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Aqueous synthesis and characterization of bovine hemoglobin-conjugated cadmium sulfide nanocrystals

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ABSTRACT

Cadmium sulfide (CdS) nanocrystals with average diameter about 5.5 nm were synthesized in aqueous solution of bovine hemoglobin (BHb) via simple biomimetic method. Powder X-ray diffraction (XRD), energy dispersive X-ray spectroscopy (EDS), transmission electron microscopy (TEM) and selected area electron diffraction (SAED) characterizations were used to determine the structure and morphology of CdS nanocrystals. It was revealed that amount of BHb, chelating of Cd^{2+} to BHb and reaction temperature were key factors in controlling shape and dispersion of CdS nanocrystals. The binding sites of BHb to CdS nanocrystals and the change of secondary structure of protein have been identified by Fourier transform infrared (FT-IR) and circular dichroism (CD) spectroscopy. It was found that conjugating of BHb with Cd^{2+} and CdS could protect nanocrystals from agglomerating. Moreover, the thermostability of BHb enhanced after conjugating with CdS nanocrystals. The interaction mechanism of BHb with Cd^{2+}/CdS was also proposed. The quantum-confined effect of CdS nanocrystals was confirmed by ultraviolet–visible (UV–vis) spectrum. The nanocrystals exhibited a well-defined photoluminescence (PL) emission feature at about 510 nm with narrow full width at half maximum (FWHM).

1. Introduction

Chalcogenide nanocrystalline materials have been attracted considerable attention due to their potential applications in catalysis, optics, electronics, ceramics and magnetic storage, which are different from those of bulk counterparts [1–5]. In the past decade, various synthetic schemes have been developed for the size- and shape-controlled synthesis of chalcogenide nanomaterials including hydrothermal process [6,7], solvothermal process [8,9], chemical bath deposition (CBD) [10], chemical vapor deposition (CVD) [11], molecular beam epitaxy [12], electrosynthesis [13], microwave-assisted method [14], sonochemical method [15], sol–gel method [16] and solid-state reaction [17]. However, in most cases, toxic organic solvent or ligand, extreme reaction conditions such as high temperature, high pressure, and special instruments were required and the procedure was complicated. More importantly, the nanocrystals prepared by these methods were of either irregular shape or size distribution. Synthetic methods for high-quality monodisperse nanoparticles in organic solvents were well-developed, but their biological applications were restricted due to the lack of water solubility and tailored surface chemistry [18]. Therefore, developing green and sustainable chemical process is of importance to nanotechnology and remains a key research challenge. Suitable water-soluble biomolecules can be chosen as templates to synthesis chalcogenide nanocrystals because of their non-toxicity and bio-compatibility, which is also a new challenging project in green chemistry.

To the best of our knowledge, biomolecules, as basic building blocks of life, have special spatial structure and fascinating self-assembly functions, which can be as matrix to control nucleation and growth of inorganic materials and thus manipulate their morphologies and optical properties [19–21]. Recently, biomolecules involving amino acids [22–25], peptides [26,27], proteins [28–35], enzymes [36,37], DNAs [38], polysaccharides [39,40], viruses [41] have been exploited to regulate the size and shape of chalcogenide nanocrystals. The obtained biomolecules-conjugated nanomaterials were bioactive and biocompatible, which resulted in many applications related to biological process such as bioengineering, biomedical imaging, bio-tagging and bio-sensing. For example, Yang groups obtained Ag_2S, PbS, CuS, ZnS, HgS nanocrystals by using proteins as structure-directing template and studied inhibition effects of protein-conjugated nanocrystals on tumor cells [28–32]. Saha groups prepared cysteine-capped CdS, ZnS

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nanocrystals and investigated the interaction between amino acids with nanocrystals [22–25]. Stupp et al. reported the synthesis of CdS nanocrystals in amphiphilic peptide template [27]. Mann et al. used self-assembled bacterial S-layer as matrix to synthesize CdS superlattices [41]. Walsh et al. demonstrated a simple and green methodology for the preparation of CuS, Ag_2S nanoparticles in dextran biopolymer and investigated their application for fluorescence imaging of biological samples [39]. Li and Mann prepared DNA-directed assembly of multifunctional nanoparticle networks using metallic and bioinorganic building blocks [42]. Among these biomolecules, proteins have been the subject of particular attention due to their nanoscale dimensions and capability to control the size and shape of chalcogenide nanocrystals. Functional groups such as thiol, carboxyl, hydroxyl and amine which are abundant in proteins can be the possible interaction sites with nanocrystals. In addition, select proteins as matrix can facilitate the synthesis of nanocrystals at or near room temperature and atmosphere, in aqueous solutions, and at or near neutral pH, which makes bioenabled synthesis inherently "green" processing [21]. Bovine serum albumin (BSA) has been widely used as stabilizing agent for the synthesis of chalcogenide nanocrystals, including Ag_2S, Ag_2Se, CdS, CdSe, CuS, CuSe, PbS, PbSe, HgS etc. [28–35]. However, there have been very few studies on biomimetic synthesis using bovine hemoglobin (BHb) as template.

Hemoglobin is a protein with responsible for oxygen carrying in the vascular system of animals, which exists as a tetramer of globin chains that is composed of two α and two β subunits [43–45]. The α subunit contains 141 amino acid residues whereas β subunit contains 146 amino acid residues. Each subunit has one redox iron heme as its prosthetics group, and the heme is located in the crevices at the exterior of the subunit. The understanding of the interaction of Hb with nanomaterials is still of great concern for successful use in nanomedicine and nanotoxicology. Some research suggested that there was a close relationship between structural variation of Hb arising from the interaction of Hb with inorganic materials and its physiological function, which could cause many diseases such as leukemia, anemia and cardiopathy [46,47]. This change of Hb configuration also has great significant practical implications for clinical diagnosis and physiological research. CdS is one of the most important II–VI semiconductor compounds with E_g of 2.42 eV, possessing excellent optical and electrical properties [48]. Nano-sized CdS has already shown vital applications in fluorescence probe, photocatalysis, solar battery, sensors, light-emitting diodes (LED) and photoelectricity devices etc. [48–50]. Taking into account the current trends towards "green chemistry", in the present investigation we synthesized CdS nanocrystals using bovine hemoglobin (BHb) as matrix by means of biomimetic strategy. Fully understanding the interaction between BHb with CdS nanocrystals is another important goal in this paper. In addition, as an important fluorescence material, the conjugation of CdS with BHb is expected to become a promising fluorescent probe which can be used to detect Hb content of serum and plasma in the field of medicine and molecular biology. As we know, the preparation of CdS nanocrystals directly conjugated with bovine hemoglobin has not been reported before.

2. Experimental

2.1. Chemicals and materials

Cadmium (II) acetate ($Cd(CH_3COO)_2{\cdot}2H_2O$, >99%) and thioacetamide (TAA, >99%) were purchased from Sinopharm Chemical Regent Co., Ltd., PR China. All chemicals were of analytical reagent grade and can be used without further purification. Bovine hemoglobin (BHb) was of electrophoretic purity, purchased from Sigma and used as received. Water was used after purification through double distillation.

2.2. Synthesis of CdS nanocrystals in BHb solution

In a typical experimental procedure, 0.67 g cadmium acetate and various amounts (0.2 g, 0.4 g and 0.8 g) of BHb were dissolved 150 mL distilled water and transferred to a 250 mL round-bottom flask. The mixed solutions of Cd (II)–BHb were kept static under N_2 atmosphere for 24 h at 37 °C. Then 0.19 g TAA was dissolved in 50 mL distilled water and added into as-prepared solutions with vigorous stirring for 20 min at 37 °C. The final concentrations of BHb were 1.0 g L^{-1}, 2.0 g L^{-1} and 4.0 g L^{-1}, respectively. After TAA addition, the color of solution gradually changed from colorless to yellow. The mixed reaction solutions were again maintained under a static condition for 3 d, then separately by high speed centrifuging at 12,000 rpm. The collected yellow solid-state products were washed with distilled water and absolute ethanol, and finally dried in a vacuum at 50 °C for 48 h.

2.3. Characterization of BHb-conjugated CdS nanocrystals

Powder X-ray diffraction (XRD) measurements were performed on a Bruker D8Advance X-ray powder diffractometer with graphite monochromatized Cu Kα (λ = 0.15406 nm). A scanning rate of 0.05 deg/s was applied to record the pattern in the 2θ range of 20–70 deg. Samples for TEM and HRTEM characterizations were prepared by depositing a drop of the as-prepared nanocrystals solution on a carbon-coated copper grid and then drying at room temperature. TEM and HRTEM measurements were made on a JEOL JEL-2010 transmission electron microscope. UV–vis absorbance spectra were recorded on a Shimadzu UV-2550 spectrophotometer. Photoluminescence (PL) measurements were carried out on a Hitachi F-7000 FL spectrophotometer. Fourier transform infrared spectra (FT-IR) were taken on a Bruker Tensor-37 spectrophotometer in the wave number range of 4000–800 cm^{-1}; the spectra were collected at 2 cm^{-1} resolution with 128 scans by preparing KBr pellets with a 3:100 "sample-to-KBr" ratio. The circular dichroism (CD) spectra of reaction systems were measured with a Jasco-810 spectropolarimeter with wavelength 190–250 nm. Thermogravimetric analysis (TGA) was performed with a TA Q-600 thermogravimetric apparatus under a stream of N_2. The products were heated at 10/min from 25 °C to 600 °C.

3. Results and discussion

3.1. Morphology and structure of BHb-conjugated CdS nanocrystals

The crystal structure and phase composition of CdS nanocrystals synthesized in BHb solution were characterized by powder XRD. Fig. 1A shows the XRD pattern of the prepared CdS nanocrystals with 2.0 g L^{-1} solution of BHb. It is observed that 2θ values of

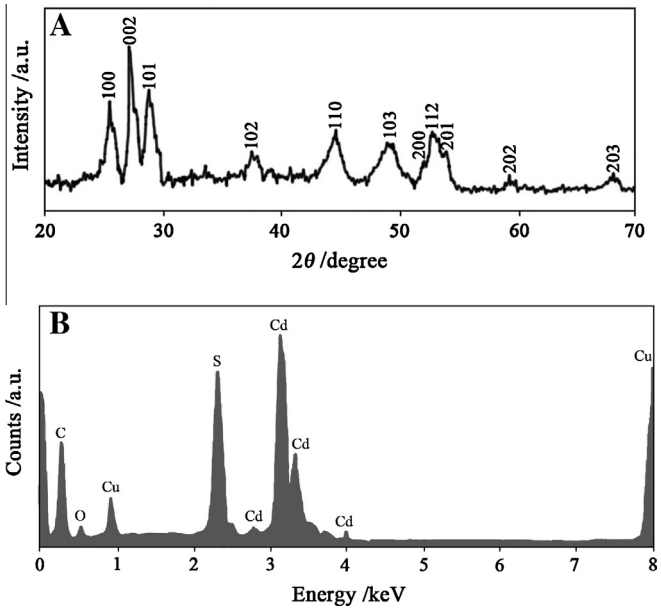

Fig. 1. (A) XRD pattern and (B) EDS spectrum of CdS nanocrystals synthesized in BHb solution.