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Synthesis of water-soluble and thermoresponsive phthalocyanine ended block copolymers as potential photosensitizer

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ABSTRACT

A novel zinc(II) tetraaminophthalocyanine ended block copolymer methoxypolyethylene glycol-*b*-poly(N-isopropylacrylamide) (MPEG-*b*-PNIPAAM-ZnTAPc) had been synthesized through an easy way. First, the diblock copolymers MPEG-*b*-PNIPAAM were synthesized by atom transfer radical polymerization (ATRP) and functionalized with one molecule of maleic anhydride (MA) by ATRP, and then reacted with ZnTAPc to form a water-soluble and thermoresponsive photosensitizer. The lower critical solution temperatures for the copolymers can be easily controlled by changing the ratio of MPEG and PNIPAAM. In aqueous solution, the thermosensitive copolymers will self-assemble into micelles and characterized by dynamic light scattering and transmission electron microscopy, and the sizes of micelles will increase with temperature increasing. In the micelles, ZnTAPc will aggregate to induce fluorescence quenching. As a photosensitizer, MPEG-*b*-PNIPAAM-MA-ZnTAPc can generated singlet oxygen species with good quantum yields ($\Phi_\Delta = 0.23$), which were believed to be the major reactive oxygen species. And the copolymer had low dark cytotoxicity and high phototoxicity based on MTT assay, so the MPEG-*b*-PNIPAAM-ZnTAPc will used in the field of photodynamic therapy and photocatalytic oxidation.

1. Introduction

Phthalocyanines (Pcs) are a class of natural macrocyclic compounds and have been used as dyes and pigments traditionally [1,2]. The ring of phthalocyanines is easy to be modified to change their physical parameters and subsequently broadening their applications [3–6]. Recently, Pcs have been used as sensing [7,8], nonlinear optics [9,10], and photovoltaic energy conversion [11]. Especially, Pcs is one of the most promising photosensitizers because of its strong absorption in far-red light and the ability to generate singlet oxygen with high efficiency [12–14]. However, Pcs' poor solubility and aggregation in aqueous solution limit its applications as photosensitizers in photodynamic therapy (PDT) and photocatalytic oxidation, especially in PDT. In the methods of improving Pcs solubility in aqueous solution, using water-soluble polymer encapsulated or graft is an effective way [15–17].

Photosensitizers physical loading by polymeric carriers [18–20] or as a core to irritate polymerization [16,21–24] are used extensively, but grafting photosensitizers on polymers directly is seldom reported. The latter approach will offer well-defined polymeric materials with precise photosensitizers' contents, avoids the unwanted release often occurred in physical loading, as well as increase its stability by chemically bonding with polymer backbone. Moreover, photosensitizers incorporated with multifunctional polymers will perform new propriety, such as when grafted with Poly(*N*-isopropylacrylamide) (PNIPAAM), the photosensitizer will be thermosensitive [25–27].

PNIPAAM is a typical thermosensitive polymer [28,29]. Below its lower critical solution temperature (LCST), PNIPAAM can dissolve in aqueous solution, while above LCST, the PNIPAAM molecules change from hydrophilic to hydrophobic to occur phase separation and precipitate from aqueous solution. In order to increase the LCST of PNIPAAM, grafting with a hydrophilic molecule, such as monomethoxy poly(ethylene glycol) (MPEG), is an effective way [30]. The ratio of PNIPAAM and MPEG can control the value of LCST. Above the LCST, the block copolymer is amphiphilic and has the ability to selfassemble in aqueous solution, below the LCST, the block copolymer is hydrophilic and can dissolve in aqueous solution [31]. Otherwise, hydrophobic molecule grafting on PNIPAAM will decrease its LCST [32].

Some efforts have introduced Pc on PNIPAAM by the method of atom transfer radical polymerization (ATRP). In their works, Pc is

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reacted to from Pc initiator to irritate NIPAAM polymerization by ATRP [33–35]. Different from this method [34], we found an efficient way to graft zinc(II) tetra-aminophthalocyanine (ZnTAPc) on MPEG-b-PNIPAAM with maleic anhydride (MA) as chain end directly. Because MA can hardly homopolymerize, using MPEG-b-PNIPAAM-Br as initiator to irritate MA by one step of ATRP and yield one molecule residing at every chain end is possible [36].

So in this work, the diblock copolymers MPEG-*b*-PNIPAAM, containing the same length of the hydrophilic MPEG but with different number of NIPANM units were synthesized by ATRP, and then the copolymers were functionalized with maleic anhydride (MA) to react with ZnTAPc to form a water-soluble and thermoresponsive polymer photosensitizers MPEG-*b*-PNIPAAM-ZnTAPc. We expected the MPEG-*b*-PNIPAAM-ZnTAPc has the abilities as follow: (1) Above the LCST, they can selfassemble into nanoparticles which will escape from capturing by the reticuloendothelial systems (RES) when used *in vivo* [37]. Below LCST, they will dissolve in water as a homogeneous solution to avoid aggregation. (2) Chemical attaching Pcs on MPEG-*b*-PNIPAAM are stable in aqueous solution that avoids the unwanted release efficiently. (3) MPEG-*b*-PNIPAAM-ZnTAPc irritated by light with an appropriate wavelength will generates enough singlet oxygen ($^1O_2$) to kill cancer cells effectively. It is expected that connecting Pcs with functional polymer in direct way is an easy method to get a promising photosensitizer to use in photodynamic therapy (PDT) in future.

2. Experimental

2.1. Materials

Monomethoxy poly(ethylene glycol) (MPEG; 99%, M_n = 1000), were purchased from Aladdin, eliminating trace water through azeotropic distillation with toluene before use. *N*-isopropyl acrylamide (NIPAM; 99%, Aldrich) was recrystallized three times from benzene/hexane (10:1 v/v) before use. Maleic anhydride (MA; 99%, Aladdin) was recrystallized three times from chloroform before use. The CuCl (99%, Aldrich) and CuBr (99%, Aldrich) catalyst was washed with glacial acetic acid and absolute alcohol and dried in vacuum and then stored under a nitrogen atmosphere. Tris[2-(dimethylamino)ethyl]amine (Me_6TREN) was synthesized according to the literature [38]. The 2,2′-bipyridine (bipy; 99%), N,N-diisopropylethylamine (DIEA; 99%), N-hydroxybenzotriazole (HOBT; 98%), 1,8-diazabicyclo [5,4,0]undec-7-ene (DBU; 99%), O-(1H-benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HBTU; 99%) and dimethyl carbonate (DMC; 99%) were purchased from Aladdin Reagent Company and used as received. All other chemicals were purchased from Sinopharm Chemical Reagent Co. and were used as received.

2.2. Process of MPEG-b-PNIPAAM-ZnTAPc synthesis

2.2.1. Synthesis of 2, 9, 16, 23-tetraamino-phthalocyanine (ZnTAPc)

The synthesis of ZnTAPc was shown in Scheme 1.

First, we used a green solvothermal method to synthesize tetra-substituted nitro zinc phthalocyanine (ZnTNPc). The mixture of 6 mmol 4-nitrophthalonitrile, 1.5 mmol of zinc(II) acetate dihydrate, and suitable amount of absolute ethanol were added in a 50 mL Teflon-lined autoclave, and then maintained at 190 °C for 3 h. After cooling to room temperature, the blue-green product was taken out and washed with hot ethanol and water to remove the residual reagents, and the ZnTNPc were dried at 100 °C in vacuum overnight. Yield: 45%. FT-IR (KBr): í (Pc) 1115, 1041, 929, 817, 757, 730 cm^{-1}; í (NO_2) 1340, 1521 cm^{-1} (Fig. 1). ^{1}H NMR (400 MHz, DMSO-d_6): ä = 7.87 (d, 4H, Pc-$H_á$), 8.26 (s, 4H, Pc-$H_à$), 8.51 (d, 4H, Pc-$H_á$) (Fig. 2A).

Second, 1.0 mmol ZnTNPc was dispersed in 15 ml DMF, then 12 mmol of sodium sulphide nonahydrate was added and stirred gently until the temperature reach to 60 °C and continued for 1 h. Then the products were poured into 150 mL water and filtered by pump, the solid product was separated and purified further using 1.0 M hydrochloric acid and 1.0 M sodium hydroxide. Finally, the complex was washed with water until free from sodium hydroxide and sodium chloride. The dark bluish green product ZnTAPc was dried in vacuum over phosphorus pentoxide. Yield: 55%. ^{1}H NMR (400 MHz, DMSO-d_6): ä = 6.23 (s, 8H, -NH_2), 7.37 (d, 4H, Pc-$H_á$), 8.42 (d, 4H, Pc-$H_á$), 8.91 (q, 4H, Pc-$H_à$) (Fig. 2B). FT-IR (KBr) of ZnTNPc and ZnTAPc were shown in Fig. 1.

2.2.2. Synthesis of the MPEG-Br

The MPEG macroinitiator (MPEG-Br) was synthesized according to a procedure in reported previously [39,40]. In brief, MPEG (2.0 g, 2 mmol) and TEA (0.40 g, 4 mmol) were dissolved in 30 ml of dry methylene chloride in a two-neck round flask. After cooling to 0 °C in an ice bath, 2-bromoisobutyryl bromide (0.69 g, 3 mmol) was added dropwise with gentle stirring, and the reaction mixture was warmed to room temperature under N_2 protection for 24 h. Then the crude mixture was poured into cold diethyl ether to precipitate. The crude products were filtered and washed with diethyl ether, and then dissolved in methylene chloride (300 mL) to wash with distilled water (3 × 50 mL). And the organic layer was dried over anhydrous magnesium sulfate and filtered. Finally, the MPEG-Br was isolated by precipitation from concentrated methylene chloride under reduced pressure into cold diethyl ether. Yield: 75%. FT-IR (KBr): ν(CH_3, CH_2) 2870 cm^{-1}, ν(C=O) 1734 cm^{-1}, ν(C-O) 1112 cm^{-1} (Fig. 3). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.94 (s, 6H, -$C(CH_3)_2$-), 3.38 (s, 3H, CH_3O-), 3.64–3.75 (m, ~96H, -CH_2CH_2O-) (Fig. 4A).

2.2.3. Synthesis of diblock copolymer MPEG-b-PNIPAAM

The diblock copolymer MPEG-*b*-PNIPAAM was synthesized by means of atom transfer radical polymerization (ATRP), which was carried out in a water/DMF 40:60 (v/v) mixture as the solvent at 25 °C with MPEG-Br/CuCl/Me_6TREN as the initiator/catalyst system and a molar feed ratio ([NIPAAM]) 2.0 M, [NIPAAM]/[MPEGMI]/[CuCl]/[Me_6TREN] = 80:1:1:1:1). Typically, NIPAAM (9.05 g, 80 mmol) and MPEG-Br (1.0 g, 1 mmol) were dissolved in 40 mL of water/DMF solvent mixture in a 100 mL Schlenk flask under magnetic stirring. After NIPAM and MPEG-Br were completely dissolved, the mixture was degassed by three freeze-pump-thaw cycles. The flask was then filled with nitrogen and immersed in a water bath that was kept at about 25 °C. The freshly prepared Cu(I) and Me_6TREN complex stock solution of 2 mL (prepared by adding degassed water of 8 mL to CuCl of 12 mmol and Me_6TREN of 12 mmol under nitrogen flow) was withdrawn *via* a syringe and quickly added to the above mixture under nitrogen flow, and the polymerization reaction was then initiated. After 1 h, the polymerization was terminated by exposing the mixture to air, diluting with THF and passed through Al_2O_3 column (basic, activated) to remove Cu complexes. The copolymer was further purified by dialyzing against distilled water for three days with a molecular weight cutoff of 3500. The white solid product was isolated by lyophilization. FT-IR (KBr): ν(C=O) 1648 cm^{-1}, ν(N-H) 1545 cm^{-1}(Fig. 3). ^{1}H NMR (400 MHz, D_2O): δ = 1.07 (s, ~576H, -CH_3), 1.51 (br t, ~192H, -CH_2-), 2.00 (br d, ~96H, -CH(C=O)-), 3.31 (s, 3H, CH_3O-), 3.63 (s, ~96H, -CH_2CH_2O-), 3.82 (s, ~96H, -CH(NH)-) (Fig. 4B).

2.2.4. Synthesis of maleic anhydride terminated MPEG-b-PNIPAAM

The terminal functionalization of MPEG-*b*-PNIPAAM was carried out in a previously dried Schlenk flask equipped with a magnetic