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Easy synthesis of highly fluorescent carbon dots from albumin and their photoluminescent mechanism and biological imaging applications

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ABSTRACT

A simple and green approach was developed to synthesize highly fluorescent carbon dots (CDs) using albumin as a carbon source in aqueous solution at room temperature. The CDs were characterized by excellent monodispersion, superior photostability, pH-independent emission, long fluorescence lifetime and high quantum yield (QY). The photoluminescent (PL) mechanism of CDs was explored by means of time-resolved PL decay, and the results revealed that PL originated from the emission of both defect state and intrinsic state. In addition, biological imaging with the application of CDs was carried out in human breast cancer Bcap-37 cell, which demonstrated that CDs were provided with an excellent biocompatiblity, low cytotoxicity and good transmembrane ability. Besides, CDs could be considered as a potential substitute for organic dyes or semiconductor quantum dots (SQDs) in biological imaging.

1. Introduction

Fluorescence remains one of the most powerful and common tools for biological research [1]. As fluorescent materials, semiconductor quantum dots (SQDs) and organic dyes were employed in biological application during the past decade [2]. However, the photostability of organic dyes is poor, and SQDs with heavy metal ions may lead to potential cytotoxicity [2]. SQDs without cadmium are considered to be low toxicity, but their shortcomings of complicated synthesis and purification process are observed [3], which restrict their biotechnological and medical applications. With the advancement in fluorescent nanomaterials, photoluminescent carbon dots (PL CDs) as new nanoparticles are emerging, which possess unique properties such as low toxicity, good aqueous solubility, great biocompatibility, excellent optical performance and low photobleaching [4–6]. The CDs have been widely used in biochemical sensing [7], fluorescent probes [8], biological imaging [9,10], photocatalytic technology [11], drug carriers [12], light emitting devices [13], energy conversion/storage devices [14] and so on.

So far, a number of approaches have been developed to prepare CDs, such as laser irradiation [15], arc discharge [16], electrochemical synthesis [17], hydrothermal method [5], chemical oxidation [18], ultrasonic treatment [19] and microwave synthesis [20]. However, complicated experimental condition and post-treatment process are involved in the majority of the methods. An energy efficient method to synthesize CDs has been reported utilizing saccharide as carbon source, but the significant drawback is low quantum yield (1.2%) [21]. In our group, a combination method of hydrothermal and microwave digestion was used to synthesize CDs using ascorbic acid as carbon source [22], nevertheless expensive apparatus and complex procedures are required in the method. On the other hand, carbon source plays a significant role in CDs preparation. Currently, various carbon sources such as fullerene [23], polyacrylamide [24], polyethylene glycol [6], polyacrylic acid [25], polyethylenimine [26] and albumin [27,28], have been reported to synthesize CDs by different methods. Up to now, further investigations which could help understand CDs in depth are quite deficient. More facile methods are required because of the easy fluorescence quenching during multi-step chemical reactions. Some important information about CDs, such as the PL mechanism of CDs, cell imaging and biological labeling, is still rare. As a consequence, further investigations based on CDs are not only challenging, but also necessary.

In this article, a novel one-step alkaline hydrolysis (AH) method is presented to fabricate CDs using albumin as source material in NaOH solution at room temperature. Comparisons among the morphology, size and optical properties of CDs prepared with different approaches were made. The PL mechanism of CDs was explored by means of time-resolved PL decay. Cytotoxicity, biocompatibility and transmembrane ability of the CDs were demonstrated.

2. Experimental

2.1. Materials

Fresh eggs were purchased from the local market. Sodium hydroxide, polyacrylic acid, polyethylenimine, polyacrylamide, polyethylene

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glycol and hydrochloric acid were purchased from Aladdin (Shanghai, China). Besides, all chemicals were of analytical grade, which could be applied without further purification. In addition, double deionized water was employed throughout all the experiments.

2.2. Synthesis of CDs

During a typical preparation, albumin (egg white) was well separated from egg with the aid of an egg separator and freeze-dried by means of a freeze drier, which served as a carbon source to produce CDs without any further purification. To prepare various CDs samples, 6.0 mL of albumin solution (25 g/L) was mixed with 1.0 mL of NaOH solution (1.0 mol/L), and then the mixture was incubated for different times at room temperature. After CDs preparation, remaining alkali was neutralized with HCl to adjust the pH of CDs solution to 7. Then the solution was purified to remove NaCl by dialysis for 24 h. The comparison between PL intensities of CDs was conducted under different incubation times in Fig. S1a, and the optimal incubation time was determined as 80 h. To determine optimum NaOH concentration (C_{NaOH}), CDs were prepared in solutions of different C_{NaOH} at room temperature for 80 h. The PL intensities of CDs prepared in different C_{NaOH} were presented in Fig. S1b, and the optimal C_{NaOH} was chosen as 1.0 mol/L.

Taking albumin as a carbon source, CDs were synthesized by making use of microwave (MW) and ultrasonic manipulation (UM) methods for comparison. In the MW method, 0.75 g of albumin was dissolved in 30 mL of water, and the albumin solution was heated in a domestic microwave oven (700 W) for 5 min. After that, the solution was cooled down to room temperature, and CDs suspension was obtained. The CDs solution was purified by centrifugation at 14,000 rpm for 15 min to remove precipitate. While in the UM method, 0.75 g of albumin was dissolved in 30 mL of water and then 5.0 mL of NaOH solution (1.0 mol/L) was added. The mixture was treated by ultrasound (180 W) for 5 h.

2.3. Quantum yield measurements

The QY of CDs was calculated with the following equation:

$$\phi_x = \phi_S \times \frac{m_x}{m_S} \times \frac{\eta_x^2}{\eta_S^2} \quad (1)$$

Quinine sulfate (QY = 54%) in 0.1 mol/L H_2SO_4 solution was regarded as the standard reference. In the equation, the subscripts *S* and *X* denote the standard and tested sample, respectively. ϕ is the QY, *m* is the slope from the plot of the integrated PL intensity *vs.* the absorbance of the sample or the standard at different concentrations, and η is the refractive index of the solvent.

2.4. Cytotoxicity assay

100 μL of human breast cancer Bcap-37 cell suspension was added to the well and incubated in a 5% CO_2 humidified incubator at 37 °C for 24 h, which were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1% penicillin streptomycin. And then, the culture medium was changed to fresh medium containing CDs (0.4–2.0 mg/mL) and cells were incubated in various CDs concentrations for 24 h. The culture medium was removed and phosphate buffered saline (PBS) was adopted to wash cells. 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5.0 mg/mL) was added per well and incubated for another 4 h, so that violet colored formazan could be formed. After the culture medium was removed by employing MTT, 150 μL of dimethyl sulfoxide was added. After shaking the resulting mixture for 10 min, the mixture absorbance at 490 nm was measured through the application of Bio-Rad model-680 microplate reader. The cell viability was obtained by means of calculating the absorbance percentage relative to control sample containing CDs. All experiments were conducted in triplicate and mean values were taken into consideration.

2.5. Biological imaging

2.0 mL of human breast cancer Bcap-37 cells was seeded in a cover glass bottom dish and cultured at 37 °C for 24 h, which were maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1% penicillin streptomycin. And then, the culture medium was changed to 2.0 mL of fresh medium containing 2.0 mg/mL CDs and cells were incubated at 37 °C for 24 h. Finally, the culture medium was removed and cells were washed with PBS three times. Under a laser scanning confocal microscopy, cell imaging was conducted with laser excitation wavelengths of 405 and 488 nm with the collection of fluorescence in blue and green regions.

2.6. Characterization

Transmission electron microscopy (TEM) image of CDs was obtained by a JEOL JEM 2100 microscope. Dynamic light scattering (DLS) measurement was performed by using Zetasizer Nano ZS90 (Malvern, UK) to obtain CDs size. Crystal structure of CDs was studied by using X-ray diffraction (Rigaku, Japan). Fourier transform infrared (FT-IR) spectra of CDs were recorded on a Perkin Elmer (FT-IR spectrum BX, Germany). X-ray photoelectron spectroscopy (XPS, ESCALAB250Xi) was adopted for the measurement of elemental composition of CDs. UV2450 spectrophotometer (Hitachi, Japan) was used to measure UV–visible spectra of CDs. F900 fluorescence spectrometer (Edinburgh, UK) was adopted to conduct fluorescence spectra of CDs. Taking CDs as fluorescence labels, cell imaging was investigated under laser scanning confocal microscopy (Leica DM6000 CS).

3. Results and discussion

TEM and high-resolution TEM images of CDs are shown in Fig. 1a and Fig. S2, respectively. The CDs with spherical shape are excellent dispersion in aqueous solution, and the average diameter is 3.2 ± 1.1 nm (Fig. 1d). Average hydrodynamic diameter of the CDs is determined as 3.6 ± 1.5 nm by DLS, which is accorded with the result obtained by TEM in the experimental errors (in Fig. 1e). To verify reliability and efficiency of this method, CDs were synthesized by employing MW and UM methods and the same carbon source. The morphology and sizes of CDs are compared in Fig. 1. Average diameters of CDs prepared with UM and MW methods are 4.1 ± 2.8 nm and 13.4 ± 8.5 nm (Fig. 1b and c), respectively. Size distributions of CDs synthesized by different methods are shown in Fig 1d. The monodispersion of CDs prepared by AH method is much better than that synthesized by UM and MW methods.

XRD was adopted to investigate crystallinity of the CDs, and there is a broad diffraction peak centered at about $2\theta = 24°$ (Fig. 2a). Considering the interlayer space in graphite (0.34 nm), these CDs show a graphitic nature with highly disordered carbon atoms [4,29,30,31].

FT-IR spectroscopy was employed to investigate the surface functional groups of CDs. In the FT-IR spectra of the CDs, there are respectively peaks at 3421, 2962, 2823, 1653 (1545), 1447 (1401) and 1239 (1076) cm^{-1} in the FT-IR spectra of CDs for O—H/N—H, C—H, S—H, C═O, C—H/C—N and C—O/C—S, as shown in Fig. 2b [21,22]. Besides, XPS was carried out to determine the elemental composition of CDs and the XPS spectrum presented four peaks of C1s, N1s, O1s and S2p (Fig. 2c), and which indicates that CDs are mainly composed of C, N, O and S. In addition, the high-resolution spectrum of C1s (Fig. 2d) displays four main peaks at 284.11, 284.56, 287.02 and 288.33 eV, which are attributed to C═C/C—C, C—N, C—O and C═O/C═N bonds, respectively. Other elements (O1s, N1s and S2p) of the high-resolution spectrum are shown in Fig. S3. The graphitic structure (C═C/C—C) of CDs was