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Photodynamic efficacy of water-soluble Si(IV) and Ge(IV) phthalocyanines towards *Candida albicans* planktonic and biofilm cultures

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ABSTRACT

Water-soluble phthalocyanine complexes of silicon (SiPc1) and germanium (GePc1) were synthesized. The absorbance of SiPc1 in water was with minor aggregation while GePc1 strongly aggregated in water. The fluorescence data in water showed low quantum yields of 0.073 (SiPc1) and 0.01 (GePc1) and similar lifetimes of 4.07 ns and 4.27 ns. The uptake of SiPc1 into *Candida albicans* cells was two orders of magnitude lower as compared to GePc1 and for both was dependent on the cell density. Fungal cells in suspension were completely inactivated after SiPc1 (1.8 μM) at soft light radiation (50 J cm^{-2}, 60 mW cm^{-2}). The fungal biofilm formed on denture acrylic resin was inactivated with 3 log after fractionated light irradiation.

1. Introduction

The antibiotic resistance of pathogenic microorganisms has forced the research efforts in the field of the photodynamic therapy (PDT) as an alternative, antimicrobial approach [1]. PDT for treatment of infections appears as a distinct technique in case of multidrug-resistant pathogens, although the fact that the method is still under research stage [2,3]. The photodynamic process utilises the photosensitizer (PS), the proper light from the visible and near IR spectra and surroundings of molecular oxygen. Upon irradiation, PS becomes excited and the absorbed energy is transferred to ground-state triplet oxygen to undergo electron alterations to highly reactive singlet oxygen (type II mechanism) which causes cell death. The alternative mechanism includes an electron or hydrogen transfer from the triplet state of PS to the cellular-associated biomolecules (type I mechanism). During the both processes the generated highly reactive oxygen species (ROSs) induce the membrane damages and consequence photoinactivation of pathogenic microorganisms. Most of PSs act by mechanism of singlet oxygen generation for the high cytotoxic effect [4].

The chemical aspects of PDT for treatment of microbial infections include the development of more efficient second generation photodynamic PS. Along with the well accepted phenothiazine and porphyrin derivatives, phthalocyanines (Pcs) have been of great interest during the last two decades [5–7]. The metal complexes of Pc (MPcs) characterize with intensive absorption maxima of the Q band around 675 nm and the red shifted fluorescence peak around 690 nm in organic solvents [8]. The Pc-molecule has strong lipophilic nature and on the other hand the flexible to tailoring chemical structure, which allows modifications. These include the substitution with suitable functional groups on the peripheral or non-peripheral positions of the macrocycle and axially to the coordinated metal ion. The substituents can strongly influence the hydrophobic nature and the overall charge distribution of MPcs. The combination of suitable bulky functional groups on the peripheral and axial positions influences on the water solubility and on the macromolecule being in monomeric state. The water-soluble MPcs are the better choice for PDT vs. their hydrophobic derivatives despite the fact of their aggregation in aqua media. The known MPcs with essential PDT activity have closed p- or d-electron configuration of the coordinated metal ions into ligand

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[9]. Apparently, have been reported well defined fluorescence quantum yields together with a long life-time of the triplet excited state of MPcs [8,9]. During the last decade, the PDT with MPcs, which are complexes of Zn(II), Al(III) and Si(IV) pass though all stages of investigations and were approved for clinical applications on a number of oncological and non-oncological conditions [10].

Recent studies on microorganisms reported different cationic substituents to the Pc-ligand that lead to an optimal hydrophobic/hydrophilic balance and charge distribution for improved uptake into microbial cells [11–14]. In addition the chemical structure is well accepted as a crucial factor for the good membrane diffusion, cellular localization and the final photodynamic response [15].

The oral infections are provoked by the increase amount of pathogenic microorganisms in the oral cavity and further formation of the biofilm on the tooth or the denture surfaces [16,17]. Our recent studies with numerous cationic, water-soluble MPcs coordinated with Zn(II), Ga(III), and In(III) metal ions and peripherally substituted with four or eight methylpyridyloxy functional group, suggested a potential value of MPcs for treatment of wide range of pathogenic microorganisms [18–20]. The inactivation of representative strains of pathogenic cells, all in planktonic phase was previously studied with MPcs (M = Ga, In, Al and Zn) [19]. However in case of biofilms of *Candida albicans* (*C. albicans*) the treatment with PDT has one limitation of the incomplete penetration depth into biomass [20]. The biofilms of *C. albicans* that were grown for 18 h on acrylic resin were not susceptible to PDT with phenothiazine dye methylene blue (MB) and also with the studied complexes of Ga(III) and In(III). The treatment of biofilms was effective only after complex of Zn(II) with four methylpyridyloxy-groups (ZnPcMe) and of Ga(III) with eight methylpyridyloxy-groups (GaPc2) [20]. Several authors reported techniques to improve the drug penetration into biofilms [21–23]. For example the combine action of PDT to destroy the polymeric matrix followed by antibiotic application [21]. The other is an ultrasound effect together with PDT to allow a low integrity of the biofilm by forming channels into the matrix [22] or the usage of selective to the pathogenic cells drug delivery nanoparticles for treatment of dental biofilms [23].

The present study aims the synthesis of tetra-methylpyridyloxy-substituted Si(IV)- and Ge(IV)-phthalocyanines (SiPc1 and GePc1) and their investigation as photodynamic sensitizers for inactivation of *C. albicans* as planktonic and biofilm cultures. Both quaternized MPcs were synthesized by modification of the previously described chemical procedure in order to obtain high yield and purity. The absorbance and the fluorescence properties were studied in water and in presence of additives that allow disaggregation, and in dependence on the temperature. The cellular uptake of SiPc1 and GePc1 into *C. albicans* cells in suspension was investigated in comparison. The penetration depth and the localization ability of water-soluble SiPc1 and GePc1 into 48 h fungal biofilm were assessed. The photodynamic responses of SiPc1 and GePc1 were compared to the photodynamically active ZnPcMe for *C. albicans* biofilms formed on denture acrylic resin.

2. Results and discussion

2.1. Synthesis

The starting compound 4-(3-pyridyloxy)phthalonitrile (**1**) was prepared by following a slightly modified procedure of Refs. [24,25]. The commercially available 4-nitrophthalonitrile and 3-hydroxypyridine were mixed in dry DMSO together with potassium carbonate as a base. The reaction mixture was stirred for five days at room temperature and the high purity product was finally isolated in a good yield (80%). The IR spectrum confirmed the presence of CN group with positioned at 2228 cm^{-1} sharp, narrow band and the aromatic ether group with characteristic vibrations at 1280 and 1253 cm^{-1}. The EI-MS spectrum showed a molecular ion peak at *m*/*z* 221 $[M]^+$ and two fragmentation ions peaks at *m*/*z* 127 $[M - C_5H_4NO^-]^+$ and at *m*/*z* 78 $[C_5H_4N]^+$.

4-(Pyridyloxy)-1,3-diiminoisoindoline (**2**) was obtained from the phthalonitrile (**1**) by bubbling with ammonia gas in the presence of sodium methoxide in methanol according to known modified procedure [26]. The attempts to purify product (**2**) by column chromatography were not successful. Hence, after solvent removal and drying under vacuum, the highly hygroscopic product was used as obtained in the next step. The reaction mixture soon after initiation turned slightly greenish. This observation is consistent with the fact that diiminoisoindolines display increased tendency towards cyclization and readily react to form phthalocyanines even at very mild conditions. The peak is very small in the ESI mass spectrum. The diiminoisoindoline is thermally not very stable and finally under heat forms the phthalocyanine. Therefore under conditions of the heating during ESI-MS also dimer as precursor of phthalocyanine is formed.

Diiminoisoindoline (**2**) was proved to be an excellent precursor for the synthesis of phthalocyanine complexes. In order to obtain SiPc1 at first the non-alkylated SiPc2 was prepared. The product (**2**) was reacted with $SiCl_4$ in anhydrous, freshly distilled quinoline (200 °C, 1 h). The formation of the SiPc2 proceeds quickly. The chlorine atoms exchange at the silicon was achieved by refluxing the reaction mixture with ammonium hydroxide at RT overnight. SiPc2 was synthesized in 54% yield. The electrospray mass spectrum of SiPc2 showed molecular ion peak at *m*/*z* 946 $[M^-]$. In the second step SiPc2 was converted into the positively charged, water-soluble derivative SiPc1. The reaction was carried out by stirring SiPc2 with an excess of methyl iodide in dry DMF. The water-soluble phthalocyanine SiPc1 was isolated in high yield (83%). The water-soluble complex GePc1 was obtained in a good yield of 88% via GePc2 (yield 77%). The electrospray mass spectrum of GePc2 showed *m*/*z* 992 $[M]^+$, 974 $[M - H_2O]^+$.[1] The ESI positive mass spectra of SiPc1 and GePc1 showed the molecular ions at *m*/*z* 251.3 $[M - 4I^-]^{4+}$ and *m*/*z* 263 $[M - 4I^-]^{4+}$, respectively.

IR spectra of SiPc1 and GePc1 showed intense bands at 1237–1278 cm^{-1} due to the presence of aromatic ether bonds (Ar–O–Ar) and the characteristic low intensity IR vibrations at 2941 and 2830 cm^{-1} and 1395 cm^{-1} of methyl groups.

The structures of all synthesized phthalocyanines were also assessed by ^1H NMR. The obtained proton spectra confirmed the structure assigned to the compounds. The presence of several regioisomers of the analysed substances resulted in a complicated multiplicity of the proton resonance signals especially in the aromatic region. SiPc1 signals in the interval 8.30–8.63 ppm could be attributed to the protons of the core aromatic structure of the phthalocyanine ring. The resonances of the aromatic protons of the R-constituents are subjected to significant deshielding effect of both oxygen from the bridge structure and the nitrogen in the heterocycle which are shifted downfield (9.00–9.77 ppm). The existence of at least two regioisomers of SiPc1 accounts for the two N-methyl group singlets at 4.47 and 4.49 ppm. The position and the shape of the hydroxyl proton signals could be quite different

[1] The mass spectrum of GePc2 has, due to various isotopes of Ge, a complicated calculated isotope pattern (including ^{13}C): *m*/*z* 988 (42.9%), 989 (27.0%), 990 (66.7%), 991 (55.1%), 992 (100.0%), 993 (54.7%), 994 (33.9%), 995 (14.4%), etc. Therefore also fragments were showed this complicated pattern. The OH groups at the Ge of GePc2 are reactive and can be protonated as we know. It is possible that in the solution of GePc2 for ESI some protonation at Ge–OH occurs followed by elimination of H_2O.