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Synthesis of poly(ethylene glycol)-graft-chitosan and using as ligand for fabrication of water-soluble quantum dots

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ABSTRACT

The synthesis of water-soluble, stable and biocompatible quantum dots (QDs) is of crucial importance for nanobiotechnology. A chitosan derivative, poly(ethylene glycol)-graft-chitosan (PEG-g-CS), was successfully synthesized and employed as ligand for the growth of CdSe QDs in aqueous solution. The bivalent Cd^{2+} ions can coordinate with multiple amino-groups, thus they act as both inter- and intramolecular cross-linking agents. When the concentration of Cd^{2+} was 0.2 mmol/L, the CdSe/PEG-g-CS aggregates formed an irregular cross-linked network; when the concentration was 1 mmol/L, a phenomenon of micro-phase separation emerged as a result of enrichment of CS phase; when the concentration was 2 mmol/L, spherical nanohybrids with the size of 30–50 nm were obtained. Moreover, a possible mechanism was proposed for the formation of CdSe/PEG-g-CS aggregates. Meantime, in vitro 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity tests against HepG2 cells were carried out, the corresponding results suggested that the CdSe QDs prepared using PEG-g-CS as ligand displayed very low cytotoxicity. Therefore, these water-soluble QD-polymer hybrids are expected to find promising applications in medical field.

1. Introduction

Due to their size in the nanometer range, quantum dots (QDs) show an interesting physical behavior that is totally different from the properties exhibited by bulk material. This effect is known as the quantum confinement effect. In recent decades, QDs have received wide interest for applications in photovoltaic cells, light-emitting diodes, biological imaging and diagnostics [1–7]. Generally, QDs are synthesized via two strategies: organic phase and aqueous phase [8–12]. There are disadvantages with both of the two methods. The QDs synthesized by organic phase method are not water-soluble due to their surface coated with a hydrophobic layer such as trioctylphosphine (TOP) and trioctylphosphine oxide (TOPO) and they cannot be directly used in biological environments as a result. Ligand exchange strategy is a common way to substitute hydrophilic molecules such as amphiphilic polymer for TOP and TOPO molecules [13–15]. QDs synthesized by aqueous phase method have the possibility to release Cd^{2+} ions especially under UV irradiation, thereby making QDs toxic to the biosystem [16]. Coating QDs with polymer is one of the ways to protect the surface of QDs [17–19]. In a word, polymer can endow QDs with new physical and chemical properties including solubility, processability, stability and biocompatibility.

Two main methodologies have been developed to prepare QD-polymer nanohybrids: “QDs first” and “polymer first” approaches. The above-mentioned methods of modifying QDs with polymer belong to the former one. However, the reported approaches were either too complicated to carry out readily or easy to lose the optical property of QDs during polymerization. The “polymer first” route involves first synthesis of functional polymer, and then utilizes the functional polymer as template or ligand to grow QDs. Murphy was the first to report using poly(aminoamine) (PAMAM) dendrimers as template to fabricate and control the size of CdS QDs [20]. Polymers that can serve as ligands have been discovered mainly based on polyanions, for example, PAA copolymers. Wang synthesized PS-b-PAA diblock copolymer stabilized CdS QDs in benzene/methanol solution, however, the intensity of fluorescence was relatively low [21]. Duxin and co-workers synthesized PEO-b-PS-b-PAA triblock copolymer and then prepared CdS QDs in THF solution, finally formed multicore spherical aggregates by dialysis against water [22]. Unfortunately, the fluorescence information of the hybrid aggregates was not reported in the paper. Therefore, it is a challenge to synthesize water-soluble QD-polymer with stable optical performance and biocompatibility.

In this paper, we synthesized poly(ethylene glycol)-g-chitosan (PEG-g-CS) amphiphilic graft copolymer, and then prepared CdSe QDs with nice fluorescence property directly in aqueous solution.

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Chitosan is a kind of natural polycation, with a repeated structure of β-(1,4)-linked 2-amino-2-deoxy-D-glucose. Since Cd^{2+} ions can coordinate with multiple amino-groups, they can form stable chelates with chitosan. To the best of our knowledge, there is rarely any report about using polycation as ligand to prepare QDs. What is more, much attention has been paid to utilize chitosan as drug or gene carriers because of its relatively good biocompatibility, biodegradability, low immunogenicity and biological activities in recent decades [23–26]. It is expected that these water-soluble QD-polymer hybrids can be applied in medical field.

2. Materials and methods

2.1. Materials

4,4′-Diisocyanatodiphenylmethane (MDI, 98%), triphenylchloromethane (TrCl, 98%) and 3-mercaptopropionoc acid (3MPA) were supplied by Sigma–Aldrich (USA) and used without further purification. Chitosan (CS, degree of deacetylation = 92.8%, average molecular weight = 6.0×10^5), phthalic anhydride, poly(ethylene glycol) 20000 (PEG20000), ethyl ether, pyridine, dibutyltin dilaurate, hydrazine hydrate (85%), acetic acid, cadmium acetate, sodium borohydride and selenium powder were purchased from Sinopharm Chemical Reagent Company (China) and used as received unless otherwise indicated. N,N-dimethyl formamide (DMF) was purchased from Nanjing Chemical Reagent Company (China) and distilled under pressure immediately before use. The dialysis bag (the intercepted molecular weight was 8000–10,000) was an American product.

2.2. Preparation of PEG-g-CS graft copolymer

The graft copolymer of poly(ethylene glycol)-g-chitosan, specifically PEG-g-CS, was synthesized by group coupling of hydroxyl end group of PEG with C6-OH of CS.

2.2.1. Complete N-phthaloylation of CS (PHCS)

Phthaloylchitosan was prepared according to the protocol of literature with minor revisions [27]. A mixture of CS (5.000 g, 31.0 mmol amino group) and phthalic anhydride (13.789 g, 93.1 mmol) in DMF (100 mL) was heated with stirring at 130 °C under a nitrogen atmosphere. After 6 h, the mixture became a clear and viscous solution. The precipitate obtained by pouring the solution into ice water was collected by vacuum filtration, successively washed completely by Soxhlet's extraction with ethanol, and dried under vacuum at room temperature.

2.2.2. Single hydroxyl end group protection of PEG (PEG-Tr)

To a solution of PEG20000 (16.000 g, 0.80 mmol) in pyridine (100 mL) was added triphenylchloromethane (0.245 g, 0.88 mmol), the mixture was stirred for 24 h at 90 °C under a nitrogen atmosphere. The solution was cooled to room temperature and precipitated by pouring into ethyl ether. The product was separated by centrifuging and washed with ethyl ether for 3 times, and then dried under vacuum at room temperature.

2.2.3. Synthesis of PEG-g-PHCS copolymer

To a solution of PEG-Tr (2.024 g, 0.10 mmol) in DMF (50 mL) was added MDI (0.025 g, 0.10 mmol), the mixture was stirred for 2 h at room temperature under a nitrogen atmosphere. Then a certain amount of PHCS (0.202 g) and micro amount of dibutyltin dilaurate (10 μL) as catalyst were added into the homogeneous solution. The mixture was stirred for another 5 h at 80 °C under a nitrogen atmosphere. The solution was cooled to room temperature and precipitated by pouring into ethyl ether. The product was separated by centrifuging and washed completely by Soxhlet's extraction with ethanol, and then dried under vacuum at room temperature.

2.2.4. Synthesis of PEG-g-CS copolymer

A mixture of PEG-g-PHCS, hydrazine hydrate and water was heated with stirring for 6 h at 100 °C under a nitrogen atmosphere. After cooling, the mixture was dialyzed against water until its pH value reached 7. The product was separated by centrifuging and then dried under vacuum at room temperature.

2.3. Synthesis of water soluble CdSe QDs

The preparation of water soluble CdSe QDs assisted by graft copolymer was performed as follows. First, a certain amount of PEG-g-CS was dispersed in water to give a stock solution of about 0.1% (w/w). Then 1% (w/w) acetic acid aqueous solution was added to convert 80% of $-NH_2$ groups to $-NH_3{}^+Ac^-$. A certain amount of $Cd(Ac)_2 \cdot 2H_2O$ was added 1 h after the protonizing reaction. The resultant solution was degassed for 1 h with N_2 gas before the injection of NaHSe aqueous solution (the molar ratio of Cd:Se was 2) which was prepared according to a previous report [28]. Immediately, the color of the solution changed into bright orange because of generation of CdSe QDs. Finally, the solution was refluxed for 1 h at 90 °C.

2.4. Characterization

All infrared spectra were obtained using a Bruker VECTOR22 FT-IR spectrophotometer. ^{1}H NMR spectra were taken by a Bruker 300 NMR spectrometer. X-ray powder diffraction diagrams were recorded with a Bruker D8 Advanced diffractometer with Cu Kα radiation (λ = 1.5406 Å) at 45 kV, 40 mA. The diffraction data were recorded for 2θ angles between 8° and 60°. Transmission electron microscopy studies were performed on a JEOL JEM-2100 electron microscope at 200 kV. Photoluminescence measurements were conducted with an FL3-TCSPC fluorescence spectrometer, excited at 365 nm.

2.5. Cell toxicity test by MTT assay

A standard MTT assay was employed to determine the cytotoxicity of CdSe/PEG-g-CS hybrids against HepG2 cells. Cells used in the experiments were routinely cultured in growth medium (DMEM, containing 10% FBS) at 37 °C in 5% CO_2 atmosphere. For MTT tests, 100 μL of PEG-g-CS (100 μg/mL) and different concentrations of CdSe/PEG-g-CS aqueous solution (in terms of CdSe concentration, diluted by the synthesized CdSe solution using PEG-g-CS as ligand, of which the concentration of Cd^{2+} was 2 mmol/L) were added to approximately 5×10^4 cells in a 96-well plate and incubated for 24 h. Cell viability was determined by incubating the cells for 4 h at 37 °C with 20 μL of MTT solution (5 mg/mL in PBS, pH 7.4). The yellow MTT is reduced only by living cells to a purple, water-soluble formazan salt. As such, the amount of formazan formed is proportional to the number of living cells. After centrifugation (1000 rpm, 10 min) and extraction in 100 μL of DMSO, the intensity of this purple salt can then be easily and rapidly quantified using a conventional microplate reader by measuring the absorbance of the cell lysate at 620 nm. The cell viable rate was calculated by the equation of $OD_{test}/OD_{control} \times 100\%$, in which OD_{test} and $OD_{control}$ are the absorbance values of the testing well (in the presence of samples) and the control well (in the absence of samples), respectively. All results were averages of 5 samples. Meanwhile, the in vitro cytotoxicity of 3MPA-CdSe (prepared according to the literature [29]) was also investigated using MTT assay for contrast.