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The effects of gold coated and uncoated zinc oxide nanohexagons on the photophysicochemical properties of the low symmetry zinc phthalocyanine

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ABSTRACT

A new low symmetry, Zn phthalocyanine monosubstituted with diethylaminoethanethiol (mDEAET ZnPc) was synthesized and characterized. This work reports on its photophysical and photochemical properties of mDEAET ZnPc alone and when conjugated to gold coated and uncoated zinc oxide nanohexagons (ZnO NHXs). The photophysicochemical properties generally improved in the presence of the ZnO NHXs. These complexes were also tested for their photodynamic antimicrobial activity against *Staphylococcus aureus* (*S. aureus*). The Pc alone showed remarkable growth inhibition even at concentrations as low as 0.05 mg/mL. The conjugates showed a high photoinactivation of *S. aureus* after 30 min at a fluence of 90 mW cm^{-2} at a concentration of 0.05 mg/mL. The ZnPc-ZnO NHX conjugates produced the best antimicrobial results.

1. Introduction

Phthalocyanines (Pcs) are a class of macrocyclic compounds which have been extensively studied for their applications in numerous fields including in solar cells [1–3], photocatalysis [4–7], photodynamic therapy (PDT) [8–10] and photodynamic antimicrobial chemotherapy (PACT) [11–13]. These applications are a result of the Pcs' strong absorption in the near infrared region of the electromagnetic spectrum, selective localization in cells as well as their efficient generation of singlet oxygen ($^1O_2$) [14].

The mode of action for PACT is similar to PDT in that light is used to activate a photosensitizer which results in the production of cytotoxic oxygen species, thereby inactivating microorganisms [11]. Some bacteria have been shown to be resistant to antibiotics, such as methicillin-resistant *Staphylococcus aureus* (*S. aureus*) (MRSA) [15–17]. Thus, the development of methods (such as PACT) to deal with multi-drug resistant pathogens are becoming increasingly explored. *S. aureus* is well known for its drug resistance [18,19] as well as causing infections in hospitals [20,21], as such it is selected for study in this work.

In this work, novel mono-diethylaminoethanethiol zinc phthalocyanine (mDEAET ZnPc) was synthesized as the photosensitizer for the inactivation of *S. aureus*, which is known for its drug resistance as stated already. DEAET Pcs containing 3, 4, 6 and 8 substituents have been studied [22–27]. A ZnPc monosubstituted with diethylaminoethanethiol is presented in this work for the first time in order to allow for only one linkage to the nanoparticles. The diethylaminoethanethiol ligand was chosen since it will allow for coordination to gold coated ZnO nanoparticles through the well-known Au–S or Au–N bonds.

Research into zinc oxide nanoparticles has become increasingly popular in biomedicine due to their high photo- and chemical stability, broad range of absorption, low toxicity, biocompatibility and biodegradability [28–30]. More importantly, ZnO nanoparticles are known to have effective antibacterial properties [31–36]. Gold was chosen as the dopant since it has been reported to improve the activity of anti-microbial agents such as toluidine blue and cofactor [37,38]. Hence it is expected that Au will improve the PACT activity of the phthalocyanine in this work. Au was also used to allow for Au–S bond formation with the phthalocyanine which is not possible with uncoated ZnO nanoparticles.

This paper shows that the low symmetry mDEAET ZnPc and ZnO NPs are more effective against *S. aureus* when conjugated than when they are employed individually. Gold coated and uncoated hexagonal shaped zinc oxide nanoparticles are employed.

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Hexagonal nanoparticles are employed since the shape of nanoparticles have been reported to influence cellular uptake of the photosensitizer [39].

2. Experimental

2.1. Materials

Zinc phthalocyanine, $AuHCl_4 \cdot 3H_2O$, dimethylaminoethane (DMAE), 1,3-diphenylisobenzofuran (DPBF) and hexadecanediol were purchased from Sigma Aldrich. $ZnNO_3$, NaOH, dicyanobenzene (**1**), zinc acetate dehydrate, nutrient broth (HG000C24.500) and nutrient agar (HG000C1.500) were purchased from MERK Chemical Ltd. *S. aureus* (ATCC 6538) was purchased from Microbiologics. 4-Diethylaminoethylsulfanyl phthalonitrile (**2**) was synthesized and characterized as reported in literature [40]. The rest of the reagents were obtained from commercial suppliers and used as received.

2.2. Equipment

Ground-state electronic absorption spectra were recorded on a Shimadzu, UV–vis 2550 UV–vis spectrometer. Fluorescence emission and excitation spectra were recorded on a Varian Eclipse spectrofluorimeter. Fluorescence lifetimes were measured using a time correlated single photon counting setup (TCSPC) (FluoTime 200, Picoquant GmbH) with a diode laser (LDH-P-670 with PDL 800-B, Picoquant GmbH, 670 nm, 20 MHz repetition rate, 44 ps pulse width). Details of the equipment have been described before [41].

Transmission electron microscope (TEM) pictures were obtained using a ZEISS LIBRA 120C transmission electron microscope at a 90 kV accelerating voltage. Energy-dispersive X-ray spectra (EDX) were acquired on a INCA PENTA FET coupled to the VAGA TESCA using 20 keV accelerating voltage.

A laser flash photolysis system was used for the determination of triplet decay kinetics. Laser flash photolysis experiments were performed with light pulses produced by a Quanta-Ray Nd: YAG laser providing 400 mJ, 90 ns pulses of laser light at 10 Hz, pumping a Lambda-Physik FL3002 dye (Pyridin 1 dye in methanol). Single pulse energy was 7 mJ. The analysing beam source was from a Thermo Oriel xenon arc lamp, and a photomultiplier tube was used as detector. Signals were recorded with a digital real-time oscilloscope (Tektronix TDS 360); the kinetic curves were averaged over 256 laser pulses. Triplet lifetimes were determined by exponential fitting of the kinetic curves using OriginPro 8 software.

X-ray powder diffraction (XRD) patterns were recorded on a Bruker D8, Discover equipped with a proportional counter and a Lynx- Eye detector, using Cu-K_α radiation (=1.5405 Å, nickel filter). Data were collected in the range from $2\theta = 10°–90°$, scanning at $1° \text{ min}^{-1}$ with a filter time-constant of 2.5 s per step and a slit width of 6.0 mm. The samples were placed on a silicon wafer slide. The X-ray diffraction data were treated using the Eva (evaluation curve fitting) software. A baseline correction was performed on each diffraction pattern by subtracting a spline fitted to the curved background.

Photo-irradiations for singlet oxygen determinations or bacterial studies were done using a General Electric Quartz line lamp (300 W). A 600 nm glass cut off filter (Schott) and a water filter were used to filter off ultraviolet and infrared radiations, respectively. An additional interference filter (Intor, 700 nm with a band width of 40 nm) was placed in the light path before the sample for singlet oxygen quantum yield determinations. Light intensities were measured with a POWER MAX 5100 (Molelectron detector incorporated) power meter and found to be 2.97×10^{16} photons s^{-1} cm^{-2} for singlet oxygen quantum yield studies and 9.43×10^{18} photons s^{-1} cm^{-2} for bacterial studies. All plate readings for the antimicrobial studies were obtained using the LEDETECT 96 computer controlled microplate reader for in vitro diagnosis from LABXIM Products.

2.3. Photophysical and photochemical studies studies

Fluorescence quantum yields (Φ_F) were determined by the comparative methods reported before [42,43] using ZnPc as a standard ($\Phi_F = 0.20$ in dimethylsulfoxide, DMSO [43] and 0.30 in dimethyl formamide, DMF [44]). Both the samples and standard were excited at the same wavelength and emission spectrum was recorded from 630 nm to 800 nm. The absorbances of the solutions at the excitation wavelength were about 0.05 to avoid any inner filter effects. The triplet state quantum yields (Φ_T) were also determined using a comparative methods [45,46], using ZnPc as a standard, $\Phi_T^{Std} = 0.65$ in DMSO [45] and 0.58 in DMF [46]. Solutions for triplet state quantum yields and lifetimes were deaerated for 15 min using nitrogen and irradiated at the Q band maxima.

Quantum yields of internal conversion (Φ_{IC}) were obtained by assuming that only three processes (fluorescence, intersystem crossing and internal conversion) are involved in the deactivation of the excited singlet state of an MPc molecule, Eq. 1

$$\Phi_{IC} = 1 - (\Phi_F + \Phi_T) \tag{1}$$

The singlet oxygen quantum yield (Φ_Δ) determinations were performed using an experimental set-up described above. Comparative methods reported in literature [47,48] were employed using ZnPc as a standard: $\Phi_T^{Std} = 0.67$ in DMSO [47] and 0.56 in DMF [48].

The fraction of the excited triplet state quenched by ground state molecular oxygen, S_Δ was calculated using Eq. (2):

$$S_\Delta = \frac{\Phi_\Delta}{\Phi_T} \tag{2}$$

2.4. Bacterial studies

2.4.1. Percent growth inhibition

The procedure to grow *S. aureus* (ATCC 6538) has been fully described in Ref. [25]. To evaluate the inactivation of *S. aureus*, different concentrations of mDEAET ZnPc or its conjugates, ranging from 0.05 mg/mL to 0.25 mg/mL were prepared in DMF. *S. aureus* is known to be resistant to DMF [49]. Harvested bacterial cells (10 μL) were added to 90 μL of nutrient broth and diluted ten-fold to produce the desired concentration for this work (1×10^8 CFU/mL). Then 10 μL of mDEAET ZnPc or its conjugates with ZnO nanohexagons (ZnO NHXs) or Au coated Zn NHXs (Au@ZnO NHXs) at the respective concentration was added and the cells irradiated at 2.12 W cm^{-2} for 20 min. After this time, 20 μL of these cell solutions were plated onto agar plates and then incubated at 37 °C on a shaking platform for 12 h. Control experiments were also performed where mDEAET ZnPc or its conjugates were not added. The colony forming unit (CFU) were determined using the Interscience Scan 500.

2.4.2. Logarithmic reduction of S. aureus with time

The inactivation of *S. aureus* was carried out by placing aliquots of 10 μL of the bacteria culture in a 96-well plate containing 90 μL of nutrient broth. The sample was diluted ten-fold to obtain a concentration of 1×10^8 CFU/mL of mDEAET ZnPc or its conjugates with ZnO NHXs or Au@ZnO NHXs (10 μL in DMF). Experiments where no photosensitizer (the control) was employed were also performed. The conjugates showed higher antimicrobial activity at