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Charge dependent photodynamic activity of alanine based zinc phthalocyanines

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ABSTRACT

In this paper, to minimize the effects of different structure, three alanine-based zinc phthalocyanines (Pcs) of differing charges were engineered and synthesized with the same basic structure. On this premise, the relationship between nature of charge and photodynamic activity was studied. Besides, further verification and explanation of some inconsistent results were also carried out. The results showed that charge can influence the aggregation state, singlet oxygen generation ability and cellular uptake of Pcs, thereby affecting their photodynamic activity. In addition, the biomolecules inside cells may interact with Pcs of differing charges, which can also influence the aggregation state and singlet oxygen generation of the Pcs, and then influence the relationship between nature of charge and photodynamic activity.

1. Introduction

Photodynamic therapy (PDT) is a promising and clinically approved noninvasive modality for several cancers [1,2]. In the PDT process, the highly reactive singlet oxygen was formed after energy transfer from an excited photosensitizer (PS) that absorbs light of an appropriate wavelength [1,3]. The singlet oxygen is lethal to cells and is responsible for irreversible photodamage to tumor tissues [4,5]. Pcs, which possess many desirable features of ideal photosensitizers, of which are intense absorption in the phototherapeutic window (600–900 nm), efficient singlet oxygen generation, high phototoxicity, high thermal and light stability, have been considered as the promising photosensitizers for PDT [6–9].

To obtain ideal Pcs used for PDT, many kinds of moieties have been introduced at the peripheral or axial positions of the Pcs. Amino acids are not only the building blocks of proteins but are also regulators of gene expression and the key metabolic pathways that are necessary for maintenance, growth, reproduction and immunity [10]. PSs conjugated with amino acids or peptide sequences attract wide attention because of their enhanced biocompatibility and anticancer activity [11,12].

Photodynamic activity of PSs can be affected by many factors, such as sensitizer structure and electrical charge [13,14]. The relationship between sensitizer structure and photodynamic activity has been studied extensively [15–18,7,19]. However, in contrast, there are few researches on the relationship between nature of charge and photodynamic activity. By studying the influences of charges on the aggregation state, cellular uptake, diffuse cytoplasmic distribution and photosensitising ability of PSs, previous studies have concluded that the cationic Pcs had improved activity over the anionic and neutral Pcs [14,20,21]. Since the structures of Pcs used by the authors are very different, the results may depend on both structure and charges, which makes it difficult to get an accurate conclusion. Furthermore, the interactions between PSs and biomolecules inside cells differ based on the charges carried by PSs, which would also affect the relationship between nature of charge and photodynamic activity. This has never been involved, thus also need studying. In addition, some inconsistent results in the previous researches need further verification and explanation.

Based on the above conceptions, in this paper, three alanine-based ZnPcs (**ZnPc1**, **ZnPc2** and **ZnPc3**) of differing charges were engineered and synthesized with the same basic structure to minimize the effects of structure. Their photophysical and photochemical properties and in vitro anticancer activities in relation to unsubstituted zinc Pc (**ZnPc**) were discussed and compared. In order to study the influences of biomolecules inside cells on the relationship between nature of charge and photodynamic activity,

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the aggregation state and singlet oxygen generation ability of the above Pcs were also measured in cell lysates. The results show that charge can influence PS's aggregation state, singlet oxygen generation ability and cellular uptake, thereby affecting their photodynamic activity. The aggregation state and singlet oxygen generation of PSs in water and in cell lysates are different from each other, which confirms the effects of biomolecules in cell and further explains the reason for the contradictory photodynamic activity of the three Pcs.

2. Materials and methods

2.1. Materials

The Pcs 2(3), 9(10), 16(17), 23(24)-tetra-((4-(N-(2-amino) propanamide) amino) phenoxy) phthalocyaninato-zinc (II) (**ZnPc2**, electrically neutral) and Quaternized 2(3), 9(10), 16(17), 23(24)-tetra-((4-(N-(2-amino) propanamide) amino) phenoxy) phthalocyaninato-zinc (II) (**ZnPc3**, electropositive) used in this work were synthesized in our laboratory [22]. The unsubstituted zinc Pc (**ZnPc**), disodium salt of 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. The HeLa cell lysates were prepared by using cell lysis buffer for western and IP which can be purchased commercially. The water was triply distilled and the pH was 6.81 at the room temperature. All chemicals were of analytical grade and used without further purification.

2.2. Instrumentation and characteristics

IR spectra were determined with IR-Spectrometer Nicolet Nexus 670. ^{1}H NMR and ^{13}C NMR spectra were determined with a Bruker Advance 400 MHz NMR spectrometer. Elemental analyses were taken with Vario MICRO, Elementar. Electronic absorption spectra were recorded on spectrophotometer Cary 5000, Varian. Fluorescence spectra were monitored with Cary Eclipse Fluorescence spectrophotometer, Varian. Zeta potential was determined with Zetasizer Nano 90 light scattering, 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 was used as light source.

2.3. Synthesis

2.3.1. 4-(3,4-Dicyanophenoxy) benzoic acid (**1**, Scheme 1)

A mixture of 4-nitrophthalonitrile (2.00 g, 11.55 mmol), 4-hydroxybenzoic acid (1.68 g, 12.13 mmol) and finely ground anhydrous K_2CO_3 (3.19 g, 23.10 mmol) in DMF (15 mL) was heated at 40 °C for 6 h under nitrogen atmosphere. The mixture was cooled to room temperature and then poured into water (150 mL). The pH of the solution was adjusted to 2–3. After filtration, the cake was washed with distilled water (3 × 30 mL). The crude product was then purified by silica gel column chromatography using petroleum ether/ethyl acetate (2:1 v/v) as the eluent to give a light gray solid (2.62 g, 85.9%). M.P. = 199 °C. IR (KBr, cm^{-1}): 3440, 3090, 2230 (CN), 1680 (C=O), 1586, 1510, 1426, 1300, 1250, 826. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 12.68 (s, 1H, COOH), 8.15 (d, 1H, 8.8 Hz, ArH), 8.03 (d, 2H, 8.8 Hz, ArH), 7.93(d, 1H, 2.4 Hz, ArH), 7.52–7.55 (m, 1H, ArH), 7.26 (d, 2H, 8.8 Hz, ArH). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 166.98, 160.29, 158.34, 136.86, 132.44, 128.14, 124.27, 123.82, 120.04, 117.35, 116.27, 115.76, 109.72. Anal. Calcd. For $C_{15}H_8N_2O_3$: C, 68.18; H, 3.05; N, 10.60. Found: C, 68.01; H, 3.11; N, 10.58.

2.3.2. 2-(4-(3,4-Dicyanophenoxy) benzamido) propanoic acid (**2**, Scheme 1)

Thionyl chloride ($SOCl_2$, 5 mL) was added dropwise to the solution of **1** (1 g, 3.78 mmol) in CH_2Cl_2 (10 mL) with 5 drops DMF at 0 °C. Then, the mixture was heated to reflux and stirring for 5 h under nitrogen atmosphere. After evaporation of the solvent, the residue was redissolved in acetone (18 mL). The solution was added to a solution of K_2CO_3 (1.05 g, 7.57 mmol) and L-alanine (0.37 g, 4.16 mmol) in water (3 mL) at room temperature. After stirring for 30 min, the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (15 mL) and washed with distilled water (3 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4. The crude product was purified by silica gel column chromatography using petroleum ether/ethyl acetate (1:3 v/v) with drops of CH_3COOH as the eluent to afford **2** as a white solid (0.84 g, 66.1%). M.P. > 200 °C. IR (KBr, cm^{-1}): 3379 (OH), 2230 (CN), 1720 (C=O), 1654 (C=O), 1603, 1485, 1256. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 11.35 (s, 1H, COOH), 8.66 (d, 1H, 7.2 Hz, NH), 7.99 (d, 2H, 8.6 Hz, ArH), 7.84 (d, 1H, 8.2 Hz, Ar), 7.39–7.42 (m, 1H, ArH), 7.31 (d, 1H, 2.0 Hz, ArH), 7.23 (d, 2H, 8.6 Hz, ArH), 4.40 (t, 1H, 7.2 Hz, CH), 1.39 (d, 3H, 7.3 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 174.72, 168.99, 168.77, 165.66, 162.03, 157.95, 135.80, 130.98, 130.39, 127.67, 125.84, 123.89, 119.75, 112.53, 48.78, 17.48. Anal. Calcd. For $C_{18}H_{13}N_3O_4$: C, 64.47; H, 3.91; N, 12.53. Found: C, 64.38; H, 4.01; N, 12.50.

2.3.3. 2(3), 9(10), 16(17), 23(24)-tetra-(4-((3-methyl) carbonylmethylaminocarboxyl) phenoxy) phthalocyaninato-zinc (II) (***ZnPc1***, Scheme 1)

A mixture of phthalonitrile **2** (0.46 g, 1.37 mmol), $Zn(OAc)_2$ (0.16 g, 0.86 mmol) and DBU (0.4 mL, 2.67 mmol) in n-pentanol (10 mL) was heated at 140 °C for 24 h under nitrogen atmosphere. After evaporating the solvent under reduced pressure, the crude green solid was thoroughly washed with CH_2Cl_2. The crude product was purified by silica gel column chromatography using CH_3OH/CH_3COOH (100:1 v/v) as the eluent to afford **ZnPc1** as a dark green solid (0.24 g, 49.7%). M.P. > 200 °C. IR (KBr, cm^{-1}): 3330, 3060, 1720 (C=O), 1650 (C=O), 1525 (NH), 1480, 1240. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 9.01 (s, 4H, NH), 8.71–8.78 (m, 8H, ArH), 8.08–8.17 (m, 8H, PcH), 7.81–7.83 (d, 4H, 7.6 Hz, PcH), 7.45–7.56 (m, 8H, ArH), 4.46–4.51 (m, 4H, CH), 1.45 (t, 12H, 5.9 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 174.71, 166.06, 160.52, 159.96, 157.78, 157.38, 139.87, 133.88, 130.46, 129.74, 129.46, 125.85, 124.62, 121.38, 119.75, 118.87, 118.59, 118.48, 114.79, 103.12, 100.10, 48.98, 48.78, 17.37. Anal. Calcd. For $C_{72}H_{52}N_{12}O_{16}Zn$: C, 61.48; H, 3.73; N, 11.95. Found: C, 60.86; H, 3.92; N, 11.80.

Compounds **ZnPc2** and **ZnPc3** were prepared in our laboratory (their structures were shown in Scheme 1) [22]. Their structures have also been fully characterized.

2.4. Charge measurement

The charges of the three ZnPcs were measured by a Malvern Zetasizer Nano 90 light scattering. The ZnPcs were dilute with distilled water. The experiments were performed three times and an average of the results was used.

2.5. Photostability assaying

Photostability studies of Pcs used in this work were carried out in water by monitoring the decrease in the Q-band absorption before and after irradiation with 665 nm LED using UV–Vis spectrophotometer.

2.6. Singlet oxygen detection

The singlet oxygen generation ability of the Pcs in solutions was determined by using the method described before [23]. Pcs