Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 4829–4835
Bioorganic & Medicinal Chemistry

Photodynamic activity of water-soluble phthalocyanine zinc(II) complexes against pathogenic microorganisms

Vanya Mantareva,[a,*] Veselin Kussovski,[b] Ivan Angelov,[a] Ekaterina Borisova,[c] Latchezar Avramov,[c] Günter Schnurpfeil[d] and Dieter Wöhrle[d]

[a]*Institute of Organic Chemistry, Bulgarian Academy of Sciences, ''Acad. G. Bonchev'' str, Bl. 9, 1113 Sofia, Bulgaria*
[b]*The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, ''Acad. G. Bonchev'' str, Bl. 26, 1113 Sofia, Bulgaria*
[c]*Institute of Electronics, Bulgarian Academy of Sciences 72, Tsarigradsko chaussee Blvd., 1784 Sofia, Bulgaria*
[d]*Institute of Organic and Macromolecular Chemistry, University of Bremen, PO Box 330 440, 28334 Bremen, Germany*

Received 9 March 2007; revised 19 April 2007; accepted 27 April 2007
Available online 6 May 2007

Abstract—Photodynamic activity of tetrakis-(3-methylpyridyloxy)- and tetrakis-(4-sulfophenoxy)-phthalocyanine zinc(II) toward the gram-positive *Staphylococcus aureus*, the gram-negative *Pseudomonas aeruginosa*, and the fungi *Candida albicans* was studied. The drug uptake dependency with an inverse behavior to the cell density was observed. The cationic photosensitizer completely inactivated *S. aureus* and *C. albicans*, and with $4\log_{10}$ *P. aeruginosa*. The photoinactivation at mild experimental conditions, such as drug dose of 1.5 μM and fluence of 50 mW cm^{-2} for 10 min irradiation time, was shown.

1. Introduction

The widespread occurrence of antibiotic-resistant microorganisms has necessitated considerable need for new antimicrobial modality treatment. Photodynamic therapy (PDT) as a method utilizes visible or ultraviolet light in combination with a photosensitizing agent to induce several phototoxic reactions, which results in cell damage or death.[1] Photodynamic reaction involves a light absorption by a photosensitizer to excite the molecule to the excited singlet state. This excited state undergoes intersystem crossing to the long-lived triplet state, which can react with molecular oxygen inducing reactive species such as singlet oxygen, superoxide, and radicals.[2] These reactive species can oxidize the surrounding bioorganic molecules, such as proteins, nucleic acids, lipids, leading to cell death. The photodynamic activity is determined by the photophysical and photochemical properties of the photoreactive dye including: (1) the lipophilicity and ionization, (2) the molecular extinction coefficient, (3) the quantum yield of triplet state formation, (4) the quantum yield of singlet oxygen formation if the reaction occurs by the type II pathway, and (5) the redox potentials of the excited states, which is important for the type I mechanism.[3]

PDT appears as a promising technique against bacteria, viruses, and fungi, and for the local treatment of infections.[4–6] Progressive development of a large number of synthetic and natural compounds for antimicrobial photodynamic therapy has occurred.[7] The first published results more than a century ago described acridine hydrochloride for the photoinactivation of microorganisms.[6,8] The phenothiazine dyes as methylene blue and toluidine blue showed promising results with bacteria and fungi.[9] Several cyanine dyes like pyrvinium and stilbazium, and merocyanines were studied for the photoinactivation of microbes in plasma and serum.[10,11] The photosensitizers with highly conjugated molecules like porphyrins and phthalocyanines possess improved optical properties.[12] Phthalocyanines, which are characterized with far red wavelength absorption (>670 nm), long triplet life time (~1 ms), and high quantum yields of singlet oxygen generation (>0.2), have been studied as drugs in microbial photodynamic inactivation.[13] An additional important factor for a good photodynamic response is drug uptake into cell originates.[14] It is determined by the charge and hydrophobicity balance of the photosensitizers. The macrocycles have flexible structures to the substitution and by addition of suitable

Keywords: Photodynamic inactivation; Phthalocyanines; Light fluence; Bacteria and fungi.
* Corresponding author. Tel.: +359 2 9606 181; fax: +359 2 8700 225; e-mail: mantareva@yahoo.com

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.04.069

substituents an improved accumulation and photodynamic activity can result.[15]

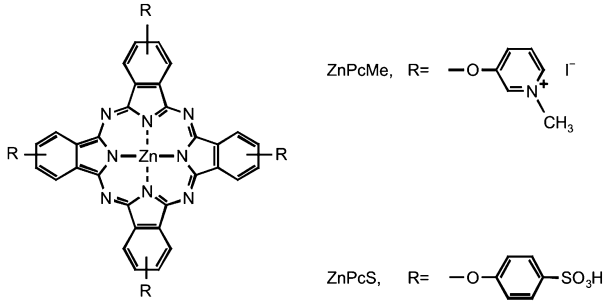
The positively charged phthalocyanines can effectively photoinactivate both, gram-positive and gram-negative bacteria.[16] The gram-positive *Staphylococcus aureus* are more susceptible to photoinactivation than gram-negative *Pseudomonas aeruginosa* due to the morphology characteristics of their membranes.[17,18] The yeast *Candida albicans* has a nuclear membrane. It was observed that the photosensitizer bound to the cell envelope and none or very little entered the cell. Thus, resulting in some resistance to the photoinactivation.[19,20] By modifying the treatment conditions such as the incubation time, surrounding solvents or detergents, drug dose, fluence rate, and light energy the photoinactivation of the resistant species can be enhanced.

The photodynamic activity of two new water-soluble phthalocyanines, one cationic tetrakis-(3-methylpyridyloxy)- and one anionic tetrakis-(4-sulfophenoxy)-phthalocyanine zinc(II), was evaluated toward the representative strains of the three main groups of human pathogens: the gram-positive *S. aureus* MS (methicillin-sensitive), *S. aureus* MR (methicillin-resistant), the gram-negative *P. aeruginosa*, and the fungi *C. albicans*. The drug uptake into the microorganisms in dependence on the cell density was evaluated. The photodynamic inactivation at different treatment conditions such as drug dose, cell–drug binding, cell density, light fluence, and irradiation time was studied.

2. Results

2.1. Phthalocyanines

Phthalocyanine zinc(II) complexes (ZnPcs) with different charges were evaluated as photodynamic agents for bacteria and fungi (Scheme 1). The diamagnetic metal ions in a closed-shell arrangement such as zinc(II) determine a high fluorescence quantum yield (0.3) and long triplet lifetimes (1 ms) with high triplet quantum yields (0.6) and therefore a high probability of energy or electron transfer, as was confirmed in our previous work.[21–24] The hydrophilic character of ZnPcs was determined by distribution coefficients of ZnPcs between octanol-1 and 0.01 M Tris-buffer, pH 7.0.[25–27] The anionic ZnPcs and the cationic ZnPcMe are well soluble in water and can easily aggregate in aqueous solutions.[21–23] The monomer formation has concentration dependence in aqua media and both ZnPcs exhibit monomer/dimer equilibrium. The aggregation can be reduced by use of a carrier system or by addition of detergents.[21] DMSO dissolves well all studied compounds and in the monomer state.[21,24]



Scheme 1. Molecular structures of substituted Zn(II)-phthalocyanines.

2.2. Phthalocyanine uptake by microorganisms

The amount of photosensitizer bound to the microbial cells with densities between 10^5 and 10^9 cells/mL by using the fluorescence spectroscopy setup was evaluated.[28] The fluorescence signal of the organic extracts from the cells was recorded for the spectral range 650–800 nm after excitation at $\lambda = 635$ nm. It was possible to determine quantitatively the phthalocyanines at a low therapeutic concentration of 1.5 μM. Figure 1a and b present the uptake of ZnPcMe and ZnPcS for methicillin-resistant *S. aureus*, *P. aeruginosa*, and for *C. albicans* at different cell densities. The phthalocyanine bound to the bacterial cells and the fungi decreased with increasing of cell density.

The uptake by methicillin-resistant *S. aureus* was two orders higher compared to *P. aeruginosa* and one order compared to the fungi *C. albicans*. An inverse dependence of the drug accumulation on the cell densities was determined. There was not any charge dependency on the uptake of the studied compound.

2.3. Photoinactivation at different cell densities

The photodynamic inactivation with the cationic ZnPcMe and the anionic ZnPcS of the studied microorganisms with different cell densities was compared (Fig. 2). Both *S. aureus* strains and *C. albicans* were successfully treated with the result of no survival at cell densities between 10^6 and 10^8 cells/mL and 6 μM ZnPcMe (Fig. 2a). In the case of *P. aeruginosa* at higher cell concentrations (10^7 and 10^8 cells/mL) the survival fraction dropped to $6\log_{10}$ and $4\log_{10}$, respectively. In contrast, the anionic ZnPcS with the same drug concentration (6 μM) was not sufficient to photoinactivate the gram-negative *P. aeruginosa* (Fig. 2b). For the yeast *C. albicans* the photoinactivated cells were only $1–2\log_{10}$, when treated with ZnPcS (Fig. 2b). The methicillin-resistant *S. aureus* treated with ZnPcS was deactivated less than $4\log_{10}$ for all tested cell densities, and $4\log_{10}$ for *S. aureus*, MS at the lowest cell density (10^6 cells/mL). The effect was negligible at higher cell densities of the treated species with the exception of the resistant strain *S. aureus* ($4\log_{10}$).

The dark toxicity of the photosensitizers was negligible at the used dye concentration ($<0.5\log_{10}$ decrease of viable cells). The experimental results for all three different control groups were comparable ($<0.5\log_{10}$ decrease of viable cells).

2.4. Light dose effect on photoinactivation

The photoinactivation with ZnPcMe was evaluated for different irradiation times after bacterial suspensions

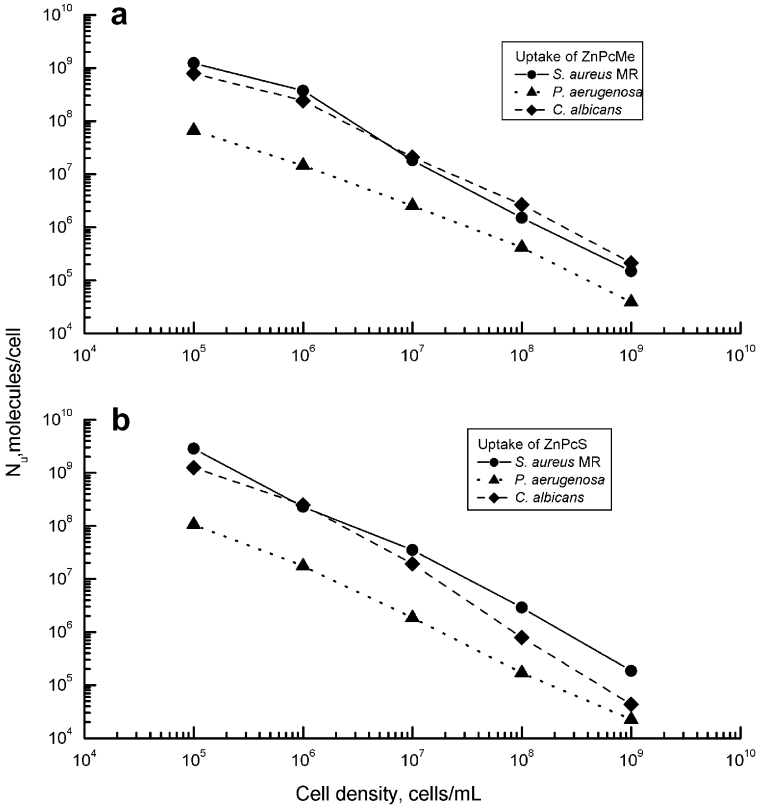


Figure 1. The number of phthalocyanine molecules bound to the microorganism's cells with different cell densities and incubated for 10 min with 1.5 μM ZnPcs: (a) ZnPcMe and (b) ZnPcS.

were washed out to remove the unbound photosensitizer (Fig. 3). As can be seen, the therapeutic response after washing was lower in comparison to the unwashed bacteria of *S. aureus*. This result suggests that the molecules non-bound to the cells can result in the bacterial photoinactivation after irradiation.

The photoinactivation of the microorganism was determined for different irradiation times, respectively, the various light doses (12, 30, and 60 J cm^{-2}). The complete photoinactivation with the cationic ZnPcMe even for the low light dose applied to *S. aureus* strains was observed (Fig. 4a). In case of the gram-negative bacteria treated with ZnPcMe at 30 J cm^{-2} a photoinactivation of 4–5 $\log_{10}$ was observed and at a dose of 60 J cm^{-2} it was 5–6 $\log_{10}$ (Fig. 4a). *C. albicans* treated with ZnPcMe at light dose 30 J cm^{-2} was fully photoinactivated. The anionic ZnPcS was active only at a dose of 60 J cm^{-2} and an effect of 3–4 $\log_{10}$ photoinactivation for *S. aureus*, MR was obtained (Fig. 4b). *P. aeruginosa* treated with ZnPcS at light dose 60 J cm^{-2} was not photoinactivated. The similar results were observed for *C. albicans* after ZnPcS treatment (Fig. 4b).

In summary, the use of the cationic ZnPcMe is preferable for the photoinactivation of wide range of different pathogenic microorganisms compared to the anionic ZnPcS. With exception of *P. aeruginosa* a total cell killing was achieved. A notable effect for *P. aeruginosa* was seen at low cell density and high light dose.

3. Discussion

Phthalocyanine zinc(II) complexes bearing different substituents (Scheme 1) were employed to evaluate the efficiency of the photodynamic inactivation of selected three classes of pathogenic microorganisms. The studied phthalocyanines exhibit the Q-band absorption at $\lambda = 675$ nm and the fluorescence maximum in DMSO at $\lambda = 690$ nm.[21,24,28] The quantum yield of singlet oxygen generation as was previously obtained by us, following the energy transfer from the excited triplet state as reactive species, was of the order of 0.55 and independent on the substituents.[21–23]

Representative strains of major human pathogens like the gram-positive and the gram-negative bacteria, and the yeast were studied. The uptake of photosensitizers by microorganisms is an important feature for the efficacy of photoinactivation.[29] The phthalocyanine uptake from the studied microorganisms was determined (Fig. 1). The results showed that the cell–drug binding for the bacteria and the fungi decreases with increasing cell density. The electronic charges of the studied phthalocyanines seem to be of not great importance. Antimicrobials like cationic phthalocyanines can bind well to bacteria walls.[30] In the case of ZnPcMe for *S. aureus* the uptake was similar to *C. albicans*, and two orders more than *P. aeruginosa* (Fig. 1a). The uptake for the yeast *C. albicans* is comparable to the bacteria *S. aureus*, but different mechanism of the photosensitizer's influx and target organelles upon irradiation occurs.[19] The found

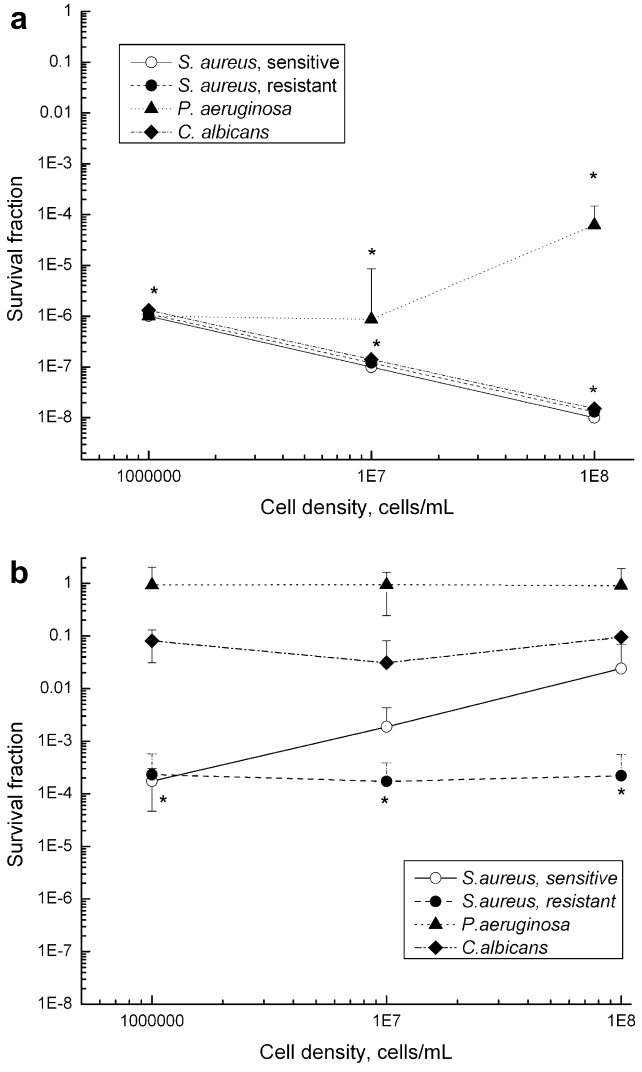


Figure 2. The photoinactivation of microorganisms with different cell densities irradiated with a fluence rate of 100 mW cm^{-2} for 10 min after 10-min incubation with 6 μM ZnPcs: (a) ZnPcMe and (b) ZnPcS (*$P < 0.05$, compared with untreated bacteria).

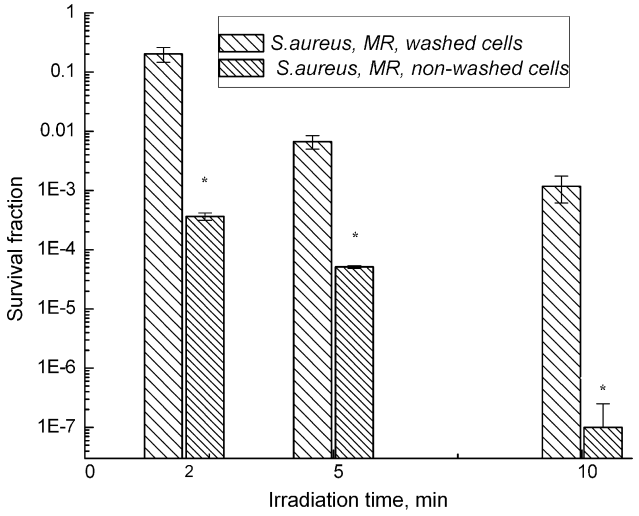


Figure 3. Photosensitizer-cell binding dependency on the photoinactivation of *S. aureus*, methicillin-resistant (10^7 cell/mL) after 10 min incubation with 1.5 μM ZnPcMe and irradiation with a fluence rate of 50 mW cm^2 for different times, respectively, the light doses (*$P < 0.05$, compared with untreated bacteria).

low amount of ZnPcMe in *P. aeruginosa* was apparently non-sufficient to cause a significant photoinactivation ($4\log_{10}$) at high light dose (60 J cm^{-2}). ZnPcS was taken up from the gram-negative bacteria cells a half of the amount compared to the uptake from gram-positive bacteria and fungi (Fig. 1b).

The photoinactivation behaviors of the bacteria *S. aureus* and *P. aeruginosa* are in agreement with the differences in their accumulation behavior in case of cationic ZnPcMe (Figs. 1a and 2a).

The most microbial species demonstrate two mechanisms of drug uptake: (1) a diffusion-controlled process and (2) an affinity-mediated binding.[18] The accumulation behavior of ZnPcMe and ZnPcS suggests that both mechanisms are involved. This was proved by washing of the unbound excess of ZnPcMe from the resistant *S. aureus.* The effect of bacterial photoinactivation was significantly decreased for all irradiation times (Fig. 3). Obviously, the unbound phthalocyanine produces after irradiation extracellular singlet oxygen, which is sufficient to cause a significant effect of the decrease of the survival cells.

The other authors pointed out that based on the interaction of photosensitizers poly-L-lysine chlorine (e6) conjugate, toluidine blue O, and rose bengal with bacteria *S. aureus, Escherichia coli* and the yeast *C. albicans*, three possibilities exist: tightly bound and penetrate into cells, only weakly attached, and non-bound.[18] Thus suggested that microbial cells have to compete for the necessary amount of photosensitizer, and therefore the cell density is an important factor. The cell density dependence was observed only in the case of *P. aeruginosa* treated with ZnPcMe (Fig. 2a). This can be due to the limited susceptibility of this strain to photoinactivation.[17] The other microorganisms were fully inactivated (Fig. 2a). In case of ZnPcS the photoinactivation was 3–$4\log_{10}$ for *S. aureus*, methicillin-resistant at all cell densities (Fig. 2b). The increasing of the cell density resulted in decreasing of the photoinactivated cells for *S. aureus*, sensitive (Fig. 2b). Probably, there is a charge dependence on the effect for the gram-positive and gram-negative bacteria treated with cationic or anionic photosensitizer.

The microbial photoinactivation dependence on the light dose was examined (Fig. 4). The threshold light dose for the bacteria and fungi was determined. For example, the resistant strains like *P. aeruginosa* and *C. albicans* irradiated in the range from 12 to 60 J cm^{-2} showed decreasing in survival ratios from $4\log_{10}$ and $3\log_{10}$ to $6\log_{10}$ and $7\log_{10}$ (Fig. 4a). Obviously, a much higher concentration of the excited dye was needed for the effective photoinactivation of both the resistant microorganisms. An exception was *S. aureus* treated with ZnPcMe with no surviving cells found at low light

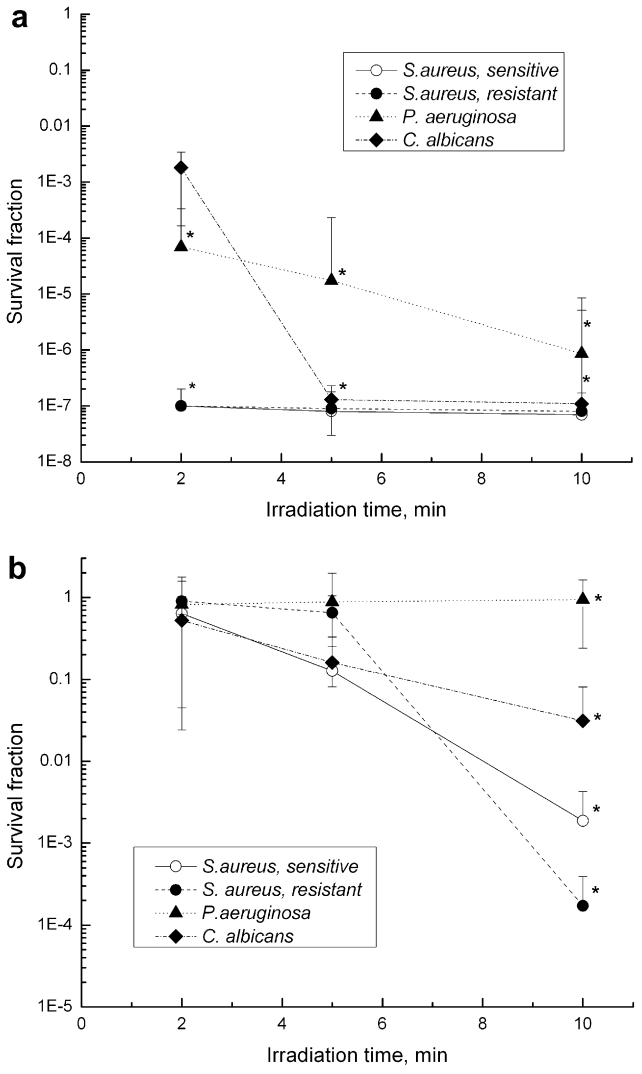


Figure 4. Effect of the irradiation time at fluence rate of 100 mW cm^{-2} on the survival of the microorganisms (10^7 cell/mL) after 10-min incubation with 6 μM ZnPcs: (a) ZnPcMe and (b) ZnPcS (*$P < 0.05$, compared with untreated microorganisms).

dose (12 J cm^{-2}). The light dose dependence was pronounced for all bacterial species when treated with the anionic ZnPcS, with the exception of *P. aeruginosa* because of the lack of any photokilling (Fig. 4b). The bacteria and the fungi treated with ZnPcS at light dose of 12 J cm^{-2} were not deactivated. At fluence rate of 100 mW cm^{-2} and an irradiance of 60 J cm^{-2} ZnPcS-inactivated both the *S. aureus* with 3–4 $\log_{10}$ but could not inactivate *C. albicans*. The higher light fluences can induce a light toxic effect, which was tested on the controls without photosensitizer.

In summary, it is pointed out that an efficient photosensitizer is capable to give a high photodynamic response even at high cell density and low light dose. This allows quantitative evaluation of the photodynamic effect after treatment of pathogenic microorganisms with different densities.

Size and morphology of bacteria and fungi influence the photoinactivation.[18,20] The great size of fungi (3–5 μm) compared to bacteria (0.5–1 μm) means that more singlet oxygen is needed for the photokilling of morphologically convoluted fungi.[20] *C. albicans* was efficiently photoinactivated with the cationic ZnPcMe (Figs. 2a), as a result of a good penetration of the positively charged photosensitizer to the fungi during irradiation as assumed.[19] A mechanistic study suggests that an active binding on the cell enveloped of *C. albicans* that caused the membrane damages upon irradiation and a massive influx of drug into the cells happened.[19]

The externally generated reactive species can induce cell damages to the outer membrane, which are significant for the effect as was shown (Fig. 3). The outer membrane is preventing the penetration of various compounds into the cells of gram-negative type bacteria.[18] Possibly, extracellular oxygen or other reactive species are responsible for the effective photoinactivation of *P. aeruginosa* (Figs. 2a and 4a). It was photoinactivated with the cationic ZnPcMe at light dose 60 J cm^{-2} (Fig. 4a) and there was not any inactivation with the anionic ZnPcS (Fig. 4b). The resistance of gram-negative species to the photosensitizing action of neutral and anionic porphyrinoids was explained with the rigid characteristic of the outer membrane located outside the peptidoglycan compartment of the wall.[20] Obviously, two orders lower drug uptake into *P. aeruginosa* compared to the other studied microorganisms leads to only 4 $\log_{10}$ photoinactivation with ZnPcMe (Figs. 1a and 2a). Nevertheless both photosensitizers showed similar uptake after 10-min incubation, the photodynamic effect was different. These observations assumed different accumulation behaviors of the opposite charged molecules (ZnPcMe and ZnPcS) into gram-negative bacteria upon irradiation.

4. Conclusions

The efficacy of PDT with tetrakis-(3-methylpyridyloxy)- and tetrakis-(4-sulfophenoxy)-phthalocyanine zinc(II) on the studied microorganisms depends on the cell density, the light dose, and the drug uptake. The cell-photosensitizer binding before irradiation depends on the cell density. The gram-negative *P. aeruginosa* can accumulate two order lower amounts of the studied photosensitizers compared to the *S. aureus* and *C. albicans*. There was not observed any charge dependence of the drug on the uptake. Photodynamic response for *S. aureus* showed full photoinactivation with the cationic ZnPcMe (1.5 μM) at fluence rate 50 mW cm^{-2} after 10-min irradiation. The gram-negative *P. aeruginosa*, and the yeast *C. albicans* can be effectively treated with ZnPcMe, but at high concentration (6 μM) and more powerful light (100 mW cm^{-2}). The anionic ZnPcS is effective for the studied bacteria and fungi, but at high drug concentration (6 μM) and light energy (60 J cm^{-2}). The gram-positive bacteria *S. aureus*, the gram-negative bacteria *P. aeruginosa* and the yeast *C. albicans* can be effectively photodynamically inactivated with the cationic tetrakis(3-methylpyridyloxy) phthalocyanine zinc(II). These results are encouraging for further investigation of the

proposed phthalocyanine for clinical PDT of bacterial infections in humans.

5. Experimental

5.1. Photosensitizers and other chemicals

The phthalocyanine zinc(II) complexes (Scheme 1), i.e., 2,9,16,23-tetrakis(3-methylpyridyloxy)phthalocyanine zinc(II) (ZnPcMe) and 2,9,16,23-tetrakis(4-sulfophenoxy)phthalocyanine zinc(II) (ZnPcS), were prepared by cyclotetramerization of the respective phthalonitriles according to the synthetical procedures previously described by Woehrle et al.[21–23] All chemicals from commercial sources were of analytical or spectroscopic grade. The organic solvents were dried and distilled before use. Stock solutions of the studied phthalocyanines (1 mM) were prepared in DMSO and stored refrigerated in the dark. The dilutions to the proper concentrations (1.5 or 6 μM) were performed in 0.01 M phosphate-buffered saline (PBS), pH 7.4, prior to the experiments. The absorbance was monitored on Shimadzu UV–vis 3000 spectrophotometer (Japan).

5.2. Bacterial strains

The following microorganisms were selected: the bacteria gram-positive *S. aureus* 1337, methicillin-resistant strain (MRSA) from the Collection of the Institute of Microbiology, Bulgarian Academy of Sciences, and *S. aureus* 509, methicillin-susceptible strain (MSSA) and gram-negative *P. aeruginosa* 1390, and the yeast *C. albicans* 74 from the National bank for industrial microorganisms and cell cultures (NBIMCC) Bulgaria. Brain heart infusion broth (Difco, BD Diagnostic Systems, Sparks, MD) was used for *S. aureus* and *P. aeruginosa*. YM medium (Difco) was used for *C. albicans*. All strains were grown aerobically overnight at 37 °C. Cells were harvested by centrifugation and were resuspended in sterile PBS. Prior to the experiments, they were diluted to cell densities of 10^9, 10^8, 10^7, 10^6, and 10^5 cells/mL.

5.3. Light sources

Irradiation of the microorganisms was carried out with an argon-pumped dye laser system (Spectra Physics, USA) at the absorption maximum of phthalocyanines (675 nm). The bacterial suspensions were placed in 1 cm disposable cuvette into thermostated cell holder at 37 °C and were magnetically stirred during illumination. The fluence rates were 100 and 50 mW cm^{-2} and were controlled with photometer equipment (Spectra Physics, USA). A red LED (Lumileads, USA) at 635 nm was used as an excitation source for fluorescence measurements.

5.4. Phthalocyanine binding to cells

The cells with densities of 10^9, 10^8, 10^7, 10^6, and 10^5 cells/mL were incubated with ZnPcMe and ZnPcS (1.5 μM) for 10 min in the dark at 37 °C by gentle stirring. The supernatants were removed and the unbound photosensitizer was washed out with PBS. After three times washing with PBS, the cells were resuspended in THF:aqueous 2% SDS (1:9) and kept in the dark. Then the samples were centrifuged, and the collected extracts were examined by fluorescence analysis. The fluorescence experimental set-up built-in from a red LED as excitation source at $\lambda = 635$ nm was previously described.[31] Fluorescence signals were obtained by collection fibers adjusted to a 90° position toward the excitation beam. The fluorescence spectra were recorded by using a fibro-optic microspectrometer (Ocean Optics Inc, USA). The detector was a high-sensitive 4096 element linear CCD-array with 600 lines/mm grating. The calibration lines were plotted for the samples with known ZnPcs concentrations. The changes in the intensity of the fluorescence signals between solutions in organic solvent and supernatants collected after incubation and cell's washing were used for indication of phthalocyanine binding to the bacteria (Fig. 5). The amount of phthalocyanine molecules bound to the bacteria or fungi was determined by using Avogadro's number for calculation of the number of Pc-molecules per cell at the respective cell density.

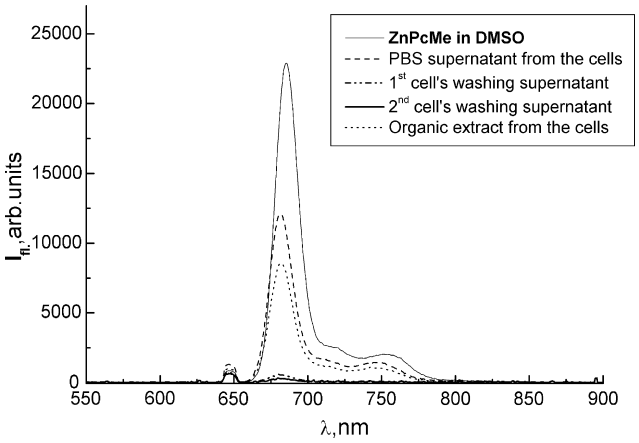


Figure 5. Fluorescence spectra of the samples taken after 10-min incubation with 1.5 μM ZnPcMe of *S. aureus*, MR (the conditions described in the inserted box) at excitation with 635 nm.

5.5. In vitro photodynamic inactivation

Bacterial suspensions with different cell densities (10^6, 10^7, 10^8, and 10^9 cells/mL) were prepared by serial dilutions with PBS. Samples of 1 mL containing the cells were incubated for 10 min with 5 μL of photosensitizer stock solution to final concentrations of 1.5 or 6 μM ZnPcs at 37 °C by gentle stirring. Then the samples were irradiated at the Q-band maximum of ZnPcs with different light doses. At fluence rate 50 mW cm^{-2} and for different exposure times were reached the light doses of 12, 30, and 60 J cm^{-2}. The control experiments with the microbial cells were as follows: (1) without photosensitizer, but illuminated, (2) with photosensitizer, but no light, and (3) only suspension (no photosensitizer, no light). After irradiation 0.1 ml samples were taken off and serially diluted (10-fold) with PBS. Aliquots were spread over agar plates made from appropriate media.

The number of colonies on each plate was counted following 24-h incubation at 37 °C for the bacterial strains and after 48-h incubation for the *C. albicans*. The survival fraction was calculated as a ratio of the PDI treated toward means of the control groups 1–3. Each experiment was carried out three times.

5.6. Statistics

Survival values are expressed as means ± standard deviation (SD). The difference between two means was compared by a two-tailed unpaired Student's test. *P* values of <0.05 were considered as significant.

Acknowledgment

This work was supported by the National Science Fund, Ministry of Education and Science (Grant B1534/05), Sofia, Bulgaria.

References and notes

1. Henderson, B. W.; Dougherty, T. J. *Photochem. Photobiol.* **1992**, *55*, 145.
2. Nunes, S. M. T.; Sguilla, F. S.; Tedesko, A. C. *Br. J. Med. Biol. Res.* **2004**, *37*, 273.
3. Jori, G.; Fabris, C.; Soncin, M.; Ferro, S.; Coppellotti, O.; Dei, D.; Fantelli, L.; Chiti, G.; Roncucci, G. *Lasers Surg. Med.* **2006**, *38*, 468.
4. Wainwright, M. *J. Antimicrob. Chemother.* **1998**, *42*, 13.
5. Hamblin, M. R.; Hasan, T. *Photochem. Photobiol. Sci.* **2004**, *3*, 436.
6. Raab, O. *Z. Biol.* **1900**, *39*, 524.
7. Jori, G. *Environ. Toxicol. Oncol.* **2006**, *25*, 505.
8. Tappeiner, HvJ. A. *Műnch. Med. Wochenschr.* **1903**, *50*, 2041.
9. Wainwright, M.; Phoenix, D. A.; Laycock, S. L.; Wareing, D. R. A.; Wright, P. A. *FEMS Microbiol. Lett.* **1998**, *160*, 177.
10. Anderson, G. S.; Gunter, W. H. H.; Searle, R. *Photochem. Photobiol.* **1996**, *64*, 683.
11. Neyndorff, H. C.; Barel, L. D.; Turafo, F.; Levy, G. J. *Transfusion* **1990**, *30*, 485.
12. Stenberg, E. D.; Dolphin, D.; Brückner, C. *Tetrahedron* **1998**, *54*, 4151.
13. Jori, G.; Tonlorenzi, D. *Photodyn. News* **1999**, *2*, 2.
14. Hamblin, M. R.; O'Donnell, D. A.; Murthy, N.; Contag, C. H.; Hasan, T. *J. Antimicrob. Chemother.* **2002**, *49*, 941.
15. Minnock, A.; Vernon, D. I.; Schofield, J.; Griffiths, J.; Parish, J. H.; Brown, S. B. *J. Photochem. Photobiol. B: Biol.* **1996**, *32*, 159.
16. Lambrechts, S. A. G.; Aalders, M. C. G.; Langeveld-Klerks, D. H.; Khajali, Y.; Lagerberg, J. W. M. *Photochem. Photobiol.* **2003**, *79*, 297.
17. Merchat, M.; Bertolini, P.; Giacomini, P.; Villanueva, A.; Jori, G. *J. Photochem. Photobiol. B: Biol.* **1996**, *32*, 153.
18. Demidova, T. N.; Hamblin, M. R. *Antimicrob. Agents Chemother.* **2005**, *49*, 2329.
19. Lambrechts, S. A. G.; Aalders, M. C. G.; van Marle, J. *Antimicrob. Agents Chemother.* **2005**, *49*, 2026.
20. Bliss, J. M.; Bigelov, E. C.; Foster, H. T.; Haidaris, G. C. *Antimicrob. Agents Chemother.* **2004**, *48*, 2000.
21. Wöhrle, D.; Iskander, N.; Graschew, G.; Sinn, H.; Friedrich, E. A.; Maier-Borst, W.; Stern, J.; Schlag, P. *Photochem. Photobiol* **1990**, *51*, 351.
22. Schneider, G.; Wöhrle, D.; Spiller, W.; Stark, J.; Schulz-Ekloff, G. *Photochem. Photobiol.* **1994**, *60*, 333.
23. Nazeeruddin, M.; Humphry, R.; Grätzel, M.; Wöhrle, D. *J. Porphyrins Phthalocyanines* **1999**, *3*, 230.
24. Spiller, W.; Kliesch, H.; Wöhrle, D.; Stark, J.; Schulz-Ekloff, G. *J. Porphyrins Phthalocyanines* **1998**, *2*, 145.
25. Mantareva, V.; Petrova, D.; Avramov, L.; Angelov, I.; Borisova, E.; Peeva, M.; Wöhrle, D. *J. Porphyrins Phthalocyanines* **2005**, *2*, 47.
26. Darwent, J. R.; Douglas, P.; Harriman, A.; Porter, J.; Richoux, M.-C. *Coord. Chem. Rev.* **1982**, *44*, 183.
27. Ishii, K.; Kobayashi, N. In *The Porphyrin Handbook*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; Academic Press: San Diego, 2003; pp 1–21.
28. Mantareva, V.; Angelov, I.; Borisova, E.; Avramov, L.; Kussovski, V. *SPIE, Int. Soc. Opt. Eng.* **2007**, *6604*, 66042 A. doi:10.1117/12.727717.
29. Segalla, A.; Borsarelli, D. C.; Braslavsky, E. S.; Spikes, J. D.; Roncucci, G.; Dei, D.; Chiti, G.; Jori, G.; Reddi, E. *Photochem. Photobiol. Sci.* **2002**, *1*, 641.
30. Scalise, I.; Durantini, E. N. *Bioorg. Med. Chem.* **2005**, *13*, 3037.
31. Borisova, E.; Angelov, I.; Mantareva, V.; Petrova, D.; Townsend, P.; Valberg, L.; Avramov, L. *SPIE, Int. Soc. Opt. Eng.* **2005**, *5830*, 399.