitizer [3], which consequently influences the efficiency of the photosensitizing action.The lifetime of the excited triplet state of a sensitizer is very sensitive to the environments that the sensitizer locates and the status of the sensitizer presents per se [4–8]. The diverse chemical and photophysical properties of porphyrins result partly from the various circumstances in which they are. In micro-heterogeneous environments such as cells and tissues, structural (such as conformation) and functional parameters (such as lifetime and quantum yield of an excited state) of a sensitizer, which are affected by microenvironment, determine the overall efficiency of the photosensitization process.

Porphyrins for PDT applications are usually classified into three major groups: hydrophobic, hydrophilic and amphiphilic sensitizers, and the amphiphilic is especially regarded as the most important type among those porphyrinic compounds [9]; one of its unique properties is it may associate strongly with plasma lipoproteins and may be incorporated into tumor cells through receptor-mediated endocytosis of low-density lipoproteins [10]. The synthesis and pharmacokinetics on amphiphilic porphyrins have been investigated extensively [11–13].

In this study, we attempted to elucidate the relationship between the microenviroments and the properties of amphiphilic porphyrin. Considering the ability to control the size and properties of the water pool, the reverse micelle is an interesting model candidate to mimic the water pockets that are often found in various bioaggregates such as proteins, membranes, and mitochondria. Here, we use bis(2-ethylhexyl)sodium sulfosuccinate (AOT) as surfactant to probe the triplet state lifetime of the porphyrins in

* Corresponding authors. Fax: +86-10-64886985.
E-mail addresses: g203@ipc.ac.cn (X.S. Wang), g203@ipc.ac.cn (B.W. Zhang).

1010-6030/03/$ – see front matter
doi:10.1016/S1010-6030(02)00429-X

AOT/*iso*-octane/water reverse micelle with different molar ratio of water:AOT, w_o (w_o = [water]/[AOT]).

2. Materials and methods

2.1. Materials

The amphiphilic porphyrins **1** and **2** (as shown in Fig. 1) were prepared according to literature procedures [14]. Chloroform, tetrahydrofuran (THF), cyclohexane, *iso*-octane (IOA) and acetone were purchased from Beijing Chemical Company (Beijing) and were used after the general purification [15]. Bis(2-ethylhexyl)sodium sulfosuccinate were purchased from Acros and purified as described in the literature [16]. Methyl viologen chloride was purchased from TCI (Japan) and used as received.

2.2. Preparation of samples

The reverse micelles were prepared by dissolving AOT in IOA and then adding the necessary volume of water to obtain the desired value of w_o, with a concentration of AOT in *iso*-octane being 0.1 mol l^{-1} throughout this paper. The porphyrin and methyl viologen chloride solutions were prepared by adding a chloroform solution of the porphyrin or methyl viologen chloride to a volumetric flask. The solvent was evaporated and the AOT stock solution at the desired w_o added to the flask. It was then sonicated for at least 30 min until the absorbance of the solution maintained.

Monolayers were prepared on a Model 622 NIMA Langmuir–Blodgett trough. Solutions of porphyrins in chloroform were applied dropwise on the clean water subphase by syringe at a subphase temperature of 20 ± 1 °C. Chloroform was allowed to evaporate for 15 min, and the floating film was then compressed at a rate of 40 cm^2 min^{-1}.

2.3. Photophysical measurements

The absorption spectra were measured with Shimadzu UV-1601PC UV-Vis spectrophotometer and the fluorescence spectra with Hitachi F-4500 fluorescence spectrophotometer. The fluorescence quantum yield (Φ_F) of a porphyrin was determined by comparing its integrated fluorescence intensity per absorbed photon to that of free base tetraphenylporphyrin (TPP) using the following relationship [17]:

$$\Phi_{F(s)} = \Phi_{F(r)} \frac{F_{(s)} n^2_{(s)} A_{(r)}}{F_{(r)} n^2_{(r)} A_{(s)}}$$

where the subscripts (s) refers to the sample and (r) to the reference; F represents the integrated area under the emission band; A denotes the absorbance of the solution at 416 nm, where the excitation wavelength was set; and n is the index of refraction of the solvent at the sodium D line at 20 °C. The $\Phi_{F(r)}$ of TPP is 0.11 in air saturated benzene [18].

The flash photolysis set-up employed the 532 nm second harmonic generation of Nd^{3+}:YAG laser as the excitation source (Quanta Ray DCR) with a pulse width (FWHM) of 8 ns and an energy of 0.64 mJ per pulse (10 Hz). The probe beam source was a 500 W cw Xe lamp. The transient signal was detected by a six-stage R456 (Hamamatsu) photo-multiplier tube, amplified by a 300 MHz dc amplifier and was finally fed into a 500 MHz digital oscilloscope (Tektronix) interfaced to a PC by GPIB board for data handling and processing. The system gives a resolution of 0.1% absorbance difference and a temporal resolution of 50 ns for a non-scattering sample. The transient difference absorption spectra were recorded point by point at intervals in the range between 420 and 680 nm after pulsed laser excitation of an Ar-saturated reverse micelle solution of porphyrin.

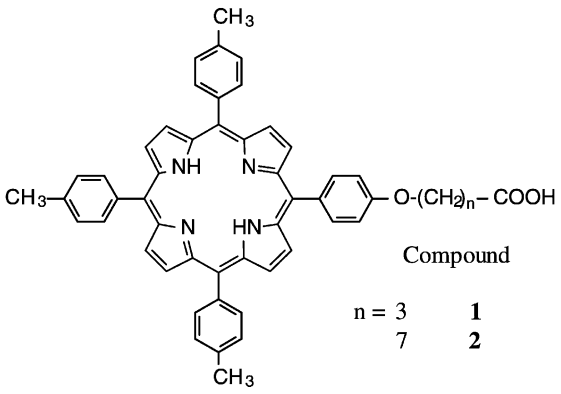

Fig. 1. Structures of compounds **1** and **2**.

3. Results and discussion

3.1. Steady-state UV-Vis absorption and fluorescence measurements

Considering that tumor necrosis can only occur when the number of absorbed photons exceeds a damage threshold [19], we choose a relatively high concentration of 2.3 × 10^{-5} mol l^{-1} for porphyrin in reverse micelle solutions.

The absorption spectra of each compound at a fixed concentration in AOT/IOA/water reverse micelles have little change upon the variation of w_o, from w_o 4 to 12, while at a fixed value of w_o, a new strong absorption (411 nm) abutting against the original band (425 nm) appears upon increasing the concentration of porphyrins studied from 1.8 × 10^{-7} to 2.3 × 10^{-5} mol l^{-1} (as shown in Fig. 2). This means that porphyrins in reverse micelles tend to form H-aggregates, while the expansion of water pool does not give any distinguished influence on absorption change. The compounds, **1** and **2** can form H-aggregate as well as in chloroform at the concentration of 2.3 × 10^{-5} mol l^{-1} and exhibit similar absorption spectra as in reverse micelle solutions (see the inset of Fig. 2), but the B-band FWHMs of compounds **1** and **2** in chloroform are 34.5 and 37 nm, respectively, broader than that in reverse micelle solutions (both 34 nm). Such a

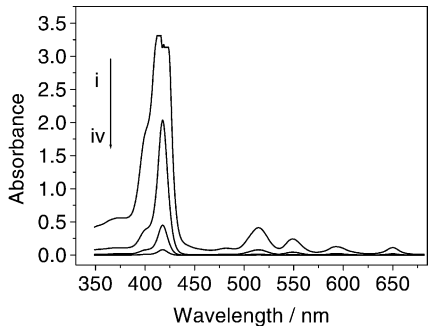

Fig. 2. Absorption spectra of compound **1** in reverse micelle solutions with the concentrations at: (i) 2.3×10^{-5}, (ii) 4.6×10^{-6}, (iii) 9.2×10^{-7}, and (iv) 1.8×10^{-7} mol l^{-1}. The inset shows the absorption of compound **1** in reverse micelles at (—) $w_o = 12$ and (---) in chloroform at the concentrations of 2.3×10^{-5} mol l^{-1}.

broadening of B-band in chloroform might be ascribed to more kinds of aggregates formed than that in reverse micelle solutions.

The formation of aggregates can also be envisaged from fluorescence excitation spectrum. The excitation spectra of compound **1** are shown in Fig. 3, at varying concentration in reverse micelle solutions. The splitting of the peaks from 420 to 410 nm and 425 nm may result from the aggregate formation as the concentration of porphyrin increases. Such a splitting is in-line with the data from UV-Vis absorption spectra. At the same time, the fluorescence intensity excited from Q band increases degressively. Such a tendency may also be ascribed to the aggregating of porphyrins [20].

Accurate fluorescence quantum yields of the free monomers and aggregates in an aggregating system are difficult to calculate, as the exact concentration of emitting species is unknown. So, the overall fluorescence quantum yields (Φ_F, contributed by both monomers and aggregates) of porphyrin solutions in reverse micelles or chloroform are measured and shown in Table 1. All the porphyrins only have very small change in Φ_F as w_o is increased from 4 to 12, indicating that water content of the reverse micelles has little influence on the excited singlet state of porphyrin, based on static fluorescence spectra.

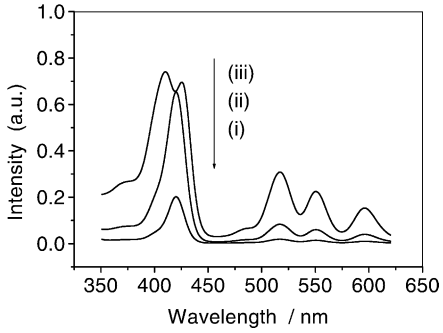

Fig. 3. Fluorescence excitation spectra of compound **2** at varying concentration in reverse micelle solutions ($w_o = 12$). The concentrations of porphyrin are: (i) 1.8×10^{-7}, (ii) 9.0×10^{-7}, and (iii) 4.6×10^{-6} mol l^{-1}. The fluorescence is probed at 658 nm.

Table 1
Fluorescence quantum yields of compounds **1** and **2** in AOT/IOA/water reverse micelles and chloroform

Solvent	Φ_F[a]	
	1	**2**
Reverse micelles at varying w_o		
4	0.059	0.037
6	0.056	0.034
8	0.058	0.035
12	0.058	0.035
$CHCl_3$	0.052	0.001

[a] The concentration of compounds **1** and **2** was 2.3×10^{-5} mol l^{-1}, and at such a concentration aggregates formed in both reverse micelle and chloroform solutions as indicated in the text. The excitation wavelength was 416 nm.

The Φ_F of compounds **1** and **2** in chloroform is even smaller than that in reverse micelles. Considering that H-aggregates of porphyrins have lower fluorescence quantum yield than their monomers [21], the higher fluorescence quantum yield in reverse micelle solutions can be ascribed to the monomeric dispersion of porphyrin inside the reverse micelles. Since the water pool radius, R_w, follows the relation [22]:

$$R_w\,(\text{Å}) = 1.5 w_o$$

and the apparent molar volume of solubilized water in AOT/IOA/water reverse micellar solution is 17.6 ml mol^{-1} at 24 °C [23], the reverse micelle concentration in the solution is ca. 3.22×10^{-3} mol l^{-1} at $w_o = 8$, nearly two orders of magnitude higher than the concentration of porphyrins in solution (2.3×10^{-5} mol l^{-1}), which may disfavor the aggregation of porphyrins greatly because in such case the probability of two porphyrin molecules in one micelle will be not more than 1×10^{-4} according to Poisson population [24]. This more or less diminish the possibility of aggregating though it is shown that the probability for aggregating is zero if the [sensitizer]/[lipid] ratio is at least 1/1000 [25].

It is worthy noting that the fluorescence quantum yield in chloroform of the compound **2** is less than that of the compound **1** by nearly 50-fold. This may be because the compound **2** has higher amphiphilicity than **1** and, as a result, readily form aggregates in $CHCl_3$ just as a surfactant does in solution. Consequently, aggregates dominate the chloroform solution of **2** and a remarkably diminished fluorescence quantum yield is exhibited. Once the compound **2** is dissolved into reverse micelle solutions, they disperse in discrete micelles so that aggregation is restricted and enhanced fluorescence quantum yield can be obtained.

3.2. Amphiphilic properties

Fig. 4 shows the surface pressure–area isotherms of the compounds **1** and **2**. Although no other surfactants were added, the films show relatively high collapse of pressures,

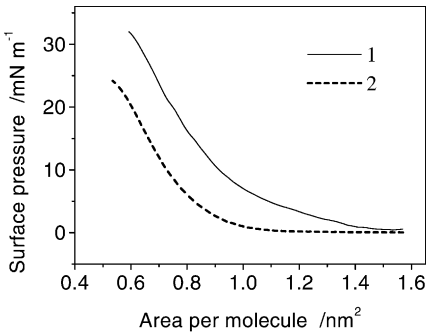

Fig. 4. Surface pressure–area (π–A) isotherms of compounds **1** and **2** at the air–water interface ($20 \pm 1\,^{\circ}$C).

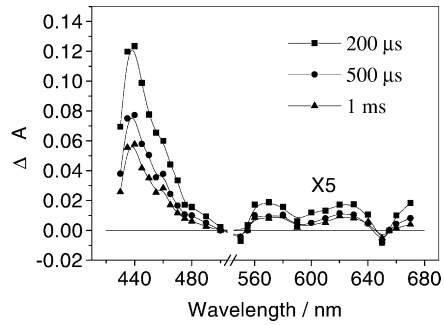

Fig. 5. Transient absorption spectra of compound **1** (2.3×10^{-5} mol l^{-1}) in reverse micelles ($w_o = 4$), measured after flashing at 532 nm. The signals between 540 and 670 nm are amplified by a factor of five.

indicating that the porphyrins possess certain amphiphilic properties. Compound **1** gives a higher collapse of pressure, but its limiting area per molecule (111 Å) is larger than that of **2** (91 Å). The difference in limiting areas per molecule suggests that the films collocate more tightly as the length of side chains increases. As there is so far no theory correlating the amphiphilicity and the collapse pressures, we can only consider that the one containing smaller area per molecule but longer side-chain length is more likely to act as a surfactant, i.e. it has the self-assembling ability which usually results from the amphiphilicity of compound. This assumption accords with the conclusion, deduced from the fact of the much lower fluorescence quantum yield of **2** with respect to **1** in chloroform, that **2** is more amphiphilic.

3.3. Laser flash photolysis

3.3.1. Triplet state lifetime in reverse micelles

The lifetimes of triplet excited state of compounds **1** and **2** in reverse micelles are much longer than that in homogeneous solution. Take compound **2** as an example: the lifetimes are 212 ns in cyclohexane and 514 ns in THF, respectively, while 24.8 μs in reverse micelles ($w_o = 6$). Two reasons may account for the extended lifetimes of compounds **1** and **2** in reverse micelles: (i) the enwrapping of the porphyrin molecules by the inert materials, i.e. the detergent molecules, restricts the aggregating of porphyrins which is expected to self-quench the excited state [26]; and (ii) increased microviscosity in reverse micelles slows down the radiationless decay rates.

The comparison of triplet state lifetimes as well as fluorescence quantum yields between in reverse micelles and in organic solutions indicates that the aggregating of compounds **1** and **2** is more or less restricted in reverse micelle solutions.

3.3.2. Location of porphyrins in reverse micelles

Porphyrins in reverse micelles locate assumedly at the interface with the carboxy inserted in polar part of the reverse micelle and the porphyrin skeleton near the apolar phase. Here, we use a water-soluble quencher, methyl viologen chloride, to detect the location of porphyrins. The results show that the triplet state lifetime of compound **1** decreases from 87.2 μs to 92.4 ns, corresponding to a quenching constant of 1.1×10^7 s^{-1}, after the addition of methyl viologen chloride (3×10^{-3} mol l^{-1}) to the reverse micelles ($w_o = 10$), while that of compound **2** only from 757 to 132 μs, corresponding to a quenching constant of 6.3×10^3 s^{-1}, in the same condition. As quenching occurring need a close contact between the chromophore and the quenching compound [27], the higher quenching efficiency of compound **1** by methyl viologen chloride means it locates closer than compound **2** to the water pool, i.e. the porphyrin skeleton of **2** is remote to the methyl viologen due to its longer side chain, implying the curling of the side chains of porphyrins in the interface can be excluded.

3.3.3. Transient absorption spectrum

Fig. 5 shows the transient absorption spectrum of compound **1** in reverse micelle solution at $w_o = 4$, measured by laser flash photolysis at 532 nm. Measurements between 515 and 545 nm were skipped to avoid the interference from the excitation. The spectrum exhibits a major peak around 440 nm which results from the triplet–triplet absorption [28], and its absorbance is 30 times larger than that above 570 nm. The bleaches at 550 and 650 nm result from the steady-state absorption of porphyrin, but there is only a base at 590 nm where exists a steady-state absorption.

Poor fitting parameters are obtained when fitting the decays at 440 nm to a mono-exponential law except the decay of compound **2** at $w_o = 6$, but the quality of the fitting was improved when a bi-exponential law was used. These profiles at 440 nm can be successfully approximated by a sum of two exponential decay components (see Table 2): one in the order of microsecond and the other in millisecond.

The bi-exponential decay may be explained in terms of the co-existence of different state for porphyrins in reverse micelle solutions. As mentioned earlier, the triplet state lifetime in reverse micelle solutions is much longer than that in THF, i.e. detergent molecules retard the aggregation of porphyrin and consequently the fast decay induced by aggregation is restrained. If we assume there exists an equilibrium between

Table 2
Parameters of triplet lifetime decays recorded at 440 nm for compounds **1** and **2** in reverse micelle solutions with different w_o

w_o	Lifetime (τ)[a] and pre-exponential factors (a)[a]				Average lifetime
	τ_1 (μs)	a_1	τ_2 (μs)	a_2	τ_a (μs)
Compound **1**					
4	233	0.48	1960	0.52	1789
6	141	0.82	1280	0.18	899
8	119	0.46	1230	0.54	1145
12	164	0.81	2210	0.19	895
Compound **2**					
4	278	0.46	1970	0.54	2606
6	24.8	–	–	–	24.8
8	229	0.48	1660	0.52	1498
12	83.7	0.49	1300	0.51	1229

[a] Derived from the fittings of lifetime decays by the equation: $I(t) = a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2)$.

monomers and aggregates, it can be reasonably concluded that the slow decay represents the monomer-dominating part and the fast one may result from the aggregate-dominating part. The higher the fraction of monomer in all porphyrin, the longer the excited lifetime will be. Thus, the bi-exponential decay may indicate there are at least two status of the porphyrin molecules in reverse micelles.

The decay of compound **2** at $w_o = 6$ is mono-exponetial and its lifetime is only 24.8 μs, rarely short compared to the others. This single and short lifetime reflects the porphyrin molecules locate in only one mode, for example, in the inner or the outer part of the interface, and collocate too close than the aggregating dominates in this case.

3.3.4. *Effect of amphiphilic property on the triplet state lifetime*

The amphiphilic property of porphyrins resulting from different side-chain length highly influences the lifetime of excited triplet state of porphyrins, an important parameter in their PDT application. The average lifetimes of porphyrin in reverse micelle solutions at w_o, τ_a (defined as

$$\tau_a = \frac{\sum_{i=1}^{N} a_i \tau_i^2}{\sum_{i=1}^{N} a_i \tau_i}$$

where a_i is the pre-exponential factor of an ith component, τ_i its lifetime, and N the number of component of the fit), can give some information about the effect of amphiphilic properties on the triplet state lifetime. As shown in Table 2, the average lifetimes of compound **2** are generally longer than those of **1** at the same w_o. For example, the lifetime of compound **1** is 1145 μs at $w_o = 8$, while that of **2** is 1498 μs.The difference in lifetime of excited triplet state may be explained in terms of the status in which the compounds **1** and **2** exist in the reverse micelles. The status of porphyrin in reverse micelle solution can be partly determined by two factors: the driving force for aggregation of porphyrins, and the dispersion ability of porphyrins in detergent resulting from the amphiphilic property. As the amphiphilic property improved, porphyrin would be easier to set its hydrophilic part in the water phase and correspondingly the hydrophobic part in the apolar phase. In another words, the more amphiphilic, the much easier the compound disperses in surfactant layer in reverse micellar solution. The intervention of detergents prevents porphyrins from aggregation and, therefore, lengthens the average lifetime of the triplet excited state of porphyrins. Based on the analysis earlier and the conjecture in Sections 3.1 and 3.2, compound **2**, a more amphiphilic porphyrin than **1**, is expected to possess longer triplet state lifetime, a fact what was really observed experimentally.

3.3.5. *Relation between triplet state lifetime and w_o*

The effect of w_o on the triplet state lifetimes of porphyrins seems to correlate very well with the concentration of micelles. The concentrations of reverse micelles in solution varies from 1.29×10^{-2} mol l^{-1} for $w_o = 4$ to 1.43×10^{-3} mol l^{-1} for $w_o = 12$, thus the local concentration of porphyrins in reverse micelles will also have such a concentration difference. The reverse micelle solution at low w_o would have low local concentration of porphyrins and the compound would consequently show the longer lifetime of excited triplet state. This is in-line with our finding that both the compounds have the longest average lifetimes at $w_o = 4$ during the variation of w_o.

It is very interesting that exceptions occurs again at $w_o = 6$. For compound **1**, the shortest triplet state lifetime appeared at $w_o = 6$ rather than $w_o = 12$, while for compound **2**, the lifetime is not only the shortest among the w_o employed, but also mono-exponential. The reason for this finding is still unclear at present.

4. Conclusions

Two amphiphilic porphyrins were studied in AOT/*iso*-octane/water reverse micelles at varying w_o, intending to mimic the relationship between microenvironments in the organism and the amphiphilic properties of porphyrins for PDT drugs. The high amphiphilic capability would promote the firm embedding of porphyrin molecules in the interfacial region of reverse micelles. Such an embedding may shift porphyrins from aggregated to monomeric form in reverse micelles and, accordingly, the slower excited state decay may increase the efficiency of the photosensitizer in PDT. The effect of amphiphilic properties on the states of porphyrins in microenvironments provides a light on the synthesis of other amphiphilic porphyrins for PDT drugs.

Acknowledgements

We thank Prof. C.H. Huang and Dr. F.Y. Li (Peking University, China) for their LB technical support, and thank the

National Natural Science Foundation of China, the Ministry of Science and Technology of China (Grant nos. 29971031, 20073050, and G2000028204) for the financial support.

References

[1] E.D. Stermberg, D. Dolphin, Porphyrin-based photosensitizers for use in photodynamic therapy, Tetrahedron 54 (1998) 4151–4202.
[2] M. Ochsner, Photophysical and photobiological processes in the photodynamic therapy of tumors, J. Photochem. Photobiol. Part B: Biol. 39 (1997) 1–18.
[3] T. Takemura, N. Ohta, S. Nakajima, I. Sakata, Critical importance of the triplet lifetime of photosensitizer in photodynamic therapy of tumor, Photochem. Photobiol. 50 (1989) 339–344.
[4] D.L. Akins, S. Özçelik, H.-R. Zhu, C. Guo, Fluorescence decay kinetics and structure of aggregated tetrakis(*p*-sulfonatophenyl)-porphyrin, J. Phys. Chem. 100 (1996) 14390–14396.
[5] R. Jasuja, D.M. Jameson, C.K. Nishijo, R.W. Larsen, Singlet excited state dynamics of tetrakis(4-*N*-methylpyridyl)porphine associated with DNA nucleotides, J. Phys. Chem. B 101 (1997) 1444–1450.
[6] S. Dhami, D. Philips, Comparison of the photophysics of an aggregating and non-aggregation aluminium phthalocyanine system incorporated into unilamellar vesicles, J. Photochem. Photobiol. Part A: Chem. 100 (1996) 77–84.
[7] M. Hoshino, N. Tezuka, M. Inamo, Photochemistry of metal-loporphyrins in polymer matrices: laser photolysis studies of chlorochromium(III) tetraphenylporphyrin in polystyrene films in the temperature range 77–300 K, J. Phys. Chem. 100 (1996) 627–632.
[8] S. Gentemann, N.Y. Nelson, L. Jaquinod, D.J. Nurco, S.H. Leung, C.J. Medforth, K.M. Smith, J. Fajer, D. Holten, Variations and temperature dependence of the excited state properties of conformationally and electronically perturbed zinc and free base porphyrins, J. Phys. Chem. B 101 (1997) 627–632.
[9] R.W. Boyle, D. Dolphin, Structure and biodistribution relationships of photodynamic senstitizers, Photochem. Photobiol. 64 (1996) 469–485.
[10] C. Schell, H.K. Hombrecher, Synthesis, self-assembling properties and incorporation of carbohydrate-substituted porphyrins into cell membrane models, Chem. Eur. J. 5 (1999) 587–597.
[11] A. Richter, E. Waterfield, A.J. Canaan, B.A. Allison, J.G. Levy, Liposomal delivery of a photosensitizer, benzoporphyrin derivative monoacid ring A (BPD), to tumor tissue in a mouse tumor model, Photochem. Photobiol. 57 (1993) 1000–1006.
[12] J.N. Weinstein, K.W. Kohn, M.R. Grever, V.N. Viswanadhan, L.V. Rubinstein, A.P. Monks, D.A. Scudiero, L. Welch, A.D. Koutsoukos, A.J. Chiausa, K.D. Paull, Neural computing in cancer drug development: predicting mechanism of action, Science 258 (1992) 447–451.
[13] M. Cornia, G. Casiragni, S. Binacci, F. Zanardi, G. Rassu, Facile entry to 5-, 10-, 15-, 20-tetra-*C*-glycosylporphyrins, J. Org. Chem. 59 (1994) 1226–1230.
[14] L. Yuan, S. Chen, H. Zhao, H. Zhu, X. Wang, Syntheses of enzyme models by appendage of metalloporphyrin to calixarene and study on their electronic spectra, Chinese J. Org. Chem. 12 (1992) 583–584.
[15] D.D. Perrin, W.L.F. Armarego, D.R. Perrin, Purification of Laboratory Chemicals, Pergamon Press, Oxford.
[16] R.D. Falcone, N.M. Correa, M.A. Biasutti, J.J. Silber, Properties of AOT aqueous and non-aqueous microemulsions sensed by optical molecular probes, Langmuir 16 (2000) 3070–3076.
[17] J.V. Casper, T.J. Meyer, Photochemitry of $Ru(bpy)_3^{2+}$: solvent effects, J. Am. Chem. Soc. 105 (1983) 5583–5590.
[18] P.G. Seybold, M. Gouterman, Porphyrins XIII: fluorescence spectra and quantum yields, J. Mol. Spectrosc. 31 (1969) 1–13.
[19] M. Patterson, B.C. Wilson, R. Graff, In vivo tests of the concept of photodynamic threshold dose in normal rat liver photosensitized by aluminum chlorosulphonated phthalocyanine, Photochem. Photobiol. 51 (1990) 343–349.
[20] F. Richelli, J. Jori, Distribution of porphyrins in the various compartments of unilamellar liposomes of dipalmitoyl–phosphatidyl choline as probed by fluorescence spectroscopy, Photochem. Photobiol. 44 (1986) 151–157.
[21] M. Kasha, H.R. Rawls, M. Ashraf El-Bayoumi, The exciton model in molecular spectroscopy, Pure Appl. Chem. 11 (1965) 371–392.
[22] M.P. Pileni, Reverse micelles as microreactions, J. Phys. Chem. 97 (1993) 6961–6973.
[23] Y. Yoshimura, I. Abe, M. Ueda, K. Kajiwara, T. Hori, Z.A. Schelly, Apparent molar volume of solubilized water in AOT/*iso*-octane/water reverse micellar aggregates, Langmuir 16 (2000) 3633–3635.
[24] K.L. Mittal, E.J. Fendler, Solution Behavior of Surfactants: Theoretical and Applied Aspects, Plenum Press, New York, 1982.
[25] C.D. Borsarelli, J.J. Cosa, C.M. Previtali, Interface effect on the properties of exciplexes formed between pyrene derivatives and *N*,*N*-dimethylaniline in reversed micelles, Langmuir 9 (1993) 2895–2901.
[26] J.D. Spikes, J.C. Bommer, Zinc phthalocyanine as a photosensitizer for biomolecules, Int. J. Radiat. Biol. 50 (1986) 41–45.
[27] N.J. Turro, Modern Molecular Photochemistry, Benjamin/Cumming, Menlo Park, CA, 1978.
[28] D.M. Tofashi, S.M.B. Costa, Absortion, fluorescence and transient triplet–triplet absorption spectra of zinc tetramethylpyridylporphyrin in reverse micelles and microemulsions of aerosol OT (AOT), Phys. Chem. Chem. Phys. 2 (2000) 5437–5444.