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Photoluminescent sensing for acidic amino acids based on the disruption of graphene quantum dots/europium ions aggregates

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ABSTRACT

A simple mix-and-detect photoluminescence method was developed for the turn-on detection of acidic amino acids. To achieve this, graphene quantum dots (GQDs), which emit both down-conversion and up-conversion photoluminescence were prepared by solvothermal synthesis. The carboxylic acid-rich surface not only increases the water solubility of the prepared GQDs, but also makes Eu^{3+}-triggered GQDs aggregation possible, thus causing the photoluminescence quenching of GQDs. The quenched photoluminescence can be recovered by the competition between acidic amino acids and GQDs for Eu^{3+}. Under optimized conditions, sensitive and specific acidic amino acids quantitation can be achieved by utilizing the changes in either down-conversion or up-conversion photoluminescence. Up-conversion mode gives a little lower detection limit than the down-conversion one. Nearly overlapped calibration curves were obtained for the two acidic amino acids, glutamic acid (Glu) and aspartic acid (Asp), thus suggesting that the proposed method can be used not only for the quantitation of individual acidic amino acids, but also for the detection of total amount of them.

1. Introduction

Amino acids are building blocks of proteins. Due to the biological importance of amino acids, there has been a continued interest in the development of reliable methods for the determination of amino acids for diagnostic and research purposes (Meesters, 2013; Ou et al., 2013; Evans et al., 2012; Zhou and Yoon, 2012; Wang and Wu et al., 2008; Zhou et al., 2010; Jia et al., 2011; Guo et al., 2010). Acidic amino acids, including glutamic acid (Glu) and aspartic acid (Asp), have two free carboxylic acid groups instead of one. Acidic amino acids are usually found on the surface of water-soluble proteins and play important roles in various biological progresses (Balcar, 2002; Ren et al., 2013; Chu et al., 2011; Samways and Egan, 2007). For example, Glu is the most abundant excitatory neurotransmitter in the central nervous system (Meldrum, 2000), and high concentration of Glu is found in cerebral ischemia injury and some neurodegenerative diseases such as Alzheimer's disease (AD), vascular dementia and so on (Brown and Nijjar, 1995; Mo et al., 2011). Asp is key to a healthy metabolism. It has a crucial role in producing energy, and proper levels of Asp are necessary for the synthesis of other biochemicals and amino acids (Galili, 2011).

The commonly used analytical methods for Glu and Asp detection are based on chromatographic technique such as high-performance liquid chromatography (HPLC), cation-exchange chromatography and micellar electrokinetic chromatography (MEKC) (Armenta et al., 2009; Rebane and Herodes, 2010; Chen et al., 2014; Yan et al., 2014). Microchip electrophoresis (MCE) and microfluidic techniques are also reported (Li et al., 2013; Cellar et al., 2005). Although sensitive, they are costly, time-consuming or require sophisticated instruments. Therefore, cost-effective, simple, rapid and sensitive detection platforms for acidic amino acids are highly desired. Photoluminescence (PL) analysis might be a good choice due to high sensitivity, selectivity and most commonly used instruments.

Recently, graphene and its derivatives have received much attention for their many unique properties (Myung et al., 2011; Hu et al., 2010; Geim, 2009), such as excellent chemical stability, environmental friendliness, water dispersibility, good biocompatibility and so on. Compared to two-dimensional graphene sheets, zero-dimensional graphene quantum dots (GQDs), which consist of graphene nanosheets in small sizes (Ponomarenko et al., 2008), possess strong quantum confinement and edge effects (Zhuo et al.,

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2012). GQDs hold great promise as a new class of fluorophores owing to their excellent photostability, good biocompatibility, low cell toxicity and tunable PL properties (Sun et al., 2013; Qian et al., 2014). Several groups have demonstrated that GQDs can be used in various applications such as photovoltaics, bioimaging, biosensing and so on (Zheng et al., 2013; Ren et al., 2010). However, the development and application of GQDs are still in early stage and more works need to be done.

Some effective efforts have been done to improve the PL properties of GQDs. For example, Pan et al. have developed a hydrothermal route to cut preoxidized graphene sheets into GQDs with blue emission (Pan et al., 2010). Shen et al. proposed a surface-passivated method to fabricate of GQDs with frequency up-converted emission (Shen et al., 2011). Using similar methods, Zhuo et al. got GQDs with excitation-independent down-conversion and up-conversion PL behaviours (Zhuo et al., 2012). Up-conversion PL refers to nonlinear optical processes, in which the sequential absorption of two or more photons leads to the emission of light at shorter wavelength than the excitation wavelength. Compared to traditional down-conversion PL, up-conversion PL has higher photochemical stability, better tissue penetration ability, and is free of background auto-fluorescence (Mai et al., 2007; Yi and Chow, 2006; Yu et al., 2009). Most studies on up-conversion PL focus on lanthanide-doped up-conversion nanoparticles, but water-soluble up-conversion nanoparticles are not easy to be achieved. Therefore, hydrophilic GQDs with up-conversion PL have become an active area of interest. Many groups have got GQDs with up-conversion luminescence (Shen et al., 2012; Cheng et al., 2010). But as far as we know, the applications of the up-conversion property of GQDs were rarely reported.

Herein, water-soluble GQDs, which emit both down-conversion and up-conversion PL, were prepared, and a simple mix-and-detect strategy was designed for acidic amino acids quantitation by utilizing the competition between acidic amino acids and carboxylic acid moieties on the prepared GQDs surface for Eu^{3+}. The results showed that both down-conversion and up-conversion PL could be used for sensitive and specific detection of acidic amino acids, and a relatively better result was given by up-conversion PL mode.

2. Experimental

2.1. Materials and instruments

Graphite powder (325 meshes) was purchased from Baichuan Graphite Co., Ltd. (Qingdao, China). $Eu(NO_3)_3$, $Gd(NO_3)_3$, $Tm(NO_3)_3$, $Tb(NO_3)_3$, $Lu(NO_3)_3$ (99.99%) was provided from Alfa Aesar. Amino acids were obtained from Sangon Biotech. Co. Ltd. (Shanghai, China). The used metal salts ($Hg(Ac)_2$, $Mg(NO_3)_2$, $Mn(Ac)_2$, $Zn(Ac)_2$, $AgNO_3$, $Ni(NO_3)_2$, $Cr(NO_3)_2$, $Co(Ac)_2$, $Cd(NO_3)_2$, $Fe(NO_3)_3$, $Ca(Ac)_2$ and $Pb(NO_3)_2$), ascorbic acid, propane diacid, oxalic acid and dimethyl sulphoxide (DMSO) were obtained from Sigma. Fetal bovine serum sample was purchased from Gibco. All chemical reagents were of analytical grade and used without further purification.

2.2. Preparation of GQDs

Graphene oxide (GO) was synthesized from natural graphite powder by a modified Hummers' method (Hummers and Offeman, 1958; Zhang and Kong, 2013). The details can be found in supporting information. The prepared GO (0.1 g) was oxidized in 80 mL mixture of concentrated H_2SO_4 and HNO_3 (v/v=3:1) for 12 h under mild ultrasonication (500 W, 100 kHZ). After addition of 600 mL deionized water, the mixture was centrifuged at 12,000 rpm for 30 min. The obtained dark brown precipitates were redissolved in deionized water, and the solution was centrifuged again to remove remaining H_2SO_4 and HCl. The obtained brown solid was sonicated in 100 mL of water, and NH_3•H_2O was added to adjust the pH to neutral. The suspension was transferred to a poly (tetrafluoroethlene) (Teflon)-lined autoclave (100 mL) and heated at 180 °C for 8 h. After cooling to room temperature, the resulting black solution was filtered through a 0.22 μm microporous membrane to obtain brown suspension. The colloidal solution was further dialyzed in dialysis bag (retained molecular weight: 3500 Da) for 24 h and GQDs were obtained.

2.3. Detection of Glu or Asp

30 μL GQDs (1 mg/mL) was mixed with 5 μL glycine–HCl buffer (50 mM, pH 3.4). After addition 0.1 mM Eu^{3+} (final concentration) and different amounts of Glu or Asp, the volume of the mixture was adjusted to 100 μL with deionized water. Then, the mixture was incubated at 37 °C for 2 h. The PL spectrum of the resulting mixture was recorded. The excitation wavelengths were set at 315 and 650 nm, down-conversion and up-conversion PL were recorded, respectively. The PL intensity at 443 nm was used for the quantitative detection of Glu or/and Asp. Excitation and emission slit widths were both set to 5 nm for down-conversion PL detection, and set to 10 nm for up-conversion PL detection. The optical path length of the quartz cell was 1.0 cm.

3. Results and discussion

3.1. Characterization of the prepared GO and GQDs

The details about synthesis and characterization of GO, the synthetic precursor of GQDs, can be found in supporting information (Fig. S1). The luminescent GQDs were obtained by a oxidation–hydrothermal synthesis–seperation process (Jin et al., 2013). Fig. S2a presents the FT-IR spectrum of GQDs, in which the characteristic absorption bands of GQDs, such as stretching vibrations of C–OH at 3453 cm^{-1}, C–O at 1114 cm^{-1} and C=C at 1628 cm^{-1}, were observed (Zheng et al., 2013). Fig. S2b presents the Raman spectra of the prepared GO and GQDs. These spectra have two typical peaks at about 1344 cm^{-1} and 1580 cm^{-1}, which correspond to D band and G band, respectively. G band is related to sp^2-hybridized carbon atoms, and D band arises from structural defects created by the attachment of oxygen-containing groups on the basal plane of graphene (Wang et al., 2008). The intensity ratio of I(D)/I(G) is an essential parameter to characterize the disorder degree of graphene materials. The prepared GO had a I(D)/I(G) ratio of 0.94, which increased to 1.52 for GODs. The increase of oxidation degree indicates that GQDs contain more oxygen-containing groups (epoxy, –OH and –COOH) on the surface, thus possessing good water solubility and the capability of coordinating with Eu^{3+}.

The optical properties of GQDs were shown in Fig. S3. The UV–vis absorption band nearly covers the whole UV region, and no clear absorption peak was observed. Correspondingly, the blue-luminescent GQDs has symmetrical and broad down-conversion PL peak, whose wavelength and intensity are dependent on excitation wavelength. The PL peak shifts to longer wavelength with increasing the excitation wavelength from 310 nm to 430 nm, and the strongest PL emission peak was observed at 435 nm when excited at 315 nm. Besides the strong down-conversion PL properties, GQDs show a clear up-conversion PL feature. The up-conversion PL of the prepared GQDs also shows an excitation-dependent behavior, i.e. a redshift of the emission wavelength was observed with the increase of excitation wavelength in the range