A photo-Responsive Porphyrin-Mn@Choles Complex for Bacteria Treatment

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Abstract

Biocompatible photo-driven producers of singlet oxygen can inhibit the growth of drug-resistant bacteria and tumors. In order to bacteria targeting generator of singlet oxygen for tumor and bacterial treatment, a metal porphyrin liposome (Phy-Mn-Ls) was prepared by the metal coordination reaction and self-assembly of porphyrin compounds with bacteria targeting polymer (HS-PEG-chol). The photo-driven production of $^1\text{O}_2$, binding with protein (BSA) and lipase, toxicity to MCF-7 breast cancer cells and inhibitory effect on the growth of *Escherichia coli* were investigated. Fluorescence analysis results show that Phy-Mn-Ls can bind to lipase, and it shows less effect on the conformation of BSA and is low cytotoxicity without irradiation. In particular, the good biocompatibility made Phy-Mn-Ls exhibit good photosensitive antibacterial activity and anti-tumor properties. The results demonstrate that the coordination of HS-PEG-chol with metal-porphyrin coordination is an effective way to develop bacteria targeting nano-complexes (Phy-Mn-Ls) for lipase affinity and photodriven bacteria treatment.

1 Introduction

The drug resistant bacteria and the effect of bactericides to environment have caused a public healthy issue [1, 2]. It is necessary to develop new bactericides for the treatment of pathogen infection [3, 4]. Photo-irradiated reactive species (such as $^1\text{O}_2$), have been reported as effective photo-driven therapy agents [5, 6]. Singlet oxygen and other reactive oxygen species (ROS) can damage protein or cause the oxidation of intracellular thiols in bacteria, which could reduce the resistance of bacteria to drug [7]. Porphyrins and boron-dipyrromethene fluorophore (BODIPY) derivatives were reported as $^1\text{O}_2$ generators for photodynamic therapy [8–10]. Photoactive Mn(II) complexes can be $^1\text{O}_2$ generators for the cancer cell treatment by attenuating the cell energy metabolism [11]. Metal porphyrins were reported as mimics of PSII for the oxygen generation [12–15]. Oxygen and $^1\text{O}_2$ can attenuate the cell metabolism. Attenuating the cell metabolism is also a promising strategy for pathogen infection [16]. Bacteria have low metabolic activity and are less sensitive to antibacterial compounds, while bacteria show strong response to nanoparticles because of their good cell membrane penetration [17–19]. Porphyrin could assemble with cholesterol-linked polyethylene glycol polymer (Chol-PEG) resulting bacteria surface targeting nanoparticles [20, 21]. Therefore, Chol-PEG conjugated porphyrin might be targeting inhibitors of bacteria. However, it is a challenge that compound is reversible affinity to protein and active to bacteria inhibition. To develop sustainable nano-generator of $^1\text{O}_2$ for bacteria treatment, thiol polyethylene glycol cholesterol (HS-PEG-chol) coordinated and assembled with Mn(II) complex of porphyrin (TAPP, scheme 1) resulting a photodriven nano-generator (Phy-Mn-Ls) of $^1\text{O}_2$ for cancer and bacteria treatment.

2 Experimental

2.1 Chemicals and instruments

1,3-Diphenyl isobenzofuran (DPBF), thiol polyethylene glycol cholesterol (HS-PEG-chol, Mw. 3400), MnCl_2 and dichloromethane (DCM), bovine serum protein (BSA), pancreatic lipase (PL), were obtained from DeWei Chemicals (Zhenjiang, China) Co., Ltd. 2-Chloro-N-(4-(10,15,20-triphenylporphyrin-5-yl)phenyl)acetamide (TAPP) was synthesized according to a reported publication [22]. Fluorescence spectra were taken on a fluorescence spectrofluorometer (VARIAN, USA), (slit width = 5 nm). The UV-vis spectra were measured on a UV-2450 spectrophotometer. FT-IR spectra were recorded by a Nicolet Nexus 470 FT-IR spectrophotometer.

2.2 Synthesis of Mn complex of 2-chloro-N-(4-(10,15,20-triphenylporphyrin-5-yl) phenyl)acetamide thiol polyethylene glycol cholesterol (Phy-Mn-Ls)

TAPP (100 mg, 0.12 mmol) was added into the ethanol solution (20 mL) of MnCl_2 (23 mg, 0.12 mmol). After the mixture was stirred at 80°C for 1 h, HS-PEG-chol (20 mg) was added, and then the mixture was stirred at 80°C for 4 h. After cooled to room temperature, the solution was purified by cellulose membrane (5000) dialysis in DMF and water, respectively. The Phy-Mn-Ls (80 mg) was obtained after concentration and dried in vacuum, yield, 60 %.

2.3 Interaction with protein and lipase

The interaction between Phy-Mn-Ls and BSA or pancreatic lipase (PL) was determined on CARY Eclipse fluorescence spectrophotometer. Firstly, BSA or PL solution (0.5 mg/mL) in PBS buffer (20 mM, pH 7.0) was prepared. Different volume of Phy-Mn-Ls in water (100 $\mu\text{g/mL}$) was mixed with proteins. After have shaken for 5 min, the emission spectra ($\lambda_{\text{ex}} = 290 \text{ nm}$; $\lambda_{\text{em}} = 340 \text{ nm}$) were determined.

2.4 Singlet oxygen determination

1,3-Diphenyl isobenzofuran (DPBF) (100 μM , 15 mL) and Phy-Mn-Ls (2 μM , 15 mL) in water were mixed; the absorption change of DPBF at 415 nm was recorded on UV-vis spectrometer every 1 min under irradiation with red LED light (4 W).

2.5 Antibacterial

The effect of Phy-Mn-Ls on *E. coli* and *S. aureus* was investigated. The single colony of bacteria was inoculated in LB broth medium (3 mL), and cultured in a vibration incubator (180 RPM) at 37°C under aerobic condition. Bacterial growth was evaluated by measuring absorbance at 600 nm (OD_{600}). Bacteria were washed with PBS and collected by centrifugation (10 min, $\sim 2000 \text{ g/m}$) for 10 min. Then the bacteria were diluted by PBS solution to $1 \times 10^5 \text{ CFU}\cdot\text{mL}^{-1}$, all instruments were sterilized before use.

2.6 MTT assay

The anticancer property of Phy-Mn-Ls was evaluated by measuring the inhibition of samples on the proliferation of MCF-7 cancer cells under normal culture conditions. Cells (4×10^4 per milliliter) were incubated into sterile 96-well plates for 24 h. Phy-Mn-Ls (100 $\mu\text{g/L}$) was diluted with culture solution to

different concentrations and then was incubated with MCF-7 cells for 24 h. Cell viability was evaluated by recording the absorption at 570 nm (MTT assay). The test was repeated for three times.

Scheme 1 Synthesis route of Phy-Mn-Ls

3 Results And Discussions

3.1 Characterization of Phy-Mn-Ls

NMR and ES-MS spectra confirm the structure of TAPP (Fig. S1, Fig. S2). TAPP coordinated with MnCl_2 , in which Mn-Cl can be replaced by HS-PEG-chol producing Phy-Mn-Ls (Scheme 1). ESR data confirm the existence of Mn(II) ion in Phy-Mn-Ls (Fig. 1). The hydrophobic interaction between TAPP and cholesterol of HS-PEG-chol makes the Phy-Mn-Ls assembled into nano-complex. The UV-vis absorption of Phy-Mn-Ls in the range of 400–450 nm shows a hypochromic effect compared to TAPP due to the hydrophobic interaction of TAPP and HS-PEG-chol. The absorption at 475 nm of Phy-Mn-Ls indicates the existence of TAPP. The content of TAPP in Phy-Mn-Ls was evaluated to be 65% according to the absorption of Phy-Mn-Ls in DCM at 475 nm. In IR spectra, the peak at $2933\text{--}2978\text{ cm}^{-1}$, 3033 cm^{-1} , 2500 cm^{-1} indicate the presence of H-C, H-C=, H-S groups in Phy-Mn-Ls.

Figure 1 (a) UV absorption spectra of TAPP (red) and Phy-Mn-Ls (black) (0.2 mg/mL, H₂O); (b) Infrared spectra of Phy-Mn-Ls (red) and TAPP (blue); (c) ESR spectrum of Phy-Mn-Ls at 298K; (d) SEM diagram of Phy-Mn-Ls

3.2 Interaction with lipase and BSA

Figure 2 (a) Fluorescence emission spectra of BSA (0.5 mg / mL) with the addition of Phy-Mn-Ls (a to g: 0, 0.02, 0.04, 0.06, 0.08, 0.1, 0.12 mg/mL), excited at 290 nm. (b) Fluorescence emission spectra of lipase (0.5 mg/mL) in the presence of Phy-Mn-Ls (a to i: 0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08 mg/mL)

Figure 3 Circular dichromatography of bovine serum protein (BSA, 1×10^{-4} mg/mL), (Red line, BSA; blue line, BSA and 2×10^{-4} mg/mL of Phy-Mn-Ls)

There are high level of lipase or fat acid synthase in most bacteria or cancer cells. The interaction of Phy-Mn-Ls with lipase was investigated by measuring the fluorescence emission change of lipase at 348 nm. The addition of Phy-Mn-Ls to lipase resulted in the decrease of fluorescence intensity at 348 nm (Fig. 2), indicating a high affinity with lipase. The decreased fluorescence emission showed that the Phy-Mn-Ls can interacted with both lipase and BSA. The affinity of Phy-Mn-Ls to BSA is a litter stronger than lipase (Fig. 3b, 3c). Do the affinity of compounds with proteins change the conformation? The effect of Phy-Mn-Ls on the conformation of BSA was evaluated by Circular dichromatography (CD). Results show that the Phy-Mn-Ls changed the band intensity of the BSA at 195 nm and 210 nm, indicating the decreased level of α -helix BSA. The spectra of BSA and BSA/Phy-Mn-Ls are similar indicating the weak effect for Phy-

Mn-Ls on the conformation of BSA (Fig. 3). The higher affinity and weak conformation change indicate protein can be good carrier of Phy-Mn-Ls.

3.3 Photodriven generation of $^1\text{O}_2$

DPBF can combine singlet oxygen with the absorption change at 415 nm [18]. The peak of DPBF decreased at 415 nm under red LED light irradiation (Fig. 4a), indicating the production of singlet oxygen. Therefore, Phy-Mn-Ls is a photo-driven singlet oxygen generator for potential photo-dynamic therapy.

Figure 4 Degrading curve of DPBF by Phy-Mn-Ls (2 $\mu\text{mol/L}$) under light (red LED light) (recorded once every minute, 7 times)

Figure 5 Toxicity of Phy-Mn-Ls to MCF-7 cells (0, 5, 10, 20, 30, 40, 50 $\mu\text{g/mL}$) in the dark environment and the blue LED light compared to the control group. ($\pm\text{SD}$, $n=4$). * ($p < 0.02$), ** ($p < 0.004$)

3.4 MTT assay and antibacteria property

The cytotoxicity of Phy-Mn-Ls was studied by measuring the cell viability of compounds treated MCF-7 cells using the MTT assay. Under red LED light irradiation, Phy-Mn-Ls did alter the viability of MCF-7 after 24 h treatment. The IC_{50} of Phy-Mn-Ls is 1.674 $\mu\text{g/mL}$. In contrast, Phy-Mn-Ls shows no toxicity to cells without irradiation (Fig. 5). The higher the concentration of the compound the more it inhibits cell proliferation, which proves that the Phy-Mn-Ls is a concentration dependent photo-responsive anticancer agent.

The effect of Phy-Mn-Ls on the inhibition of *E. coli* was investigated (Fig. 6). Phy-Mn-Ls inhibited the bacteria proliferation under light irradiation; but the antibacterial potency of Phy-Mn-Ls was weak without irradiation which indicated that the Phy-Mn-Ls was a LED light responsive antibacterial agent. In the absence of Phy-Mn-Ls, LED light has little inhibition effect on bacterial growth. Therefore, Phy-Mn-Ls interact with lipase and produced singlet oxygen to inhibit bacteria under irradiation.

Figure 6 The inhibition of *Escherichia coli* by Phy-Mn-Ls under dark (black line) and red LED light (4W) irradiation (red line) ($n=3$, $\pm\text{SD}$)

4 Conclusions

A biocompatible nanocomplex (Phy-Mn-Ls) was synthesized and used as a photodynamic agent for both cancer and bacteria treatment. The porphyrin and cholesterol groups in Phy-Mn-Ls make it can bind with lipase, which are highly expressed in most bacteria. The Phy-Mn-Ls can produce $^1\text{O}_2$ under red LED light irradiation. Meanwhile, Phy-Mn-Ls show less effect on the conformation of BSA indicating BSA can be carrier of Phy-Mn-Ls. In particular, Phy-Mn-Ls show good inhibition on the proliferation of both MCF-7 cancer cells and *E. coli* bacteria under irradiation, while it is less toxicity to cells without irradiation. Results indicate that the Phy-Mn-Ls is an environmental friendly photoresponsive agent for bacteria treatment.

Declarations

Acknowledgements

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Figures

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Figure 1

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