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Cyclodextrin type dependent host-guest interaction mode with phthalocyanine and their influence on photodynamic activity to cancer

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ABSTRACT

Three host-guest complexes of phthalocyanines (Pc) with α-, β- or γ-cyclodextrins (CDs) were prepared and their interaction modes, reactive oxygen species (ROSs) generation ability and in vitro anticancer activities were studied and compared. After forming complex with CD, the aggregation degree of Pc was greatly decreased and the water solubility and photodynamic activity was sharply increased. Computer modeling results indicated that the interaction modes between Pc and CDs were varied with different kinds of CD. Especially, the complex of Pc and β-CD has superior stability, ROSs generation ability, and anticancer activity to other complexes.

1. Introduction

Photodynamic therapy (PDT) is a clinical modality for cancer treatment. The general procedure of PDT involves the systemic, local, or topical administration of non-toxic photosensitizers (PSs) followed by selective irradiation using optical fiber, with appropriate wavelength and power, at tumor position. In the presence of oxygen, PSs can transfer the absorbed photon energy to surrounding oxygen molecules, generating ROSs, especially singlet oxygen ($^1O_2$), to cause cancer cell apoptosis and necrosis. Simultaneously, selective irradiation at tumor position can avoid the side-effect of PDT to healthy surrounding tissues. (Chen, Tian, He & Guo, 2015; Huang et al., 2012; Lu, He & Lin, 2015) In recent years, Pcs have attracted great attention in the field of PDT. Their high $^1O_2$ generation ability and strong absorption in the red overlaps the region of maximum light penetration in tissues makes them ideal candidates for PDT. However, most of the Pc derivatives are hydrophobic and prone to aggregation in water, which could reduce their $^1O_2$ generation ability and PDT efficiency. (Jia, Yang, Li, Liu & Xue, 2013) Therefore, finding effective ways to improve their water solubility and reduce their aggregation degree in water is very important for their PDT application.

CDs are cyclic oligosaccharides composed of D-glucopyranoside units, which were linked by glycosidic bonds. They can modify the physicochemical and biological characteristics of low-soluble drugs through the formation of CD: drug host-guest complexes. (Francisco, Otero-Espinar, & Blanco-Mendez, 2014) Many researches indicated that the binding constants between CDs and PSs (1:1 or 2:1) are rather low, which induced separation of PS and CD molecules in deliver process, making the host-guest complex far from being an ideal system for the drug transport of Pc or other PSs with similar structure, such as porphyrin. (Kralova et al., 2010) To solve this problem, researchers used CDs to modify Pcs to improve their solubility and PDT activity and got satisfied results. (Lourenço et al., 2014) However, the modification approach involves complicated synthetic steps, which are not general and not always possible. In this respect, finding ways to improve the stability of their host-guest complex can be considered as a simple and promising strategy for solving this problem.

Our previous research indicated that hydroxypropyl-beta-cyclodextrin (HP-β-CD) can form stable host-guest complex with tetra-1, 2-diethylamino substituted zinc (II) phthalocyanine (ZnPc) at the mole ratio of 4:1. Multiple hydrogen bonds can form between $-NH_2$ groups of ZnPc and $-OH$ groups of HP-β-CD. Besides, the

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hydrogen bond can also form between the −OH groups of adjacent CD molecules. Systemic studies indicated that such 4:1 complexes have good dispersal ability, low aggregation degree and satisfied PDT activity. (Lu et al., 2014) However, the type of CDs, including α-, β- and γ-CDs, would have a great influence on the interaction mode and strength between the host and guest molecules because of their different hole and full size. In this manuscript, we compared the different interaction modes between Pc and various CDs. In addition, we analyzed the influences on their PDT activity from these different interaction modes.

2. Materials and methods

2.1. Chemicals

All solvents were used after purification according to the reported literature. 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) and 9,10-anthracenedipropionic acid (ADPA) were obtained from Sigma-Aldrich. α-, β- and γ-CDs were all from Sigma-Aldrich. Hoechst 33342 and 2′7′-dichlorofluorescin diacetate (DCFH-DA) were from Beyotime. Dulbecco's modified eagle media (DMEM) and fetal bovine serum (FBS) were from GIBCO. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazoliumbromide (MTT) were from Amosco.

2.2. Characterization

Ultraviolet-Visible (UV–vis) absorption spectra were measured with a Varian Cary 5000 spectrophotometer, where the light path length was 1 cm. Fluorescence spectra were recorded on a Cary Eclipse fluorometer. The fourier transform infrared (FTIR) spectra were recorded on a Nicolet Nexus 670 FT-IR infrared spectrometer. Thermogravimetry-differential thermal analysis (TG-DTA) was taken on Perkin Elmer Diamond TG-DTA analyzer in N_2 atmosphere at a heating rate of 10 °C min^{-1}. The size of samples was measured by dynamic light scattering (DLS) method with Malvern Zetasizer Nano 90 measurements in aqueous system. Transmission electron microscope (TEM) images were got by a Hitachi H-7650 Transmission Electron Microscope with 120 kV accelerating voltage. Morphology and fluorescence inside cells were monitored by a Nikon Ti Fluorescence microscope. ^{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 New ultrafleXtreme MALDI TOF/TOF, Bruker. The interaction between ZnPc and CDs was performed using the Discovery Studio 2.1 (DS 2.1) software package (Accelrys, USA).

2.3. ZnPc synthesis

The synthesis of ZnPc is depicted in Scheme S1 as previous reports. (Wang et al., 2013) All necessary solvents were analytical grade and used after purification according to the reported procedure. All reactions were carried out under nitrogen atmosphere unless otherwise stated. Structures of all of the compounds were verified by spectroscopic analyses, including ^{1}H NMR, ^{13}C NMR, FTIR, mass spectra, and elemental analyses (Supporting information, Fig. S1).

2.4. Host-guest complex preparation of CDs and ZnPc

The host-guest complex of ZnPc and CD were prepared by following process. Taking the host-guest complex of α-CD and ZnPc as example, in 10 mL methanol, ZnPc (150 μL, 1×10^{-3} M in methanol) and α-CD (300, 450, 600, 750 or 900 μL, 1×10^{-3} M in Mili-Q water) were mixed by vigorous magnetic stirring. Then, the mixture was heated at reflux with stirring for 5 h under 80 °C. After all of the solvent has evaporated at 40 °C, the 2:1, 3:1, 4:1, 5:1 and 6:1 complex of ZnPc and α-CD was got. The complexes of ZnPc and other CDs (β-CD and γ-CD) were obtained by same process. These dried complexes can be re-dissolved in Mili-Q water for further experiments. The result complex can be well dispersed in aqueous solution for a long time. Free ZnPc control was prepared by dissolving ZnPc in Millipore™ water using traces of ethanol (<5‰) as latent solvent. The physical mixtures of ZnPc and varies of CDs were prepared as follows. The solid power of ZnPc and different types of CD were mixed in mortar at molar ratio of 1:4, separately. The mixtures were homogenized by triturating until they formed the homogeneous physical mixtures.

2.5. Cellular uptake ability

Human cervical carcinoma (HeLa) cells were incubated with drugs (drug concentration was 5 μM, which was calculated by ZnPc) for 2, 4, 6 and 24 h in the dark, separately. After respective incubation, the drug remaining in the medium was detected by UV–vis absorption spectra and their concentration was calculated. All cellular uptake amounts were calculated according to the standard curves.

2.6. ROSs generation ability

Extracellular $^1O_2$ generation ability was studied using the disodium salt of ADPA as detection sensor. ADPA can be bleached by $^1O_2$ to its corresponding endoperoxide, which can be monitored spectrophotometrically by recording the absorbance intensity decrease at 380 nm (λ_{max} of ADPA). ADPA was added into 3 mL drug aqueous solution, including free ZnPc and host-guest complexes. These mixtures were irradiated with a 665 nm LED and their UV–vis spectra were recorded every 30 s.

Intracellular generation of ROSs was measured by using an oxidation-sensitive fluorescent probe, 2′7′-dichlorofluorescin diacetate (DCFH-DA). ROSs oxidized DCFH-DA to the highly fluorescent DCF (2′7′-dichlofluorescein, DCF). HeLa cells were seeded in 6-well plates at 1×10^5/mL density. After incubation with drugs (5 μM) for 4 h, cells were treated with 10 μM DCFH-DA. The control experiment used cells incubated in serum free DMEM and DCFH-DA without drug. After 30 min incubation, cells were washed 3 times with PBS and then exposed to light. Immediately after light exposure, cell images were acquired using an inverted fluorescence microscope.

2.7. In vitro PDT studies with tumor cells

The chromatin damage in cancer cells was detected using Hoechst 33342 as probe. HeLa cells were plated on 6-well corning plate at the density of 1×10^5 cells/well in DMEM (containing 10% FBS) in incubator (5% CO_2, 37 °C) for 24 h. Then, serum free medium with different drugs, including free ZnPc and host-guest complexes (drug concentration was 5 μM, which was calculated by ZnPc) was replaced on the plates, separately. After 4 h incubation, medium with Hoechst 33342 was replaced on the plates and incubated for 30 min. Then, cells were washed with PBS (pH = 7.4) for 3 times and monitored by fluorescence microscopy.

The toxicity activities of drugs were carried out as follows. Cells (5×10^5 cells/well) were seeded in 96-well plates. 24 h later, serum free medium with drugs, including free ZnPc, CDs and host-guest complexes. For dark toxicity study, cancer cells (HeLa cells) and non-tumoral cells (human embryonic kidney 293 cells) were incubated for 24 h and their survival percent were studied by MTT assay. For light toxicity study, after 4 h incubation by drugs (drug concentration was 5 μM, which was calculated by ZnPc), the cells were