Journal of Luminescence 196 (2018) 100–110

Contents lists available at ScienceDirect

Journal of Luminescence

journal homepage: www.elsevier.com/locate/jlumin

One-pot solid phase pyrolysis synthesis of highly fluorescent nitrogen-doped carbon dots and the interaction with human serum albumin

Xingjia Guo[a], Liping Xu[a], Lizhi Zhang[a], Hongyan Wang[b], Xiangming Wang[a], Xuehui Liu[a], Jie Yao[a], Aijun Hao[c,*]

[a] College of Chemistry, Liaoning University, Shenyang 110036, PR China
[b] Jingzhou Products Quality Supervision and Testing Institute, Jinzhou 121000, PR China
[c] College of Pharmacy, Liaoning University, Shenyang 110036, PR China

ARTICLE INFO

Keywords:
Fluorescent nitrogen–doped carbon dots
Human serum albumin
Spectroscopic methods
Interaction

ABSTRACT

One kind of fluorescent nitrogen–doped carbon dots (denoted as NCDs) was successfully fabricated by a simple and facile one–pot solid phase pyrolysis process using citric acid and glycine as raw materials. The obtained NCDs under the optimal synthetic conditions have a fluorescence quantum yield up to 35.6% and show a spherical shape with an average diameter of about 3.0 nm. Moreover, NCDs can emit strong blue fluorescence and the surfaces when excited at 345 nm and their surfaces were decorated with lots of carboxyl, hydroxyl and amine groups. In order to further elucidate the effect of NCDs to protein, steady state/time-resolved fluorescence, UV–vis absorption, circular dichroism (CD), and Raman spectroscopic techniques as well as cyclic voltammetry (CV) were employed to explore the interaction of the as-synthesized NCDs with human serum albumin (HSA) in the simulated physiological environment. The experimental data reveal that NCDs could quench the intrinsic fluorescence of HSA via the formation of a ground-state complex with the association constants of the order of 10^4 L/mol. The values of thermodynamic parameters obtained at three different temperatures suggest that the binding reaction of NCDs with HSA mainly driven by hydrophobic forces and hydrogen bonds was spontaneous. Furthermore, the displacement experiments confirm that the binding of NCDs primarily took place in site I of HSA. The average distance between NCDs and tryptophan (Trp) residue of HSA was estimated to be 2.3 nm according to the theory of Förster non-radiation energy transfer. Finally, the analysis of synchronous fluorescence, three-dimensional fluorescence (3D), Raman, and CD spectra manifests that HSA underwent some conformational changes by unfolding the polypeptides of protein and increasing the polarity of the microenvironment surrounding both Trp and tyrosine (Tyr) residues in the presence of NCDs.

1. Introduction

As the main serum protein in the circulatory system of human blood, HSA is composed of three structurally similar domains (I, II, and III), and each domain can be divided into subdomains A and B. As is known to all, the primarily regions of ligand binding to HSA are situated in hydrophobic cavities in subdomains IIA and IIIA on HSA, namely so-called site I and site II [1]. HSA molecules possess lots of important physiological functions including transportation, distribution, and delivery of endogenous and exogenous substances [2]. The ligands binding to HSA can significantly affect many properties of ligands in vivo, such as absorption, distribution, metabolism, excretion, stability and toxicity [3]. Moreover, the interactions of these ligands with HSA may also alter the protein structure and affect the normal physiological functions of protein [4,5].

Xu et al. firstly discovered fluorescent carbon dots (CDs) in 2004 [6]. As new quantum-sized carbon-containing fluorescent nanoparticles, CDs have exhibited promising applications in drug delivery, bio-sensing and bio-imaging because they possess many attractive properties, such as excellent optical performances, good biocompatibility, and low toxicity [7,8]. Therefore, it is imperative to study the effects of CDs on proteins or cells in biological systems. To the best of our knowledge, there are many reports on the cytotoxicity of CDs to cells [9,10], but relatively few studies on the interactions of CDs with proteins [11,12]. Fluorescent CDs obtained via different synthetic methods and raw materials possess not only different optical properties, but also different particle sizes and surface chemical properties, so each kind of CDs will exhibit different effects on protein structure and function. Study on the interaction of CDs with HSA will help us to better understand the pharmacokinetic behavior of CDs and further elucidate

* Corresponding author.
E-mail address: haoaj2012@hotmail.com (A. Hao).

https://doi.org/10.1016/j.jlumin.2017.12.029
Received 12 June 2017; Received in revised form 12 November 2017; Accepted 12 December 2017
Available online 14 December 2017
0022-2313/ © 2017 Elsevier B.V. All rights reserved.

their biological effects on protein at molecular level.

In the present paper, nitrogen–doped carbon dots were successfully synthesized by one–pot solid state pyrolysis process using citric acid and glycine as carbon and nitrogen resources. The morphology, chemical structure and optical properties of the synthesized NCDs were characterized using transmission electron microscopy (TEM) and X-ray diffraction (XRD) techniques, X-ray photoelectron spectrometry (XPS), Fourier transform infrared (FTIR), UV–vis absorption and fluorescence spectroscopy. And then the binding of NCDs to HSA was systemically investigated by spectroscopic and electrochemical methods. The fluorescence quenching mechanism and binding characteristics were analyzed. The conformational change of HSA induced by NCDs was also explored. The study is expected to provide some useful information for the safe applications of NCDs in biomedical fields.

2. Experimental

2.1. Materials

Citric acid, glycine, Tris, hydrochloric acid (HCl), and sodium chloride (NaCl) were purchased from Sinopharm Chemical Reagent Co. (China). HSA (fatty acid free, purity > 98%) was purchased from Sigma-Aldrich Chemical Co., Ltd. (Milwaukee, WI, USA) and used without further purification. Phenylbutazone and ibuprofen were obtained from the National Institute for Food and Drug Control (China). All other chemicals used in this experiment are of analytical reagent grade. Double-distilled deionized water was used throughout.

2.2. Synthesis and purification of NCDs

In order to obtain the highly fluorescent NCDs, three main influence factors including reaction temperature, heating time, and the dosage of glycine, which will affect the photoluminescence (PL) intensity of the synthesized product, have been optimized by single-factor test.

Typically, 0.50 g of citric acid and 0.60 g glycine were completely mixed, and then transferred into a 50 mL Teflon-line stainless steel autoclave. Teflon-line stainless steel autoclave was heated at 200 °C for 3 h in a constant temperature drying oven. Afterwards, the vessel was allowed to cool down to room temperature. In order to remove the excess precursors and other impurities, the obtained brown solution was filtered with a 0.22 μm filter membrane, and then further purified by dialysis for 2 days. Finally, the solid power of product was obtained by freeze drying and stored at 4 °C before further experiments.

The molar concentration of NCDs solution was calculated in terms of mean molecular weight of 1500 [12].

2.3. Characterization of the synthesized NCDs

The characterization of size and morphology for NCDs sample was performed on a JEM-100SX transmission electron microscope (JEOL Ltd., Japan) at an acceleration voltage of 100 kV. The samples for TEM observation were prepared by drying a drop of NCDs solution dispersed on an amorphous carbon-coated copper grid. Powder XRD pattern was recorded via a Bruker D8 Advance X-ray diffractometer using Cu K_α radiation, and the scanning range is from 10° to 80° with a scanning rate of 10°/min. All fluorescence spectra were obtained on a Cary Eclipse 300 FL spectrophotometer (Varian Company, USA) equipped with a thermostat bath and a 1.0 cm quartz cell, and the silt widths for both excitation and emission monochromators were set at 5 mm while the scanning rate was 1200 nm/min. FTIR spectra were recorded on a Nicolet Avatar 330 FTIR spectrometer (Thermo Electron Corporation, USA) using the pressed KBr discs and the scanning wavelength number range was from 4000 to 400 cm^{-1}. XPS measurements were performed on an ESCALAB 250 XPS system (Thermo Fisher Scientific, UK) using Al Kα (hν = 1486.60 eV) radiation. UV–vis absorption spectra were collected on a TU-1800PC UV–vis spectrophotometer (Varian Company, USA) and 1.0 cm quartz cell was used. Zeta potential measurements were carried out on a Zetasizer Nano ZS analyzer (Malvern, England). All the above measurements were performed at room temperature except where otherwise specified.

2.4. Spectral and electrochemical measurements for the interaction between NCDs and HSA

The stock solutions of 2.41×10^{-5} mol/L HSA and 9.12×10^{-4} mol/L NCDs were prepared in water. Only the working solution for the CD measurement was prepared with phosphate buffer solution (0.05 M, pH 7.40), the other working solutions were prepared with Tris-HCl buffer solution (0.05 M Tris, 0.15 M NaCl, pH 7.40). The stock solutions of 2.41×10^{-6} mol/L phenylbutazone and 2.41×10^{-6} mol/L ibuprofen were prepared by dissolving appropriate amount of them in a small amount of methanol and then diluting with water.

Fluorescence titration spectra of HSA excited at 295 nm were measured at 298, 304, and 310 K, respectively, and the scanning wavelength range was from 300 to 500 nm. 3 mL of 2.41×10^{-6} mol/L HSA solution was titrated with successively adding 10 μL of 9.12×10^{-4} mol/L NCDs solution with a micro-injector and the resulting concentration of NCDs changed from 0 to 1.82×10^{-5} mol/L with an interval of 3.04×10^{-6} mol/L. Each HSA + NCDs mixture solution was incubated at one specified temperature for 10 min prior to spectral measurement. Furthermore, the synchronous fluorescence spectra of HSA after adding various amounts of NCDs were scanned from 220 to 350 nm (Δλ = 15 nm or Δλ = 60 nm) at 298 K. 3D fluorescence spectra of HSA solution in the absence and presence of NCDs were obtained at room temperature. The scanning range of the excitation wavelength is from 200 to 350 nm with an increment of 5 nm and the emission wavelength was from 200 to 600 nm.

Time-resolved fluorescence spectra were obtained using a DeltaFlex time-correlated single photon counting (TCSPC) spectrometer (HORIBA Jobin Yvon IBH Ltd., UK). The concentration of HSA was fixed at 2.41×10^{-6} mol/L and the concentrations of NCDs changed from 0 to 4.82×10^{-6} mol/L.

UV–vis absorption spectra of HSA, NCDs, and HSA + NCDs mixture solutions were respectively measured in the wavelength range from 200 to 500 nm.

All CV measurements were carried out with conventional three-electrode electrochemical testing system. The working electrode was the NCDs modified glassy carbon electrode (GCE). $Hg/HgCl_2$ electrode and platinum wire served as the reference electrode and the counter electrode, respectively. The electrolyte solution was 5.0×10^{-3} mol/L $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ and 1.0×10^{-2} mol/L KCl. For NCDs/GCE preparation, 5 μL of NCDs solution was dropped onto the surface of GCE (chitosan was added to the NCDs solution to increase the bonding force of NCDs on the electrode surface). After drying with cold air, the electrode was washed by water and then NCDs/GCE was obtained. For CV measurement, different amounts of HSA were added continuously into the electrolyte solution and then the mixture solution was stirred for 5 min. The reaction system was incubated for 5 min prior to each testing. CV was measured at a scan rate of 50 mV/s. All CV measurements were repeated three times with different NCDs/GCE at room temperature. The concentration of HSA varied from 0 to 4.82×10^{-7} mol/L with an interval of 1.21×10^{-7} mol/L.

Raman spectra of HSA solution with and without NCDs were recorded on an InVia Raman spectrometer (Renishaw, USA) using a 633 nm laser and the scanning range is from 2400 to 1000 cm^{-1}.

CD spectra of HSA solution (1.65×10^{-7} mol/L) after adding different concentrations of NCDs were scanned on a J-810 automatic recording spectropolarimeter (Jasco, Japan) in 1.0 cm quartz cell and the scanning range is from 190 to 250 nm. The scan rate was set at 200 nm/min with a response time of 2 s. Each CD spectrum was the average of three scans. The molar ratios of HSA to NCDs in the working solution were fixed at 1:0, 1:10 and 1:20, respectively.

Download English Version:

https://daneshyari.com/en/article/7840330

Download Persian Version:

https://daneshyari.com/article/7840330

Daneshyari.com