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Tumor microenvironment-responsive charge reversal zinc phthalocyanines based on amino acids for photodynamic therapy

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ABSTRACT

Amino acid substituted zinc phthalocyanines which are capable of reversing their charge from negative to positive in response to tumor microenvironment have been prepared by the isoelectric pHs regulation and charge reversal sensitivity enhancement. The charge reversal abilities of the prepared zinc phthalocyanines were judged by their isoelectric pHs and zeta potential values. The UV–Vis spectra, singlet oxygen generation ability, cellular uptake and *in vitro* anticancer ability of the prepared zinc phthalocyanines were investigated to study the influences of charge reversal on photodynamic activities. Charge reversible tetra- and octa-substituted glutamic acid ZnPcs showed enhanced singlet oxygen generation ability, cellular uptake and photocytotoxicity towards cancer cells, and were considered as promising photosensitizers for photodynamic therapy.

1. Introduction

Cancer is a major public health problem in the world. Every year, over eight million casualties worldwide are due to cancer [1,2]. Despite enormous efforts, there are still few existing anticancer drugs can meet the multiple requirements of effective transmission, high selectivity and excellent anticancer activity at the same time. Charge which is closely relate to blood transmission, uptake mechanisms, intracellular fate and activity of drugs has attracted people's considerable attention [3–6]. Generally, neutral particles are prone to aggregate or precipitate in water [7]; positively charged particles have enhanced water solubility, cellular uptake rates and anticancer activity [3–6,8,9], but can cause severe serum proteins inhibition and then induce hemolysis or get cleared from the body [3,10,11]; negatively charged particles have good blood transmission performance, while show poor cellular uptake [5,9,12]. Hence, ideally drugs should be negatively charged under normal physiological conditions to ensure effective blood transmission, while be positively charged in tumor tissues to obtain better cellular uptake and anticancer activity. Thus, the preparation of anticancer drugs which can switch their charge from negative to positive in response to tumor microenvironment will be very meaningful.

It is well known that the pH value in tumor tissue (~6.5) is more acidic than in blood and normal tissue (~7.4) [13–15]. On this basis, Shen, Wang and Lee have reported several tumor acidity activated charge reversal macromolecules containing β-carboxylic acid amide groups [11,16,17]. Na has reported charge-switchable nanoparticles containing imidazole and carboxyl groups [18]. However, most of the above charge reversible systems were based on macromolecules or nanoparticles, charge-conversion drug molecules have been reported rarely [19]. Furthermore, the development of alternative pH sensitive drugs that can accurately and efficiently respond to the tumor microenvironment is still great challenges because of the small pH difference between normal and tumor tissues. Photodynamic therapy (PDT) is a well consolidated, promising new treatment method for cancer [20–22]. The final therapeutic outcome of PDT is largely determined by photosensitizer (PS) [23,24]. Due to the unique properties which are easy modification, strong absorption in phototherapeutic window (650–800 nm), low dark toxicity and high phototoxicity, phthalocyanines (Pcs) have been considered as promising PSs for PDT [25,26].

In this study, we have prepared tumor microenvironment responsive zinc phthalocyanines (ZnPcs) which are capable of

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reversing their charge from negative to positive for PDT. We chose amino acids (AAs) as the pH sensitive moieties to conjugate with ZnPcs because they are typical of pH activated charge reversal compounds that are negatively charged when environmental pH (pHe) is above isoelectric pH (pI) and positively charged when pHe is under pI. The AAs based zinc phthalocyanines (**AAZnPcs**) are also expected to have good biocompatibility and high water solubility due to the presence of AAs which contain carboxyl and amino groups [27,28]. To ensure the prepared **AAZnPcs** are capable of reversing their charge from negative to positive in response to the tumor microenvironment accurately, the pIs of **AAZnPcs** were adjusted to fall within the range of pH 6.5–7.4 by regulating the spaces between amino and carboxyl groups (Scheme 1a). After that, the charge reversal sensitivity of the candidate **AAZnPcs** were adjusted by increasing the number of suitable AA groups (Scheme 1b).

2. Experimental

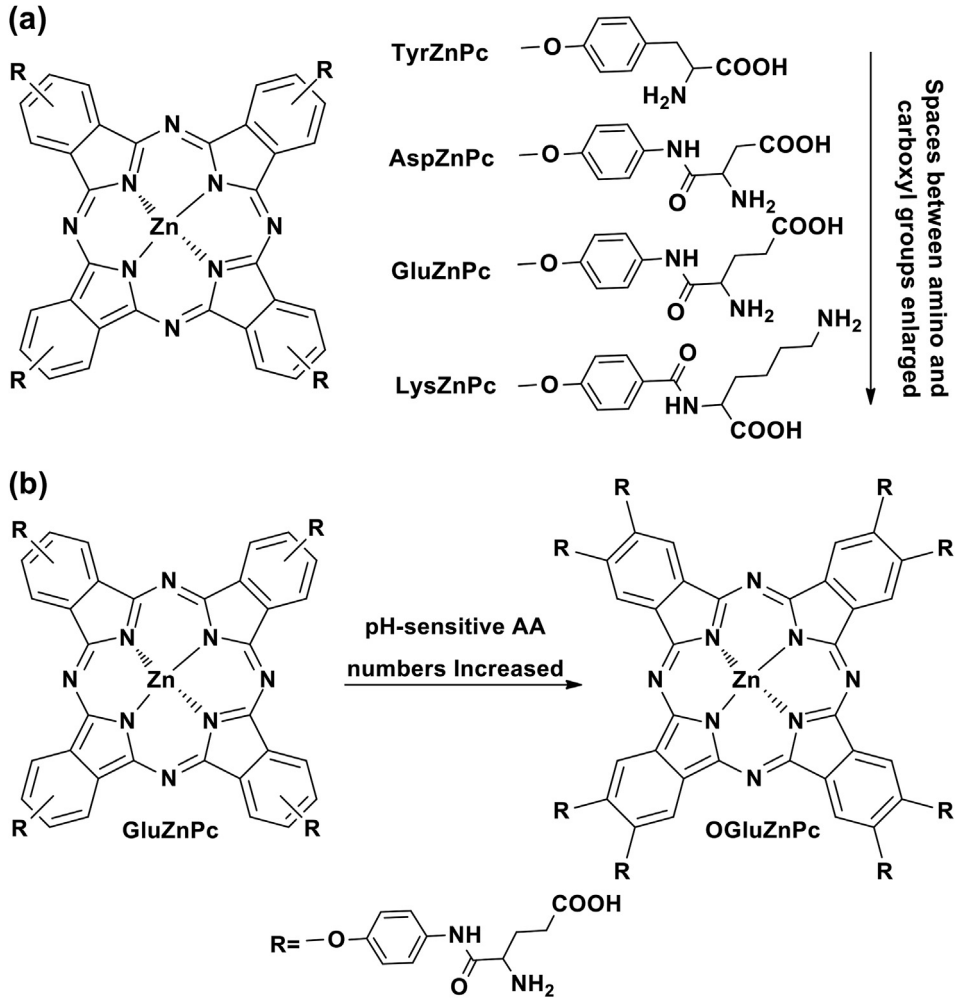
2.1. Materials and instrumentation

All necessary solvents were used after purification according to the reported literature [29]. All chemical reagents were obtained from commercial suppliers and used as received unless otherwise stated. 1, 8-diazabicyclo-[5.4.0] undec-7-ene (DBU), 9, 10-anthracenedipropionic acid (ADPA) and [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] (MTT) were obtained from Sigma–Aldrich. Dulbecco's modified Eagle's medium (DMEM) was purchased from Gibco. Chromatographic purifications were performed on silica gel columns (300–400 mesh, Qingdao Haiyang Chemical Co., Ltd, China). Infrared spectra was measured in KBr pellets on IR-Spectrometer Nicolet Nexus 670. ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker Advance 400 MHz NMR spectrometer. Elemental analyses were taken with Vario MICRO, Elementar. MS spectra were obtained on Agilent 1290 Infinity LC/6460 QQQ MS and new ultraflextreme MALDI TOF/TOF, Bruker. UV–Vis spectra were recorded on spectrophotometer Cary 5000, Varian. Fluorescence spectra was measured with Cary Eclipse Fluorescence spectrophotometer, Varian. Zeta potential was measured with Zetasizer Nano ZS90, Malvern. pH was measured with FE20/EL20 pH meter, Mettler Toledo. Cell morphology changes were observed under a Zeiss Observer fluorescence microscope. A 665 nm LED lamp (power density was 96 mW cm^{-2}) was used as light source.

2.2. Synthesis

2.2.1. Ethyl 2-((tert-butoxycarbonyl) amino)-3-(4-(3, 4-dicyanophenoxy) phenyl) propanoate ***(1)***

Under nitrogen atmosphere, a mixture of Boc-L-tyrosine ethyl ester (1.00 g, 3.23 mmol), 4-nitrophthalonitrile (0.62 g, 3.56 mmol) and anhydrous K_2CO_3 (0.90 g, 6.47 mmol) in 15 mL N, N-dimethylformamide (DMF) was heated at 40 °C for 5 h. After being cooled



Scheme 1. (a) Schematic of pI adjustment of **AAZnPcs** by increasing the spaces between amino and carboxyl groups (b) Schematic of charge reversal sensitivity adjustment by increasing numbers of AA groups.