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Fabrication of aggregation induced emission active luminescent chitosan nanoparticles via a "one-pot" multicomponent reaction

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ABSTRACT

Chitosan based nanomaterials have been extensively examined for biomedical applications for their biodegradability, low toxicity, biological activity and low cost. In this work, a novel strategy for fabrication of luminescent polymeric nanoparticles (LPNs) based on aggregation induced emission (AIE) dye and water soluble chitosan (WS-Chitosan) were firstly developed via a highly efficient mercaptoacetic acid (MA) locking imine reaction. In this multicomponent reaction (MCR), MA serves as "lock" to connect 9,10-Bis(aldehydephenl)anthracene dye (An-CHO) and amino-containing WS-Chitosan under mild reaction conditions. The obtained WS-Chitosan@An-CHO LPNs show strong yellow emission and great water dispersibility. Biological evaluation results demonstrated that synthetic luminescent polymeric nanoparticles possess desirable cytocompatibility and distinct imaging properties. Therefore, we have developed a facile and useful method to fabricate AIE active nanoprobes with desirable properties for various biomedical applications. This strategy should be a general and easy handling tool to fabricate many other AIE dye based materials.

1. Introduction

In recent years, growing attentions have been paid to develop bioprobes based on luminescent polymeric nanoparticles (LPNs) for wide biomedical applications (Liu et al., 2015, 2016; Nie, Xing, Kim, & Simons, 2007; Wang, Yuan, et al., 2014). Fluorescent imaging has been regarded as one of the useful tools to monitor the biological behavior process existed in complicated intracellular system with strong sensitivity and absolute inviolability (Kang, Ha, Yun, Yu, & Chang, 2011), and have been successfully applied as a versatile way for disease diagnosis (Liu, Tu, Zhu, Ma, & Chen, 2013), tracking and treatment (Shi, Liu, Geng, Tang, & Liu, 2012). So far, traditional luminescent probes were prepared based on some luminescent materials such as inorganic quantum dots (Wu et al., 2003; Zhang, Wang, Liu, et al., 2013) organic dyes (Wang et al., 2005), organic dye-loaded silica nanoparticles and fluorescent proteins (Heilemann et al., 2008), which were extensively applied in labeling cells and tissue specimens. Furthermore, the unique features such as high photostability and sensitivity of these luminescent materials make them allow real-time imaging of important intracellular molecules. Unfortunately, some vicious drawbacks existed in above-mentioned luminescent materials have become obstacle for their progress in cell labeling application. For example, inorganic quantum dots (Qdots) suffer from high toxicity when they were accumulated in cellular environment owing to the existence of heavy metal ions (Derfus, Chan, & Bhatia, 2004), which would cause terrible influence for human healthy. While dye-loaded silica nanoparticles possess poor biodegradability although they are highly luminescent and homogeneous (Roy et al., 2005). The green fluorescent protein also encounter high cost, easy enzyme degradation, small stokes shifts, terrible photostability and tedious transfection process and transfection efficiency (Romantschuk et al., 2000), limiting their potential use in biomedical imaging application in spite of overcoming poor degradable problems bring from Qdots and fluorescent silica nanoparticles. Meanwhile, traditional organic dyes such as fluoresceins and rhodamines often suffer from luminescence quenching phenomena when presented in solution with high concentration or aggregated state, which was also called aggregation caused quenching (ACQ) effect. Therefore, in order to conquer these problems of poor biodegradability, toxi-

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city and ACQ effect, novel fluorescent materials with better optical and biological properties should be developed.

Aggregation induced emission (AIE) dyes were firstly reported by Tang et al. in 2001 (Luo et al., 2001). AIE dyes emitted strong fluorescence in their aggregated state while non-fluorescence or weak fluorescence in dispersed state, which could effectively solve ACQ effect of conventional organic dyes. Since discovery, a number of AIE fluorogens such as siloles (Wang, Zhang, Zhang, Zhu, & Tang, 2010), cyano-substituted diarylethene (Zhang, Liu, et al., 2013; Zhang, Ma, et al., 2013), tetraphenylethene (Chi et al., 2012; Wan et al., 2016; Wan, Wang, et al., 2015; Wan, Wang, He, et al., 2015; Wu et al., 2012), tripheneylethene (Chen et al., 2012; Li et al., 2011; Zhang et al., 2010, 2011; Zhang et al., 2014b), distyrylanthracene derivatives (Wang et al., 2013; Zhang, Zhang, Yang, et al., 2013) have been synthesized and drastically investigated for their potential applications in fields such as bio/chem sensors, cell imaging and ion probes due to their excellent AIE characteristics, biodegradability and cytocompatibility. Up to now, some strategies for fabricating luminescent probes based on AIE dyes have drawn increasing attentions for their potential medical applications. Generally, two major methods for constructing LPNs based on AIE dyes have been developed, which contained non-covalent methods and covalent methods (Huang et al., 2015; Liu, Zhang, Yang, Deng, et al., 2014; Liu, Zhang, Yang, Liu, et al., 2014; Zhang, Zhang, Wang, et al., 2013; Zhang, Zhang, Yang, Hui, et al., 2014). The non-covalent methods for preparing LPNs were that embedding the AIE dyes into the biocompatible amphiphilic polymers to obtain water dispersible luminescent nanoparticles. Other silica nanoparticles encapsulation and bovine serum albumin (BSA) implantation methods were also used to construct AIE active LPNs (Qin et al., 2012; Zhang, Wang, Zhu, et al., 2013; Zhang, Zhang, Yang, Liu, et al., 2014). Nevertheless, the easy leakage of AIE dyes and separation of surface coating from LPNs have become serious restriction of non-covalent systems. On the other hand, covalent strategies to prepare LPNs could be summarized that reversible addition fragmentation chain transfer polymerization (Zhang et al., 2014d), cross-linked Schiff based dynamic bonds (Wan, He, et al., 2015; Yang et al., 2013; Zhang, Zhang, Wang, et al., 2013), emulsion polymerization (Zhang et al., 2014c) and anhydride ring-opening reaction (Zhang et al., 2014a). Based on these techniques, numerous AIE active LPNs with uniform size, great biocompatibility, high water dispersibility and strong luminescence have been fabricated and utilized for cell imaging. Although the impressive progress was made for fabrication of AIE active LPNs in methodology, more fabricated strategies with great stability, high-efficiency, short reaction time and environmentally friendly (catalyst free, room temperature condition and air atmosphere) and multifunctional potential will receive more pursuit from scientists.

The water-soluble chitosan (named as WS-Chitosan) based luminescent nanoparticles have been intensively researched for biomedical applications due to their favorable features such as biocompatibility, non-toxicity and degradable potential (Qi, Xu, Jiang, Hu & Zou, 2004). In this contribution, we report a robust synthesis protocol to prepare ultra-small cross-linked WS-Chitosan@An-CHO LPNs with uniform size, high water dispersibility, great biocompatibility and strong luminescent property via a “one-pot” multicomponent reaction (MCR).

2. Experimental

2.1. Materials and methods

All chemical agents were of analytical grade and used as received without any further purification. All the chemicals such as chitosan (deacetylation ≥ 95%, viscosity: 100–200 mpa s), hydrogen peroxide (30 wt.%), ammonium persulphate (98%, 228.2 Da), anthracene (98%, 178.3 Da) and terephthalaldehyde (98%, 134.13 Da) were purchased from Aladdin Chemistry Co., Ltd (Shanghai, China). The dialysis bags (Size: 5 M, cut-off: 7000 Da) are purchased from Biotopped Co., Ltd (Beijing, China). All the other commercially available reagents were analytical grade and used without further purification.

^{1}H NMR spectra were recorded on Bruker Avance-400 spectrometer with D_2O and $CDCl_3$ as the solvents. The synthetic polymers and materials were characterized by Fourier transform infrared spectroscopy (FT-IR) using KBr pellets. The Fourier transform infrared (FT-IR) spectra were supplied from Nicolet5700 (Thermo Nicolet corporation). Transmission electron microscopy (TEM) images were recorded on a Hitachi 7650B microscope operated at 80 kV. The TEM specimens were got by putting a drop of the nanoparticle ethanol suspension on a carbon-coated copper grid. The size distribution of WS-Chitosan@An-CHO LNPs in phosphate buffered saline (PBS) was determined by dynamic laser scattering using a Zeta Plus particle size analyzer (Zeta Plus, Brookhaven Instruments, Holtsville, NY). The fluorescence data were obtained from the Fluorescence spectrophotometer (FSP, model: C11367-11), which purchased from Hamamatsu (Japanese).

2.2. Synthesis of 9,10-bis(bromomethyl)anthracene (Br-An-Br)

The synthesis route of An-CHO was displayed in Scheme S1. At first, the intermediate products of 9,10-bis(bromomethyl)anthracene and 9,10-bis(diethylphosphorylmethyl)anthracene were synthesized by previous report (Zhang, Wang, Zhu, et al., 2013). For the preparation of 9,10-Bis(bromomethyl)anthracene: the mixture of anthracene (7.6 g, 40 mmol), $(CH_2O)n$ (4.8 g), $AlCl_3$ (5.0 g) and solution of 33% HBr in acetic acid (60 mL) was stirring at 60 °C for 6 h. The obtained solid was put into water and stirring 30 min to remove residual reactants, filtration and dry in vacuum one night. The residue product was recrystallized in toluene three times to obtain yellow solid. Yield: 7.8 g (54%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.38 (m, 4H), δ 7.65 (m, 4H), δ 5.54 ppm (m, 4H).

2.3. Synthesis of 9,10-bis(diethylphosphorylmethyl)anthracene ($(OEt)_2(O)P$-An-$P(O)(OEt)_2$)

The 9,10-Bis(bromomethyl)anthracene (1.2 g, 3.3 mmol) was mixed with triethyl phosphate (10 mL). Resulting mixture was refluxed at 180 °C for 8 h in order to obtain the pure product. The solvent was then removed at vacuum and the residue product was purified by a column chromatography on silica gel using ethyl acetate/CH_2Cl_2 as the eluent. Yield: 1.05 g (68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.38 (m, 4H), δ 7.6 (m, 4H), 4.25 (d, 4H), 3.8 (m, 8H), 1.06 ppm (t, 12H).

2.4. Synthesis of 9,10-bis(aldehydephenl)anthracene (CHO-An-CHO)

The 9,10-bis(aldehydephenl)anthracene with unique AIE feature was synthesized by follows: synthetic 9,10-bis(diethylphosphorylmethyl)anthracene (1.37 g, 3 mmol), terephthalaldehyde (1 g, 7.5 mmol) and t-BuOK (896 mg, 8 mmol) were mixed and stirring in dry tetrahydrofuran (THF) solution for 6 h at room temperature. The THF solution was removed by rotary evaporation and the residual solid was wash with water and extract with ethyl acetate for three times. The obtained organic layer was dried with anhydrous magnesium sulfate and purified by a column chromatography on silica gel using ethyl acetate/petroleum ether (1/10) as the eluent. The result solid was faint yellow. Yield: 0.8 g