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Low-temperature rapid synthesis of nitrogen and phosphorus dual-doped carbon dots for multicolor cellular imaging and hemoglobin probing in human blood

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ABSTRACT

Heteroatom doping of carbon dots (CDs) can efficiently tune the structure of electronic energy levels and modulate the photochemical properties of CDs. However, the reported synthesis routes of heteroatom-doped CDs are complicated and rigorous. Herein, a simple and rapid low-temperature heating strategy was applied to synthesize fluorescent nitrogen and phosphorus dual-doped CDs (N,P-CDs) using sucrose as carbon source, and 1,2-ethylenediamine (EDA) and phosphoric acid as dopants, respectively. These N,P-CDs exhibit excellent fluorescent stability over wide pH range solutions (4–11), ultrahigh ion strength (3 M KCl), and longtime UV light irradiation (3 h continuously), which makes these N,P-QDs promising good candidates for fluorescent probes. These N,P-CDs showed excellent biocompatibility, multicolor cellular imaging, and non-cytotoxicity to human hepatocellular carcinoma HepG2 cells even at 500 μg/mL levels for 48 h incubation. These N,P-CDs exhibited a strong blue emission and a sensitive response to hemoglobin. The N,P-CDs based fluorescent probe was then applied to sensitively determine hemoglobin with a detection limit of 0.29 nM. Notably, it was also applied for label-free detection of hemoglobin in human urine samples and human blood samples, which indicated its potential applications in clinical diagnosis and other biologically related study.

1. Introduction

Carbon dots (CDs) are a fascinating class of "zero-dimensional" carbon nanomaterials that comprise of discrete, quasi-spherical nanoparticles with a diameter less than 10 nm [1–3]. As a rising star of carbon-based fluorescent nanomaterials, CDs have attracted tremendous attentions for a wide variety of diverse promising bioapplications [4–7], because of their high chemical inertness, low toxicity, excellent biocompatibility, and cell membrane permeability [8]. Heteroatom doping of CDs is an emerging field of carbon-based nanomaterials science [9,10]. The facile access of CDs makes heteroatom doping available, and such doping can tune the structure of electronic energy levels and local chemical properties of CDs efficiently [11]. Up to now, masses of efforts have been made to prepare nitrogen (N)-doped, sulfur (S)-doped, phosphorus (P)-doped, N,S-doped, or N,P-doped CDs from natural biomass, amino acid, peptide, and other small molecule [9–14]. It is well-known that N-doped CDs can not only improve fluorescence properties of CDs but also allow to provide available functional groups for target sensing [15]. Other larger atoms (S or P) have the most preferable influences on the polarizability of carbon-based nanomaterials and thus on their performance in biological applications [11,12]. Therefore, the electronic characteristics of CDs can be altered by doping with nitrogen and phosphorus atoms by creating more active sites and unanticipated properties [14]. Although many approaches are reported for preparing nitrogen and phosphorus dual-doped CDs (N,P-CDs), some synthetic pathways suffer from the application of harsh synthesis conditions and tedious processes, such as rigorous synthesis temperature (all higher than 130 °C) [14,16–18] and complicated heating process (heating rate 1.5 °C/min to 180 °C) [16]. Therefore, it is still a great challenge and inevitable to achieve simple, rapid, and cost-efficient synthesis routes in one strategy.

Hemoglobin (Hb), which is the main tetrameric metalloprotein composed of a globular protein part and four iron-containing heme parts, plays important role in transporting molecular oxygen in

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Scheme 1. Schematic illustration of N,P-CDs synthesis.

mammalian blood [19]. The amount of Hb is associated with several clinical disease, so the easy and accurate quantitative analysis of Hb in clinical diagnostics is essential to assess the extent of diseases [20]. In the past few years, the design and development of fluorescent CDs probes for Hb is becoming an important project because of their simplicity, easy monitoring, rapid response, and high sensitivity. Barati et al. achieved highly selective detection of Hb (with a detection limit of 0.4 nM) by CDs synthesized through one-step hydrothermal method [21]. Our group proposed a Hb assay with a 0.12 nM detection limit using CDs-based fluorescent probe, which was then applied for the fluorescence detection of Hb in human urine samples and human blood samples [22]. Indeed, most of the fluorescent probes for Hb are signaled by fluorescence quenching originating from the special binding interaction between Hb and active sites on the surface of CDs. Therefore, preparing sites rich CDs that are prone to binding with Hb is still imminently desired in the detection of trace Hb in organisms.

Herein, a simple and rapid low-temperature heating approach has been designed to obtain the fluorescent N,P-CDs that use sucrose as carbon source, and 1,2-ethylenediamine (EDA) and phosphoric acid as dopants, respectively (Scheme 1). The synthesis temperature is reduced as close to the room temperature as possible, which makes this method more simple and convenient. The morphology, surface functional groups, chemical state, and fluorescent properties of N,P-CDs were investigated extensively. Due to the doping of nitrogen and phosphorus atoms, N,P-CDs exhibited sufficient water-solubility and excellent fluorescent properties in ultrahigh ion strength, wide pH range solutions, and longtime UV light irradiation. N,P-CDs show negligible cytotoxicity and excellent biocompatibility, which is of crucial importance for many biomedical applications. Moreover, the label-free N,P-CDs-based fluorescent probe showed a sensitive response to Hb in ultrawide concentration range of 1–6000 nM with the detection limit of 0.29 nM. This proposed fluorescent probe based on N,P-CDs was further applied for the detection of Hb in real human samples, which broadened the biological applications of carbon-based nanomaterials significantly.

2. Experimental section

2.1. Reagents and materials

Sucrose, EDA, phosphoric acid, and quinine sulfate were purchased from Aladdin Chemistry Co., Ltd (Shanghai, China). Hb, amino acids, and proteins were obtained from Sigma Reagents Company (St. Louis, MO, USA). Human hepatocellular carcinoma HepG2 cells were obtained from Guangxi Medical University, China. All biological reagents were purchased from Thermo Fisher (Suzhou) Instrument Co., Ltd (Suzhou, China). All other chemicals were obtained from Shanghai Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All chemicals were of the highest commercially available purity and were used as received without further purification. Ultrapure water with resistivity of 18.2 MΩ cm was prepared from a Millipore-Q Academic purification system (Bedford, MA) and was used in all experiments.

2.2. Synthesis of N,P-CDs

A low-temperature heating approach was used to prepare N,P-CDs (Scheme 1). Firstly, sucrose (1 g) and phosphoric acid (2.0 mL) with 2.0 mL ultrapure water were mixed uniformly. The mixture was heated at 80 °C for 50 min, then the heat was removed and the solution was cooled to room temperature gradually. The resulting dark brown mixture was placed into the ice-water bath. Then, EDA (6.0 mL) was added into the mixture under vigorous stirring, and the brown mushy N,P-CDs solution was obtained after the acid-base neutralization process between EDA and phosphoric acid. Subsequently, 40 mL ultrapure water was added to dilute the solution. The crude product was filtered through a 0.22 μm cellulose filter paper and then subjected to dialysis for 72 h in a dialysis bag with a cut-off molecular weight of 500 Da (Shanghai Green Bird Science & Technology Development Co., China). The khaki solid N,P-CDs products were obtained under vacuum freeze-drying for 48 h. Finally, the obtained N,P-CDs were stored at 4 °C for later applications.

2.3. Characterization of N,P-CDs

UV–vis absorption spectra were recorded on Shimadzu UV-3600 Plus UV-VIS-NIR spectrophotometer (Shimadzu, Japan). All fluorescence spectra were recorded on Thermo Scientific Lumina fluorescence spectrometer (Thermo Fisher Scientific, USA). Fourier transform infrared spectroscopy (FT-IR) spectra were performed on Thermo Nicolet iS10 spectrometer (Thermo Fisher Scientific, USA). Transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM) images were recorded on JEM 2100 LaB6 high-resolution transmission electron microscope (JEM, Japan). The sample for TEM characterization was prepared by placing a drop of N,P-CDs solution on carbon film-coated copper grid and dried at room temperature. X-ray diffraction (XRD) was recorded on Brucker D-8 advance powder X-ray diffractometer (Brucker, Germany). X-ray photoelectron spectroscopy (XPS) was acquired from ESCALAB 250Xi X-ray Photoelectron Spectroscopy (Thermo Fisher Scientific, USA). Elemental analysis was obtained using Elementar Analysensysteme Vario EL/Micro Cube organic element analyzer (Elementar Analysensysteme GmbH, Germany). Confocal fluorescence microscopy images