ELSEVIER

Journal of Photochemistry and Photobiology A: Chemistry 91 (1995) 193–198

Journal of PHOTOCHEMISTRY AND PHOTOBIOLOGY A: CHEMISTRY

A comparison of the photoproperties of zinc phthalocyanine and zinc naphthalocyanine tetrasulfonates: model sensitizers for the photodynamic therapy of tumors

John D. Spikes [a], Johan E. van Lier [b], Jerry C. Bommer [c]

[a] *Department of Biology, University of Utah, Salt Lake City, UT 84112, USA*

[b] *MRC Group in the Radiation Sciences, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Que. J1H 5N4, Canada*

[c] *Porphyrin Products, Inc., PO Box 31, Logan, UT 84321, USA*

Received 1 February 1995; accepted 2 June 1995

Abstract

Phthalo- and naphthalocyanines are of interest as sensitizers for the photodynamic therapy of tumors because of their strong absorption in the 680 and 760 nm ranges respectively. Both zinc phthalocyanine and naphthalocyanine tetrasulfonates ($ZnPcS_4$ and $ZnNcS_4$) were aggregated and photochemically inactive in aqueous buffer of pH 7.4, while in 10 mM cetyl pyridinium chloride in buffer they were monomeric and active. Therefore all these studies were carried out using the buffered detergent. The triplet lifetimes of $ZnPcS_4$ and $ZnNcS_4$ under argon were 490 and 110 μs respectively, with oxygen bimolecular quenching constants of 4.2×10^9 and 2.0×10^8 M^{-1} s^{-1} respectively. Triplet decay curves in argon, air and 100% oxygen were first order, suggesting that there was little back reaction of the triplet states with oxygen as has been observed with some naphthalocyanines. The quantum yield of singlet oxygen generation by $ZnPcS_4$ was 0.70 and that for $ZnNcS_4$ was 0.25. Both compounds sensitized the photo-oxidation of furfuryl alcohol, cysteine, histidine, methionine, tryptophan, tyrosine and guanosine; $ZnPcS_4$ was three times more efficient than $ZnNcS_4$. These reactions were 50% inhibited by about 0.5 mM azide, suggesting the involvement of singlet oxygen. Both sensitizers photobleached on illumination, with quantum yields of 1.7×10^{-5} for $ZnPcS_4$ and 4.2×10^{-3} for $ZnNcS_4$.

Keywords: Naphthalocyanine; Photobleaching; Photodynamic therapy; Photosensitization; Phthalocyanine; Singlet oxygen

1. Introduction

Photodynamic therapy (PDT), a new modality for the treatment of tumors, is presently undergoing extensive clinical trials [1]. As part of the continuing research in this area, there has been a major search for better photosensitizers. This has resulted in part in a large number of studies on a wide array of phthalocyanine (Pc) and naphthalocyanine (Nc) derivatives; this area has been extensively reviewed [2–7]. The Pcs and Ncs have strongly absorbing Q bands in the 680 and 760 nm ranges respectively, where light penetrates efficiently into tissues. Many of these compounds are relatively non-toxic, can be synthesized in high purity and generate singlet oxygen (which appears to be the main mediator for tumor destruction in PDT) in medium to high yields. Some Pc and Nc derivatives are efficiently taken up by mammalian cells and by model tumors in rodents and sensitize cell killing and tumor destruction [2–7].

Only a few reports comparing the photoproperties of homologous Pc–Nc pairs have been published [8,9]. The study reported here describes a survey of the photophysics, photosensitizing properties and photodegradation behavior of zinc phthalocyanine tetrasulfonate ($ZnPcS_4$) and zinc naphthalocyanine tetrasulfonate ($ZnNcS_4$). The structures of these compounds are shown in Fig. 1.

2. Experimental details

2.1. Materials

Zinc(II) phthalocyanine tetrasulfonate was obtained from Porphyrin Products, Inc., Logan, UT, and the $ZnNcS_4$ was prepared by the sulfonation of Zn naphthalocyanine followed by extensive reverse phase chromatography [10]. Both compounds were of high purity, as confirmed by reverse phase high performance liquid chromatography (HPLC) and an oxidative degradation assay [10]. The other chemicals used were of the highest purity available and were used as received, except for furfuryl alcohol (FA) which was distilled under low pressure and stored in a refrigerator under nitrogen.

SSDI 1010-6030(95)04129-X

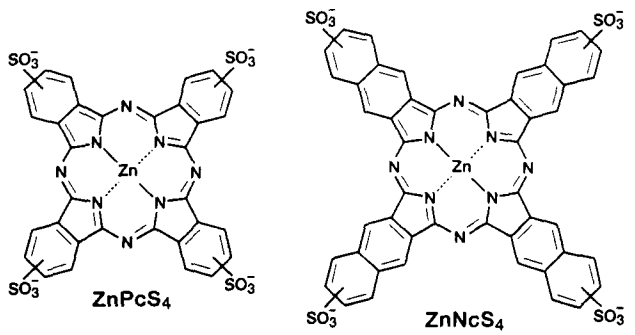

Fig. 1. Structures of zinc phthalocyanine tetrasulfonate ($ZnPcS_4$) and zinc naphthalocyanine tetrasulfonate ($ZnNcS_4$).

2.2. Photophysical measurements

The triplet–singlet difference spectra, triplet lifetimes and triplet quenching constants were determined as described elsewhere [11,12] with an on-line, computerized flash kinetic spectrophotometric system at the Center for Fast Kinetics Research (CFKR), University of Texas at Austin. Flash excitation was from a Quantel YG 481 *Q*-switched Nd:YAG laser. Singlet oxygen ($^1\Delta_g$) production was measured, after pulsed laser excitation of the sensitizers at 355 nm in air-saturated D_2O, by following the time course of emission by this species at 1.27 μm [11,12]. Some flash measurements were also made at the Center for Photochemical Sciences, Bowling Green State University, Bowling Green, OH. Absorption and fluorescence measurements were made with a Perkin–Elmer Lambda 9 spectrophotometer and an MPF-66 fluorescence spectrophotometer.

2.3. Oxygen, hydrogen peroxide and photobleaching measurements

Illumination for oxygen uptake and photobleaching experiments was provided by a 500 W quartz–halogen source coupled with bandpass filters corresponding to the long wavelength absorption peaks (Q bands) of the sensitizers (filters from Corion Corp., Holliston, MA; bandwidth 10 ± 2 nm at 50% peak transmission). Illumination at the Q band peaks was used since the photochemical behavior of $ZnPcS_4$ is altered by irradiation in the near UV [13]. Incident light fluence rates varied from 1 to 10 mW cm^{-2} depending on the experiment. A recording oxygen electrode system was used to measure the quantum yields of oxygen uptake during the sensitized photo-oxidation of substrates [11,12]; the quantum yield was defined as the initial rate of uptake of oxygen molecules divided by the initial rate of absorption of photons by the reaction system. Quantum yields of singlet oxygen production by the illuminated sensitizers were estimated using saturating concentrations of FA as substrate and rose bengal as a standard [12,14]. Hydrogen peroxide accumulation during photo-oxidations was measured by injecting 50 μl of catalase (24 000 units ml^{-1}) into the reaction mixture in the oxygen electrode vessel after the oxygen concentration in the mixture was reduced to about zero and recording the oxygen evolved. Photobleaching of sensitizers was measured spectrophotometrically after various periods of illumination; the quantum yield of photobleaching was defined as the initial rate of disappearance of sensitizer molecules divided by the initial rate of absorption of photons [14,15]. The errors in the measurements of the quantum yields of oxygen uptake during the photo-oxidation reactions and for sensitizer photobleaching were approximately ± 10%.

3. Results and discussion

3.1. Sensitizer solubilities and ground state spectra

Both $ZnPcS_4$ and $ZnNcS_4$ at 5 μM appeared to dissolve in 0.1 M Na phosphate buffer of pH 7.4 (B) to give spectra with low Q band peaks at 634 and 705 nm respectively, as shown in Fig. 2 (dotted curves). In B containing 10 mM cetylpyridinium chloride (B–CPC), a cationic detergent, the Q bands narrowed, increased in height and shifted to 679 and 762 nm, with molar absorption coefficients of 1.4×10^5 and 9.8×10^4 l cm^{-1} mol^{-1} respectively (full curves in Fig. 2). UV absorption peaks were at 348 nm for the Pc and at 331 and 385 nm for the Nc. These data suggest that both sensitizers are aggregated in B but monomerize in the detergent. Sulfonated Pcs are usually monomerized in organic solvent–water mixtures and by cationic detergents [16]. In the present work, triton X-100, a non-ionic detergent, partially monomerized the sensitizers, but sodium dodecyl sulfate, an anionic detergent, had no effect. Sulfonated zinc naphthalocyanine with an average of about three sulfonic acid groups per molecule ($ZnNcS_3$) is aggregated in water but monomerizes in ethanol–water (90:10 vol.%), methanol–water (95:5 vol.%), 10 mM hexadecyltrimethylammonium chloride (CTAC, a cationic detergent) and 3% fetal calf serum [8,9,17]. Other compounds used in the present studies, including substrates and inhibitors, had no significant effects on the absorption spectra of the sensitizers. Buffer concen-

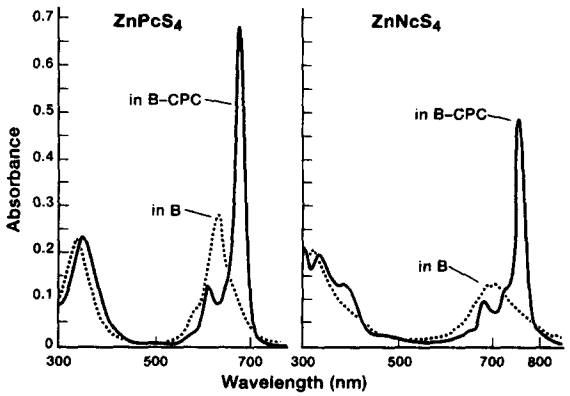

Fig. 2. Absorption spectra of 5 μM $ZnPcS_4$ and $ZnNcS_4$ as measured in a 1.0 cm cuvette in 0.1 M Na phosphate buffer of pH 7.4 (B; dotted curves) and in B containing 10 mM cetylpyridinium chloride (B–CPC; full curves).

tration over the range 0–0.44 M, or buffer pH from 5.3 to 11.4, also had little effect on the spectra. All the studies described in this paper were carried out with B–CPC as solvent unless indicated otherwise, since only the monomeric sensitizers were photochemically active. In general, aggregated photosensitizers are inactive owing to the rapid decay of their singlet excited states by internal conversion to the ground state [16].

3.2. Fluorescence

Neither sensitizer was appreciably fluorescent in B, again suggesting that they are aggregated in this solvent. In B–CPC, $ZnPcS_4$ had an emission peak at 688 nm, while $ZnNcS_4$ emitted at 770 nm (uncorrected). Neither FA nor the biomolecules used in this work had any effect on the fluorescence. It has been reported, however, that aggregated $ZnNcS_3$ in water emits weakly at 860 nm; this emission disappears upon the addition of a cationic detergent [8].

3.3. Triplet state properties

No significant transients were observed on flashing the sensitizers in B. The triplet–singlet difference spectra of 10 mM sensitizers flashed in B–CPC, as measured 1.2 μs after flashing in air at 355 nm, are shown in Fig. 3. The minima corresponded closely to the ground state absorption peaks of the sensitizers, while the broad triplet absorption peaks were at about 470 nm for $ZnPcS_4$ and 610 nm for $ZnNcS_4$; all triplet decays were measured at these wavelengths. For $ZnNcS_3$ the triplet peak is reported to be at 580 nm as measured in 100 mM CTAC [8]. The triplets of $ZnPcS_4$ and $ZnNcS_4$ decayed to the ground state by first-order processes in both oxygen and argon. This is in contrast with the behavior of some metallosilicon derivatives of Nc, in which the triplet decay in the presence of oxygen is biphasic owing to reversible energy transfer between the triplet and oxygen [18,19]. This can occur since the triplet energies of these Ncs are close to the triplet energy of $^1\Delta_g$ singlet oxygen. In the present work the triplet decays of both sensitizers were also first-order in the presence of azide, cysteine, FA, guanosine, histidine and tryptophan. However, with both sensitizers in the presence of 1,4-benzoquinone (BQ) there was an initial fast, first order decay followed by a very slow decay. Benzoquinone accepts an electron from the triplet states of some ZnPcs to give the radical cation of the Pcs [20,21]; thus the slowly decaying species observed in the present work may be radicals of this type.

The triplet lifetimes of the sensitizers in air and nitrogen and the bimolecular quenching constants for the triplets by oxygen, BQ and a variety of organic and biomolecules are listed in Table 1; the lifetime of the triplet Pc was about four times greater than that of the Nc. It has been found that the triplet lifetime of degassed Zn phthalocyanine trisulfonate ($ZnPcS_3$) is 180 μs and that for $ZnNcS_3$ is 115 μs; the triplet lifetimes of the corresponding aluminum derivatives are 500 and 200 μs respectively [8]. In the present work the quenching constant of oxygen for the $ZnPcS_4$ triplet was about six times greater than for the $ZnNcS_4$ triplet. In contrast, BQ quenched the triplets of both sensitizers with approximately the same efficiency (quenching constants of 4.2×10^8 and 5.8×10^8 M^{-1} s^{-1} for the Pc and Nc respectively, Table 1). The quenching constant of BQ for triplet ZnPc is 5×10^8 M^{-1} s^{-1}, as measured in aqueous dimethylacetamide [20]. The other compounds examined (Table 1) had quenching constants below 5×10^5 M^{-1} s^{-1}; thus at the concentrations used they would probably not compete effectively with oxygen or BQ for reaction with the triplet sensitizers.

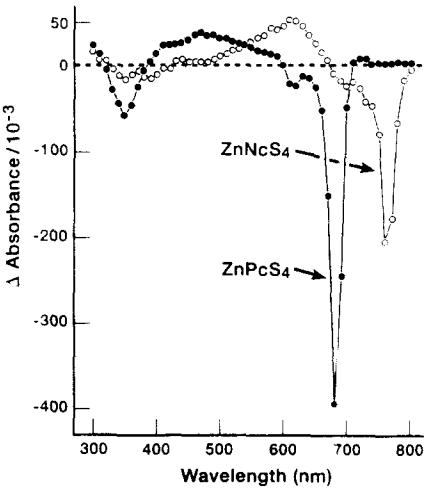

Fig. 3. Triplet–singlet difference spectra (10 nm resolution) of $ZnPcS_4$ and $ZnNcS_4$ as measured 1.2 μs after flashing at 355 nm. The reaction mixtures were 10 μM in sensitizer, 0.1 M in Na phosphate buffer of pH 7.4, 10 mM in cetylpyridinium chloride and 0.24 mM in oxygen (air saturated). The temperature was 25 °C.

Table 1
Lifetimes of triplet $ZnPcS_4$ and $ZnNcS_4$ as measured in air and under argon [a]. The bimolecular quenching constants of oxygen for the triplets are listed. Also, the quenching constants for 1,4-benzoquinone (BQ) and several other compounds as measured under argon are shown

	$ZnPcS_4$	$ZnNcS_4$
Triplet lifetime (μs) in air	2.9	18
Triplet lifetime (μs) under argon	490	110
Bimolecular quenching constant (M^{-1} s^{-1}) for O_2	1.2×10^9	2.0×10^8
Bimolecular quenching constant (M^{-1} s^{-1}) for BQ [b]	4.2×10^8	5.8×10^8

The bimolecular quenching constants for Na azide, cysteine, furfuryl alcohol, guanosine, histidine, methionine and tryptophan were below 5×10^5 M^{-1} s^{-1} as measured under argon

[a] The reaction mixtures were 10 μM in sensitizer, 0.1 M in Na phosphate buffer of pH 7.4 and 10 mM in cetylpyridinium chloride. The samples were flashed at 355 nm and the triplet decays were measured at 470 nm for $ZnPcS_4$ and 610 nm for $ZnNcS_4$. The temperature was 25 °C.

[b] For the initial fast decay component (see text).

3.4. Photogeneration of singlet oxygen

Singlet oxygen ($^1\Delta_g$), as measured by the near-IR (1.27 μm) emission of this species, was produced by both the Pc and the Nc following flash excitation in air-saturated D_2O containing B–CPC. Little IR emission was observed in the absence of the detergent. Emission was completely quenched by 10 mM Na azide, an effective quencher of singlet oxygen (quenching constant about $(2–3) \times 10^8\ M^{-1}\ s^{-1}$ [22]; data not shown). It is reported that four different silicon derivatives of Nc show the characteristic singlet oxygen emission on illumination in dimethylformamide; however, Zn 2,3-Nc does not [23].

The lifetime of singlet oxygen in D_2O containing B–CPC was found to be about 55 μs in the present experiments. The reported lifetimes in other detergents in D_2O (including brij 35, cetyltrimethylammonium bromide, sodium dodecyl sulfate and triton X-100) range from 32 to 57 μs [24]. Some Pcs and Ncs quench singlet oxygen efficiently [25]. However, in the present work the singlet oxygen lifetime of 55 μs observed in the presence of 5 μM $ZnPcS_4$ and $ZnNcS_4$ suggests that there was little quenching by these sensitizers under the conditions used.

The quantum yields of singlet oxygen generation by the illuminated Pc and Nc were estimated in comparison with that of rose bengal. The technique used involves the measurement of the quantum yields of oxygen uptake during the photo-oxidation of FA as a function of FA concentration. Furfuryl alcohol was used as substrate since it reacts chemically with singlet oxygen with good efficiency but physically quenches singlet oxygen with very low efficiency. It does not react with superoxide or hydrogen peroxide and does not appear to react with free radicals [12,14]. Fig. 4 shows the quantum yields of oxygen uptake with $ZnPcS_4$, $ZnNcS_4$ and rose bengal as a function of FA concentration: the yields leveled off at higher FA concentrations. At this point it is assumed that all the singlet oxygen produced is trapped by reaction with FA. Thus the ratios of the quantum yields of the photosensitized oxygen uptake at saturating FA concentrations will be the same as the ratios of the quantum yields of singlet oxygen generation by the sensitizers [12,14]. The quantum yield of singlet oxygen photogeneration by rose bengal is 0.75 [26]. Using this value, the yields for $ZnPcS_4$ and $ZnNcS_4$ were calculated from the data in Fig. 4 to be 0.70 and 0.25 respectively as measured in B–CPC. The yields measured in B were less than 0.005 (data not shown). On illumination in benzene, bis(tri-*n*-hexysiloxy)silicon 2,3-naphthalocyanine generates singlet oxygen with a quantum yield of 0.19 [18] and the yield with sulfonated Al and Zn naphthalocyanines in deuterated ethanol–water (9:1) is 0.3 [17]. Four different silicon derivatives of Nc were shown to generate singlet oxygen with quantum yields ranging from 0.22–0.38 when illuminated in dimethylformamide; however, Zn 2,3-Nc in the same solvent did not [23]. Many porphyrins and chlorins, when monomeric in solution, have

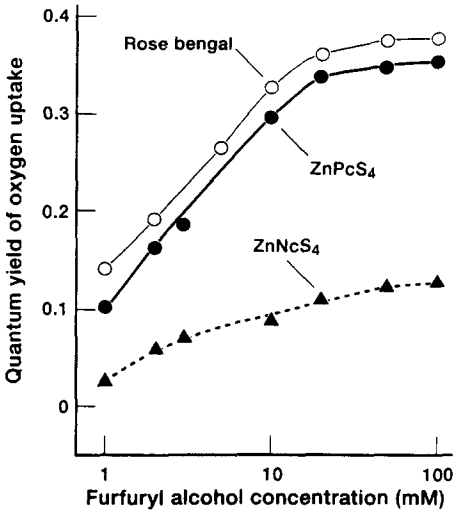

Fig. 4. Quantum yields of oxygen uptake during sensitized photo-oxidation of furfuryl alcohol as a function of FA concentration. The sensitizers were $ZnPcS_4$, $ZnNcS_4$ and rose bengal (as a standard). The reaction mixtures were 5 μM in sensitizer, 0.1 M in Na phosphate buffer of pH 7.4 and 10 mM in cetylpyridinium chloride with $ZnPcS_4$ and $ZnNcS_4$; rose bengal was in buffer without the detergent. The oxygen concentration was 0.22 mM (air saturated). Excitation was at the Q band peaks of the sensitizers. The temperature was 25 °C.

quantum yields of singlet oxygen production in the 0.4–0.8 range [12,14].

3.5. Kinetics of furfuryl alcohol photo-oxidation

The rate of oxygen uptake during the $ZnPcS_4$-sensitized photo-oxidation of FA in B–CPC was linear with time of illumination from 0.22 M oxygen (air saturated) down to a very low oxygen level. In contrast, the rate of oxygen uptake with $ZnNcS_4$ decreased progressively with time of illumination; this apparently results from the more rapid photobleaching of the Nc (see below). Furfuryl alcohol was not photo-oxidized appreciably with either sensitizer in the absence of detergent.

Azide effectively inhibited the photo-oxidation of FA; yields were decreased 50% by 0.50 and 0.48 mM azide with the Pc and Nc respectively. Since azide did not quench the sensitizer triplets appreciably (Table 1), this suggests that the photo-oxidation of FA was mediated by singlet oxygen. The BQ concentrations required to decrease the yields of FA photo-oxidation by 50% were 0.43 and 0.11 mM for $ZnPcS_4$ and $ZnNcS_4$ respectively. Since BQ does not react appreciably with singlet oxygen [27], this indicates that BQ probably inhibits FA photo-oxidation by quenching the sensitizer triplets, thus decreasing the efficiency of singlet oxygen generation (Table 1). The quenching constant of BQ for the $ZnNcS_4$ triplet is greater than that for ground state oxygen, whereas the converse is true for $ZnPcS_4$ (Table 1). This may account for the observation that BQ is a more effective inhibitor of the sensitized photo-oxidation of FA by the Nc than by the Pc.

The lifetime of singlet oxygen in D_2O and in detergent-containing D_2O is about 10–15 times longer than in H_2O [24]. With both sensitizers the quantum yields of oxygen uptake in D_2O containing B–CPC and a high concentration of FA (10 mM) increased only slightly (by about 20%) over those in H_2O, while at a low FA concentration (0.02 mM) the yields increased approximately eightfold. Thus the singlet oxygen lifetime under the reaction conditions used appears to be a rate-limiting factor only at low substrate concentrations.

Hydrogen peroxide was produced during the photo-oxidation of FA with both sensitizers. The peroxide yields, defined as the number of moles of peroxide accumulating divided by the number of moles of oxygen taken up, were 0.77 with $ZnPcS_4$ and 0.81 with $ZnNcS_4$. These values are similar to those observed with photosensitizing porphyrins [14].

3.6. Sensitized photo-oxidation of biomolecules

The quantum yields of oxygen uptake during the Pc- and Nc-sensitized photo-oxidation of several types of biomolecules that are known to be photo-oxidized with other types of sensitizers [28] are given in Table 2 in comparison with the data for FA. The yields were 2.4–3.0-fold greater with $ZnPcS_4$ than with $ZnNcS_4$. This correlates well with the observation that the quantum yield of singlet oxygen generation by the Pc is 2.8-fold greater than that of the Nc (Fig. 4). The photo-oxidation of the biomolecules as sensitized by both sensitizers (Table 2) was inhibited by low concentrations of azide, similarly to the inhibition of FA photo-oxidation as described above (data not shown). This suggests that under the reaction conditions used all the compounds listed in Table 2 were photo-oxidized largely via a singlet oxygen (type II) mechanism by both sensitizers. However, under some conditions free-radical (type I) mechanisms can also be involved. Triplet Pcs can efficiently donate electrons to some compounds, such as BQ, giving the radical cation of the Pc and the semireduced BQ radical [20,21]. Triplet Pcs can also accept electrons from substrates such as cysteine, giving the radical anion of the Pc plus the semioxidized cysteine radical [16]. These radicals might react with and alter biomolecules. Aromatic amino acids (tryptophan and tyrosine) in solution are oxidized by both type I and type II mechanisms with Al and Ga Pc tetrasulfonates as sensitizers [29]. Also, the photo-oxidation of 2′-deoxyguanosine (as a model for DNA) proceeds by both type I and type II mechanisms with di- and tetrasulfonated Al and Zn Pcs and tetrasulfonated Al Nc as sensitizers; the type II pathway predominates [30]. In general, Pc- and Nc-sensitized photo-oxidations in biological systems are probably mediated by singlet oxygen. However, at low oxygen concentrations and with the sensitizer closely associated with the biological substrate, type I mechanisms might become significant.

Table 2
Quantum yields of oxygen uptake during $ZnPcS_4$- and $ZnNcS_4$-sensitized photo-oxidation of furfuryl alcohol and selected biomolecules [a]

Substrate	Quantum yield of oxygen uptake	
	With $ZnPcS_4$	With $ZnNcS_4$
Furfuryl alcohol	0.16	0.058
Cysteine	0.056	0.022
Histidine	0.11	0.043
Methionine	0.033	0.014
Tryptophan	0.062	0.026
Guanosine	0.013	0.005

[a] The reaction mixtures were 5 μM in sensitizer, 0.1 M in Na phosphate buffer of pH 7.4, 10 mM in cetylpyridinium chloride, 0.22 mM in oxygen (air saturated) and 2.0 mM in substrate. The temperature was 25 °C.

3.7. Self-sensitized photobleaching of $ZnPcS_4$ and $ZnNcS_4$

Preliminary measurements were made of the initial quantum yields of the self-sensitized photobleaching of $ZnPcS_4$ and $ZnNcS_4$ (defined as the initial rate of disappearance of sensitizer molecules divided by the initial rate of absorption of photons). Absorption measurements were made at the Q band peaks. The sensitizers were illuminated at the Q band peaks in air-saturated B–CPC. $ZnPcS_4$ photobleached rather slowly, with a quantum yield of 1.7×10^{-5}, while $ZnNcS_4$ bleached about 250 times faster (quantum yield 4.2×10^{-3}). When illuminated in B, both sensitizers were about 100-fold more resistant to photobleaching than in B–CPC, suggesting that the sensitizer monomers are significantly more light sensitive than the aggregated forms.

The quantum yield of the photobleaching of $AlPcS_3$ is reported to be 1.1×10^{-6}, while that for $AlNcS_3$ is 3×10^{-3}, with illumination at the Q bands in air-saturated methanol–water (95:5 vol.%); thus the Nc in this homologous pair of Al derivatives is more than three orders of magnitude more sensitive to light than the Pc [8]. The yield for $AlPcS_3$ photobleaching in D_2O is 8.8-fold greater than in H_2O, suggesting the involvement of oxygen in the bleaching process [8]. Sulfonated Al Nc does not bleach on illumination at the Q band peak in oxygen-free 95% aqueous ethanol. In air, β-carotene, an efficient singlet oxygen quencher [22], inhibits the bleaching of sulfonated Zn Nc illuminated at the Q band. Again these studies indicate the participation of singlet oxygen in the bleaching process [9]. Also, tetrasulfonated palladium porphyrin (a singlet oxygen generator) sensitizes the photodecomposition of sulfonated Al and Zn Ncs in reactions that are inhibited by β-carotene [9].

These limited studies suggest that, as for porphyrins and chlorins [14,15], the mechanisms of the photobleaching of Pcs and Ncs are probably complex. Sensitizer bleaching can be an advantage or a disadvantage in the PDT of tumors. Too rapid bleaching could reduce the photosensitizer concentration to below the phototoxic level. Some photobleaching, however, might reduce the sensitizer concentration in the normal tissues around the tumor to below the phototoxic level, while phototoxic concentrations could still remain in

the tumor (reviewed in Ref. [14]). Thus, in evaluating new photosensitizers for therapy, it is important to examine their photobleaching behavior.

Acknowledgments

This work was supported in part by American Cancer Society Grant DHP-73A, the Medical Research Council of Canada and the Utah Laser Institute. The flash photolysis measurements were performed at the Center for Fast Kinetics Research (CFKR), University of Texas, Austin, with the much appreciated help of S.M. Hubig and S.J. Atherton; the Center is supported by the Biomedical Research Technology Program of the Division of Research Resources of the National Institute of Health (RR00886) and by the University of Texas. Some flash measurements were also carried out in the laboratory of M.A.J. Rodgers, Center for Photochemical Sciences, Bowling Green State University, Bowling Green, OH, with the kind assistance of A.J. McLean. Pat Meekins provided skilled technical assistance.

References

[1] B.W. Henderson and T.J. Dougherty (eds.), *Photodynamic Therapy*, Dekker, New York, 1992.
[2] J.E. van Lier and J.D. Spikes, in *Photosensitizing Compounds: Their Chemistry, Biology and Clinical Use*, Wiley, Chichester, 1989, p. 17.
[3] J.E. van Lier, in D. Kessel (ed.), *Photodynamic Therapy of Neoplastic Disease*, Vol. 1, CRC Press, Boca Raton, FL, 1990, p. 279.
[4] I. Rosenthal, *Photochem. Photobiol., 53* (1991) 859.
[5] E. Ben-Hur, in B.W. Henderson and T.J. Dougherty (eds.), *Photodynamic Therapy*, Dekker, New York, 1992, p. 63.
[6] B. Paquette and J.E. van Lier, in B.W. Henderson and T.J. Dougherty (eds.), *Photodynamic Therapy*, Dekker, New York, 1992, p. 145.
[7] N. Brasseur, T.-L. Nguyen, R. Langlois, R. Ouellet, S. Marengo, D. Houde and J.E. van Lier, *J. Med. Chem., 37* (1994) 415.
[8] I. McCubbin and D. Phillips, *J. Photochem., 34* (1986) 187.
[9] N.C. Yates, in *NATO ASI Ser., Ser. H, 15 (Photosensitisation)*, Springer, Berlin, 1988, p. 365.
[10] B. Paquette, H. Ali, R. Langlois and J.E. van Lier, *Photochem. Photobiol., 51* (1990) 313.
[11] C.R. Lambert, E. Reddi, J.D. Spikes, M.A.J. Rodgers and G. Jori, *Photochem. Photobiol., 44* (1986) 595.
[12] J.D. Spikes, N.L. Krinick and J. Kopeček, *J. Photochem. Photobiol. A: Chem., 70* (1993) 163.
[13] Y. Nishimura, Y. Kaneko, T. Arai, H. Sakuragi, K. Tokumaru, M. Kiten, S. Yamamura and D. Matsunaga, *Chem. Lett.*, (1990) 1935.
[14] J.D. Spikes, *Photochem. Photobiol., 55* (1992) 797.
[15] J.D. Spikes and J.C. Bommer, *Photochem. Photobiol., 58* (1993) 346.
[16] J.R. Darwent, P. Douglas, A. Harriman, G. Porter and M.-C. Richoux, *Coord. Chem. Rev., 44* (1982) 83.
[17] N.C. Yates, J. Moan and A. Western, *J. Photochem. Photobiol. B: Biol., 4* (1990) 379.
[18] P.A. Firey, W.E. Ford, J.R. Sounik, M.E. Kenney and M.A.J. Rodgers, *J. Am. Chem. Soc., 110* (1988) 7626.
[19] W.E. Ford, B.D. Rihter and M.A.J. Rodgers, *J. Am. Chem. Soc., 111* (1989) 2362.
[20] T. Ohno, S. Kato and N.N. Lichtin, *Bull. Chem. Soc. Jpn., 55* (1982) 2753.
[21] M.E. Daraio, P.F. Aramendia and E. San Roman, *J. Photochem. Photobiol. A: Chem., 77* (1994) 41.
[22] B.M. Monroe, in A.A. Frimer (ed.), *Singlet O_2*, Vol. I, CRC Press, Boca Raton, FL, 1985, p. 177.
[23] S. Marengo, D. Houde, N. Brasseur, T.L. Nguyen, R. Ouellet and J.E. van Lier, *J. Chim. Phys., 91* (1994) 1211.
[24] A.A. Gorman and M.A.J. Rodgers, in J.C. Scaiano (ed.), *Handbook of Organic Photochemistry*, Vol. II, CRC Press, Boca Raton, FL, 1989, p. 229.
[25] A.A. Krasnovsky Jr., M.A.J. Rodgers, M.G. Galpern, B. Rihter, M.E. Kenney and E.A. Lukjanetz, *Photochem. Photobiol., 55* (1992) 691.
[26] E. Gandin, Y. Lion and A. Van de Vorst, *Photochem. Photobiol., 37* (1983) 271.
[27] M.-T. Maurette, E. Oliveros, P.P. Infelta, K. Ramsteiner and A.M. Braun, *Helv. Chim. Acta, 66* (1983) 722.
[28] R.C. Straight and J.D. Spikes, in A.A. Frimer (ed.), *Singlet O_2*, Vol. IV, CRC Press, Boca Raton, FL, 1985, p. 91.
[29] G. Ferraudi, G.A. Arguello, H. Ali and J.E. van Lier, *Photochem. Photobiol., 47* (1988) 657.
[30] J.-L. Ravanat, M. Berger, F. Benard, R. Langlois, R. Ouellet, J.E. van Lier and J. Cadet, *Photochem. Photobiol., 55* (1992) 809.