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Characterization, catalyzed water oxidation and anticancer activities of a NIR BODIPY-Mn polymer

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ABSTRACT

To obtain near-IR absorbing biomaterials as fluorescence cellular imaging and anticancer agents for hypoxic cancer cell, a nano NIR fluorescence Mn(III/IV) polymer (PMnD) was spectroscopically characterized. The PMnD shows strong emission at 661 nm when excited with 643 nm. Furthermore, PMnD can catalyze water oxidation to generate dioxygen when irradiated by red LED light (10 W). In particular, the PMnD can enter into HepG-2 cells and mitochondria. Both anticancer activity and the inhibition of the expression of HIF-1α for PMnD were concentration dependent. Our results demonstrate that PMnD can be developed as mitochondria targeted imaging agents and new inhibitors for HIF-1 in hypoxic cancer cells.

1. Introduction

In hypoxic condition, cancer cells promote appropriate responses such as anaerobic metabolism and increased oxygen delivery so that they can survive and even proliferate in a hypoxic environment. Hypoxia-inducible factor (HIF) is a common link between O_2 availability, malignant progression, and changes in cancer metabolism [1,2]. HIF regulates the energy metabolism by triggering a switch from mitochondrial oxidative phosphorylation to anaerobic glycolysis, increasing the expression of genes that encode glycolytic enzymes and glucose transporters [3]. Moreover, HIF-1 controls the expression of a variety of genes, the protein products of which play crucial roles in the acute and chronic adaptation to oxygen deficiency. Loss of HIF-1 activity dramatically decreases tumor growth, vascularization, and energy metabolism [4–5]. Hence, targeted inhibition of HIF-1α is an important pathway to cancer therapy [6,7]. Manganese (Mn) is an essential metal found in a variety of biological tissues and plays important role as a cofactor in many enzymatic reactions, including the anti-oxidant enzyme superoxide dismutase, as well as enzymes involved in neurotransmitter synthesis and metabolism in the brain [8], which is necessary for normal functioning of a variety of physiological processes including amino acid, lipid, protein and carbohydrate metabolism [9]. This makes manganese compounds biocompatible entities which are less harmful to the patients and are therefore more safe [10]. Manganese(II) complexes have been found to induce apoptosis and autophagy by targeting mitochondria through generation of ROS and disruption of mitochondrial membrane transition pore [11–14]. Moreover, Mn(II) complexes with a boradiazaindacenes (BODIPY) group have been reported as fluorescence image and photo-active anticancer complexes [15].

Boradiazaindacenes (BODIPY, or difluoroboradipyrromethenes) have been as very popular fluorophores with high quantum yields, long-wavelength absorption and fluorescence emission [16]. The absorption and emission bands associated with the S1 state of the unmodified BODIPY parent complex lie at ca. 500 nm. Red-shift absorbing BODIPY derivatives can be obtained by the introduction of aromatic or heteroaromatic rings as substituents at the 3-, 5- and/or 1-,7-positions on the pyrrole moieties [17]. Near-IR absorbing BODIPY derivatives can be fluorescence cellular imaging agents. Recently, fluorescence manganese-complexes have been reported as potential fluorescence imaging (FI) agents and mitochondria target anticancer complexes [18]. However, the low solubility of BODIPY derivatives limits their application in cells. PEG nanoparticles are biocompatible, neutral, hydrophilic molecules in biological fluids, which help to improve the dispersity and blood circulation of the complexes they are bound to [19,20]. To develop a new kinds of NIR light driven dioxygen generator as anticancer agents for hypoxic cancer cell, herein, a NIR fluorescence manganese polyethylene glycol nanoparticle, [PEGMn$^{III/IV}$Cl(DBA)$_2$] (labelled as PMnD, DBA = diethyl 5-((4-(5,5-difluoro-3,7-bis((E)-4-methoxystyry)-1,9-dimethyl-5H-4λ,5λ-dipyrrolo[1,2-c:2′,1′-f][1,3,2]diazaborinin-10-yl)benzyl)oxy)isophthalate), Scheme 1), was investigated for photo-driven water oxidation, the anti-proliferative activities and fluorescence imaging potentials.

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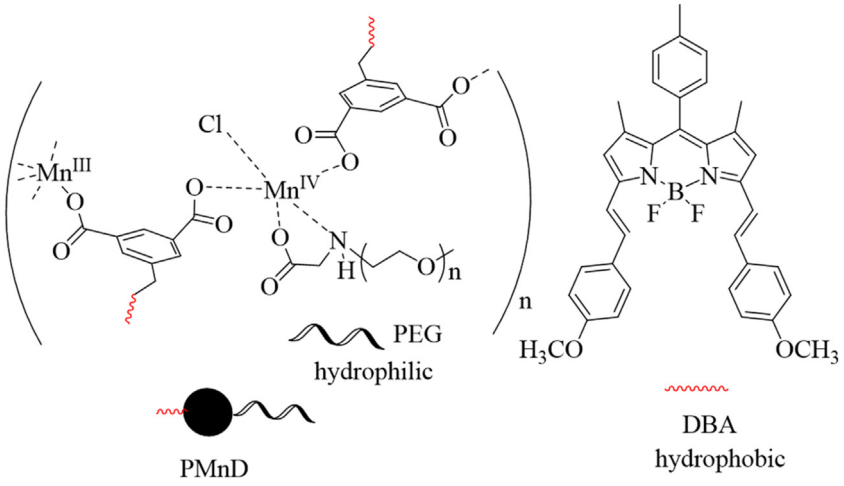

Scheme 1. The structures of DBA and PMnD.

2. Experimental

2.1. Materials and Measurements

Chemicals (AR purity), unless otherwise indicated, were purchased from Sinopharm Chemical Reagent Co., Ltd. Anisic aldehyde (98%), 4-dimethylaminobenzaldehyde (99%), 2,4-dimethylpyrrole (98%) were purchased from Energy Chemical Reagent Co., Ltd. 8-(3-Chlorobenzyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene [21] and PEG_{2000}-$NHCH_2COOH$ [22] were synthesized according to literature procedures, respectively. FT-IR characterizations were performed using a Nicolet Nexus 470 FT-IR spectrophotometer in the wave number range of 4000–400 cm^{-1}. The electronic absorption spectrum was recorded using a UV-2450 UV–vis spectrophotometer at room temperature. The concentration of manganese in PMnD was measured in triplicate using an atomic absorption spectrometer. Fluorescence measurements were performed on a fluorescence spectrofluorometer Model CARY Eclipse (VARIAN, USA), a 1.0 cm quartzcell (ex = 460 nm, slit width = 5 nm). The electrospray mass spectra (ES-MS) were determined on a Finnigan LCQ mass spectrograph. TEM was performed at room temperature on a JEOLJEM-200CX transmission electron microscope using an accelerating voltage of 200 kV.

2.2. Synthesis of Diethyl 5-((4-(5,5-difluoro-3,7-bis((E)-4-methoxystyry)-1,9-dimethyl-5H-4λ,5λ-dipyrrolo[1,2-c:2′,1′-f] [1,3,2]diazaborinin-10-yl)benzyl)oxy)isophthalate (DBA)

8-(3-Chlorobenzyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a–diaza-s-indacene (372 mg, 1.0 mmol) was dissolved in tetrahydrofuran (THF) (10 mL) under nitrogen at room temperature. K_2CO_3 (166.1 mg, 0.20 mmol) and 5-hydroxy-terephthalate (336 mg, 1.4 mmol) in THF (5 mL) was added. The solution was stirred at 70 °C for 6 h, and then concentrated to remove THF. The mixture was extracted with chloroform (20 mL). The organic layer was washed with water (3 × 10 mL), dried with sodium sulfate, and then concentrated to give brown solids after column chromatography on silica gel. Next, the brown solids (413.6 mg, 0.68 mmol) was dissolved in toluene (10 mL) under nitrogen at room temperature, 4-methoxybenzaldehyde (258.4 mg, 1.90 mmol), 4-methylbenzenesulfonate (22.4 mg, 0.13 mmol) and piperidine (18.7 mg, 0.22 mmol) were added. The reaction was stirred at 120 °C for 8 h. The solvent was removed under the vacuum and the residue was dissolved in chloroform. The organic layer was washed with saturated sodium chloride (3 × 10 mL), dried with sodium sulfate and concentrated to give blue solid (220.3 mg) after column chromatography on silica gel. Yield. 40.3%. MS (EsI^+): Calcd for $C_{48}H_{45}BF_2N_2O_7$ $[M + H]^+$ m/z = 811.70, Found m/z = 746.3 ($-BF_2 + 2H$). Found: C, 71.11; H, 5.60; N, 3.48, O, 13.81; B, 1.33; F, 4.69. Calcd. (%) for $C_{32}H_{34}BCl_3FeF_2N_5O$: C, 71.01; H, 5.32; N, 3.37; O, 13.62; B, 1.56; F, 5.12. ^1H NMR (400 MHz $CDCl_3$). δ = 8.32 (s.1H), 7.86–7.85 (d, J = 4 Hz, 2 H), 7.64 (s,1H), 7.60–7.58 (m, 7H), 7.38–7.36 (d, J = 8 Hz, 2 H), 7.24 (s,1H), 7.20 (s, 2 H), 6.95–6.93 (d, J = 8 Hz, 4H), 6.62 (s, 2 H), 5.28 (s, 2 H), 4.44–4.39 (q, J = 8 Hz, 4H), 3.86 (s, 6H), 1.44–1.41 (m, 12 H). UV–vis (CH_2Cl_2/nm) (ε × 10^4, $dm^3 \cdot mol^{-1} \cdot cm^{-1}$): 229 (3.7), 316 (2.2), 371 (6.9), 593 (3.9), 643 (10.5). IR (KBr, ν/cm^{-1}): 3079, 2967, 2919, 2838, 1716, 1602, 1535, 1484, 1369, 1301, 1113, 987, 821, 765, 717.

2.3. Synthesis of PMnD Nanoparticles

PEG_{2000}-$NHCH_2COOH$ (103 mg, 0.05 mmol) was dissolved in ethanol (10 mL) followed by addition of $MnCl_2 \cdot 4H_2O$ (152 mg, 0.07 mmol). The solution was stirred at 100 °C for 6 h and a yellow solid (PEG-Mn) was obtained, was purified with dialysis membrane in water for 12 h and sample was dried under the vacuum. Next, the DBA (80.3 mg, 0.1 mmol) in THF (3 mL) was mixed with NaOH (8 mg, 0.2 mmol) in water (2 mL), the solution was heated at 40 °C for 3 h and a solution of PEG-Mn (50.4 mg) in DMF (2 mL) was added. The mixture was stirred at 100 °C for 6 h and product was purified with dialysis membrane in water for 12 h, resulting in blue PMnD isolate.

2.4. Cytotoxicity Testing

The cytotoxicity assays were measured with HepG-2 cells in normal culture conditions. HepG-2 cells were seeded at a density of 4×10^{-4} cells mL^{-1} into sterile 96-well plates. The PEGMn, DBA and PMnD nanoparticles were added in DMSO and diluted with culture media. After 24 h, compounds were added into the cultured HepG-2 cells for 24 h. Cell viability was determined by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenpyltetra-zolium bromide (MTT) assay measuring the absorbance at 570 nm. Each test was performed in triplicate. Morphological change of HepG-2 cells was taken using Nikon Ti-E2000 microscope with live cell system.

2.5. Cell Imaging

HepG-2 cancer cells (hepatoma cancer cell) was inoculated into culture plate with 2.4×10^4 cells in each well and incubated for 24 h. Compounds were purified with semipermeable membrane and diluted to an appropriate concentration with culture solution respectively, and then inoculated with HepG-2 cancer cells for 4 h at 37 °C. The medium was washed by PBS buffer to remove the free compound before detecting