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Effect of bovine serum albumin and single walled carbon nanotube on the photophysical properties of zinc octacarboxy phthalocyanine

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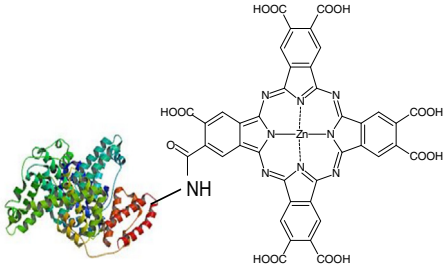
HIGHLIGHTS

- A conjugate between zinc octacarboxy phthalocyanine and bovine serum albumin was formed.
- The conjugate was adsorbed onto single walled carbon nanotubes.
- The conjugate gave larger singlet oxygen quantum yields than the phthalocyanine alone.

GRAPHICAL ABSTRACT

A conjugate between zinc octacarboxy phthalocyanine and bovine serum albumin was adsorbed onto single walled carbon nanotubes and resulted in improved singlet oxygen quantum yield when compared to the phthalocyanine in the absence of bovine serum albumin.



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ABSTRACT

This work reports on the photophysical parameters of the conjugate between zinc octacarboxy phthalocyanine (ZnOCPc) and bovine serum albumin (BSA) represented as ZnOCPc–BSA (**1**) which was further adsorbed onto single walled carbon nanotubes (SWCNT) represented as (ZnOCPc–BSA–SWCNT **2**). ZnOCPc (without BSA) was also adsorbed on SWCNT represented as ZnOCPc–SWCNT (**3**). The presence of BSA resulted in the increase in singlet oxygen quantum yield (Φ_Δ) for **1** (at $\Phi_\Delta = 0.44$) and **2** (at $\Phi_\Delta = 0.41$) compared to $\Phi_\Delta = 0.21$ for ZnOCPc alone. For complex **3** which did not contain BSA singlet oxygen quantum yield decreased.

Introduction

Metallophthalocyanines (MPcs) have the ability to generate cytotoxic species such as singlet oxygen that rapidly kill tumor cells through photodynamic therapy (PDT) [1–4]. Although MPcs have gained world recognition for use as photosensitizers in PDT and are in different phases of clinical trials [5], the problem of selectivity remains a challenge.

Studies have shown that albumin binding proteins (ABPs) interact with normal endothelial and tumor cells [6]. Serum albumins are the most abundant plasma proteins and their most important biological function is the transport of free fatty acids, although they can also bind a broad range of molecules [7]. Bovine serum albumin (BSA) belongs to the family of serum albumins which are the major soluble protein constituent of the circulatory system, and have several physiological functions including use as transport and depot protein for varieties of compounds [8]. It has been reported that Pcs covalently conjugated to biomolecules possess improved selectivity towards cancer cells [9]. Hence in order to

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improve cancer selectivity and biocompatibility, this work reports on the linking of zinc octacarboxy phthalocyanine to BSA. There have been a number of studies for non-covalent linking of BSA to Pcs [10–12]. Only a few covalently linked Pc-BSA conjugates have been reported [9,13,14]. In this work BSA is covalently linked to octacarboxy zinc phthalocyanine (ZnOCPc). The conjugate was further adsorbed onto single walled carbon nanotubes (SWCNTs). SWCNTs are employed as drug carriers, which could also absorb light in the near infrared region [15–18], thus killing cancer cell through photothermal therapy (PTT) effect. Zhang et al [19] have reported on the double cancer therapy system using PDT and PTT on cancer cell line, where ZnPc is the PDT agent and SWCNT is the PTT agent. The photophysical properties of covalently linked conjugates of SWCNT with Pc derivatives have been reported [20–24]. There have also been some reports on the photophysical behavior of phthalocyanines in the presence of BSA [25,26]. Triplet lifetimes of phthalocyanines are reported to be enhanced in the presence of BSA [25,26]. However the photophysical behavior of Pc-BSA conjugates in the presence SWCNT is reported for the first time.

In this study zinc octacarboxy phthalocyanine (ZnOCPc) was linked to BSA represented as ZnOCPc–BSA (**1**) which was further adsorbed onto SWCNT represented as ZnOCPc–BSA–SWCNT (**2**). Experiments were also done when ZnOCPc was adsorbed on SWCNT represented as ZnOCPc–SWCNT (**3**). ZnOCPc was chosen due to its monomeric behavior [27] (since aggregates are photoinactive) and the fact that ZnPc derivatives are in clinical trials for PDT [4].

Experimental

Materials

1-Ethyl-3-(3-dimethylaminoproplyl) carbodiimide hydrochloride (EDC), 9,10-N-hydroxysuccinimide (NHS), lipophilic sephadex LH-20 microbeades, pyromellitic anhydride and bovine serum albumin (BSA) were from Sigma Adrich. Single-walled carbon nanotubes (SWCNTs, 1–5 nm in diameter and 1–5 μm in length) were obtained from Nanolab. Phosphate buffer pH = 9 solutions was prepared using appropriate amounts of Na_2HPO_4 and KH_2PO_4. Aluminum sulphonated metallophthalocyanine (AlPcSmix, containing mixtures of differently substituted derivatives) [28] and zinc octacarboxy phthalocyanine (ZnOCPc) [29] were synthesized according to literature methods.

Equipment

Absorption spectra were recorded on a Shimadzu UV–Vis 2550 spectrophotometer and fluorescence emission and excitation spectra on a Varian Eclipse spectrofluorimeter using a 360–1100 nm filter, the absorbance ranged between 0.04 and 0.05 at the excitation wavelength for all samples. X-ray powder diffraction (XRD) patterns were recorded on a Bruker D8 Discover equipped with a Lynx-Eye Detector, using Cu Kα radiation (=1.5405 Å, nickel filter). Data were collected in the range from 2θ from 5° to 100°, scanning at 1° min^{-1} with a filter time-constant of 2.5 s per step and a slit width of 6.0 mm. Details have been provided before [30]. Infrared spectra were recorded on a Perkin–Elmer Universal ATR Sampling accessory spectrum 100 FT-IR spectrometer. Raman spectra were obtained using a Bruker RAM II spectrometer (equipped with a 1064 nm Nd:YAG laser and a liquid nitrogen cooled germanium detector). Solid samples diluted with KBr were used.

Fluorescence lifetimes were measured using a time correlated single photon counting (TCSPC) setup (FluoTime 200, Picoquant GmbH). Details have been provided before [31]. Transmission electron microscopy (TEM) images were obtained using a Zeiss Libra TEM 120 model operated at 90 kV.

Laser flash photolysis experiments were performed to determine the triplet decay kinetics. The excitation pulses were produced by a tunable laser system consisting of an Nd:YAG laser (355 nm, 135 mJ/4–6 ns) pumping an optical parametric oscillator (OPO, 30 mJ/3–5 ns) with a wavelength range of 420–2300 nm (NT-342B, Ekspla) as the detector as described in detail before [31].

The time resolved phosphorescence of singlet oxygen at 1270 nm was used to determine the singlet oxygen quantum yield of **1**, **2** and **3** in aqueous solution, details have been described before [32]. The singlet oxygen phosphorescence signal was compared with that of AlPcSmix.

Synthesis

Synthesis of ZnOCPc–BSA (*1*)

ZnOCPc (20 mg, 0.02 mmol) in pH buffer 9.0 was stirred together with EDC (10 mg, 0.05 mmol) and NHS (5.8 mg, 0.05 mmol) for 2 h to activate the carboxy group of ZnOCPc. Then BSA (20 mg) was added and the solution was further stirred at room temperature for 24 h, according to a modified literature procedure [19]. The solid product was precipitated out of solution using ethanol followed by centrifugation. The solid product was repeatedly washed with ethanol and further purified using size exclusion chromatography. ZnOCPc–BSA **1**: UV–Vis (buffer 9): λ_{max} nm 687, IR [(ATR) υ_{max}/cm^{-1}]: 3362 and 2820 (N—H str.), 1560 (C=O amide and N—H bend).

Synthesis of ZnOCPc–BSA–SWCNT (*2*)

ZnOCPc–BSA (**1**) were immobilized onto SWCNT–COOH according to literature methods for immobilization of other Pcs [33] with slight modification: 20 mg of SWCNT–COOH was ultrasonicated for 20 min in 5 ml of pH 9 buffer to give a brown colored suspension, 20 mg of ZnOCPc–BSA was then added to the solution resulting in a blue suspension. The mixture was stirred for 4 days resulting into a dark-blue suspension, indicating the adsorption of ZnOCPc–BSA onto SWCNT–COOH. The reaction mixture was precipitated out of solution using ethanol and the solid products were obtained by centrifugation. The conjugate was further purified using size exclusion chromatography. ZnOCPc–BSA–SWCNT **2**: UV–Vis (buffer 9): λ_{max} nm 691, IR [(ATR) υ_{max}/cm^{-1}]; 3300-3118 (N—H str.), 1564 (C=O, N—H bend). [Raman υ_{max}/cm^{-1}]: 2554 (G*), 1592 (G), 1285 (D).

Synthesis of ZnOCPc–SWCNT (adsorbed) (*3*)

ZnOCPc was immobilized onto SWCNT–COOH, using similar procedure as explained for complex **2** above. ZnOCPc–SWCNT **3**: UV–Vis (buffer 9): λ_{max} nm 687, IR [(ATR) υ_{max}/cm^{-1}]; 3490-3158 (N—H str.), 1578 (C=O and N—H bend). [Raman υ_{max}/cm^{-1}]: 2549 (G*), 1591 (G), 1283 (D).

Photophysical and photochemical parameters

Triplet (Φ_T), singlet oxygen (Φ_Δ) and fluorescence (Φ_F) quantum yields for **1**, **2** and **3** were determined using the comparative methods as described before [34–37]. Aluminum sulphonated phthalocyanine (AlPcSmix) was used as a standard for triplet quantum yield (Φ_T = 0.44 [37]) and singlet oxygen quantum yield ($\Phi_\Delta^{Std} = 0.34$) [38]) in water. ZnPc in DMSO was employed as a standard for Φ_F (=0.20 [36] in DMSO) determination and employing the refractive indexes of the solvents. Singlet oxygen (Φ_Δ) was determined using the time resolved phosphorescence decay curve at 1270 nm as described before [32,39].