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Carbon quantum dots as fluorescence resonance energy transfer sensors for organophosphate pesticides determination

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ABSTRACT

Carbon quantum dots (CQDs) obtained from natural organics attract significant attention due to the abundance of carbon sources, varieties of heteroatom doping (such as N, S, P) and good biocompatibility of precursor. In this study, tunable fluorescence emission CQDs originated from chlorophyll were synthesized and characterized. The fluorescence emission can be effectively quenched by gold nanoparticles (Au NPs) via fluorescence resonance energy transfer (FRET). Thiocholine, which was produced from acetylthiocholine (ATC) by the hydrolysis of butyrylcholinesterase (BChE), could cause the aggregation of Au NPs and the corresponding recovery of FRET-quenched fluorescence emission. The catalytic activity of BChE could be irreversibly inhibited by organophosphorus pesticides (OPs), thus, the recovery effect was reduced. By evaluating the fluorescence emission intensity of CQDs, a FRET-based sensing platform for OPs determination was established. Paraoxon was studied as an example of OPs. The sensing platform displayed a linear relationship with the logarithm of the paraoxon concentrations in the range of 0.05–50 μg L^{-1} and the limit of detection (LOD) was 0.05 μg L^{-1}. Real sample study in tap and river water revealed that this sensing platform was repeatable and accurate. The results indicate that the OP sensor is promising for applications in food safety and environmental monitoring.

1. Introduction

Carbon quantum dots (CQDs) attract significant attention as potential substrate for biosensing compared to conventional semiconductor QDs due to their attractive advantages including excellent water-solubility, good biocompatibility, low toxicity, simple synthetic routes and tunable fluorescence emission (Baker and Baker, 2010; Lim et al., 2015). The CQDs synthesized from natural organics hold great promise for sensing agent due to the abundance of carbon sources and varieties of heteroatom doping (such as N, S, P). Particularly, citrate (Yang et al., 2011), ascorbic acid (Wu et al., 2012), candle soot (Ray et al., 2009), soy milk (Zhu et al., 2012), orange juice (Sahu et al., 2012), chicken eggs (Wang et al., 2012) and gelatin (Liang et al., 2013) have been used as precursors to synthesize CQDs. These "green" CQDs demonstrate distinguished properties as fluorescence probes in chemical sensings, such as metal ions (Li et al., 2015; Yu et al., 2015; Zhang et al., 2015), glucose (He et al., 2015; Wang et al., 2015), glutathione (Gu et al., 2015) and organophosphates pesticides (Hou, et al., 2015a, 2015b; Cai et al., 2015; Yang et al., 2015).

Owing to their inherent advantages mentioned above, CQDs also receive significant interest as fluorescent probes in fluorescence resonance energy transfer (FRET) based fluorescent biosensors (Barati et al., 2015). Such FRET-based sensing platform usually contains a fluorophore probe and a nano quencher to form an acceptor-donor FRET pair (Sapsford et al., 2006). When the absorption band of the quencher overlaps with the fluorescence emission band of the donor, the FRET induced fluorescence quenching occurs effectively. Gold nanoparticles (Au NPs) is an ideal nano quencher to establish FRET-based sensing platform owing to their extremely larger extinction coefficient (about 10^8 cm^{-1} M^{-1} or more) and the broad absorption spectrum which overlap with the emission of CQDs (Moores and Goettmann, 2006; Shang and Dong, 2009). Currently, significant efforts have been made to apply such FRET-based sensing platforms for sensitive detection of various analytes such as H_2O_2 and organophosphorus pesticides (OPs) (Du et al., 2014; Long et al., 2015).

OPs are in widespread use for crop protection due to their high effectiveness and relatively low persistence (Mulchandani et al., 2011; Tse et al., 2004). OPs exert highly acute toxicity on human because they irreversibly inactivate cholinesterase by broken down acetylcholine to choline, including butyrylcholinesterase (BChE) and acetylcholinester-

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ase (AChE) (Amitai et al., 1998; Zhang et al., 2014). Enzyme inhibition can cause acetylcholine accumulation in the synapse, thus resulting in fatal consequences (Van Dyk and Pletschke, 2011; Quinn, 1987; Jarv, 1984; Pope, 1999; Ray, 1998). Considering the potential toxicity towards unintended targets (Tse et al., 2004), it is of significant promise to establish fast and sensitive methods for detection of OPs. Current OP detection methods include liquid/gas chromatography coupled with various detectors (Yao et al., 1991; Sherma, 1993 ; Albanis et al., 2004; Leandro et al., 2006), enzyme-linked immunosorbent assays (ELISAs) (Gabaldón et al., 2007; Qian et al., 2009), and electrochemical analysis (Chen et al., 2012; Du et al., 2011; Wang et al., 2011). Generally, these methods (Yao et al., 1991; Sherma, 1993; Albanis et al., 2004; Leandro et al., 2006; Gabaldón et al., 2007; Qian et al., 2009) are laborious and time-consuming and unsuitable for point-of-care detection. To improve the sensitivity of detection, different kind of nanomaterials have been used such as quantum dots, nanoprobe (Wang et al., 2008; Liu and Lin, 2007; Du et al., 2010).

Very recently, Au NPs combined with florescence probes such as rhodamine B, CdTe quantum dots and upconversion nanoparticles have been used for the determination of OPs (Liu et al., 2012; Guo et al., 2014; Long et al., 2015). In this work, CQDs were used for the detection of OPs. Compared with the florescence probes mentioned above, CQDs have the attractive advantages including low toxicity, simple synthetic routes and do not need the excitation light of NIR light. The CQDs were synthesized from commercial chlorophyll and could be effectively quenched by Au NPs via FRET. Based on the FRET between CQDs and Au NPs, a sensitive and convenient sensing platform for OPs determination was developed. The principle of the proposal is illustrated in Scheme 1. Acetylthiocholine (ATC) can be hydrolyzed by BChE to produce thiocholine, which causes the aggregation of Au NPs and the corresponding recovery of the FRET-quenched fluorescence emission. The catalytic activity of BChE is irreversibly inhibited by OPs, resulting in the decrease of Au NPs aggregation and then the reduced recovery of FRET-quenched fluorescence emission. Thus, by evaluating the fluorescence emission intensity of CQDs, a FRET-based sensing platform for determination of OPs was established.

2. Experimental

2.1. Materials and instruments

Hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4 \cdot 3H_2O$), BChE (≥300 U mg^{-1} protein from equine serum), acetylthiocholine iodide (≥98%), iron chloride ($FeCl_3$, ≥98%), magnesium chloride ($MgCl_2$, ≥98%), calcium chloride ($CaCl_2$, ≥98%) paraoxon (≥90%), cysteine (≥97%) and chlorophyll (≥90%) were purchased from Sigma-Aldrich (USA). Analytical pure potassium chloride (KCl), glucose, sodium chloride (NaCl) and hydrochloric acid (HCl) was obtained from Avantor Performance Materials (USA). Trimethylol aminomethane (≥99.8%) were bought from Fisher Scientific UK Ltd and adjusted with 0.1 mol L^{-1} HCl. River water was collected from the local river and tap water was obtained in our laboratory. ELISA 96-well flat-bottom plates were obtained from Fisher Scientific (Pittsburgh, PA). UV–vis measurements and fluorescence emission were obtained on a TECAN XFluor multifunctional microplate reader (Leica Microsystems Inc., IL, USA). Dynamic light scattering (DLS) and zeta potential were performed on a Zeta Sizer Nano ZS. Transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) images were measured with a Philips CM200UT transmission electron microscope.

2.2. Synthesis of CQDs

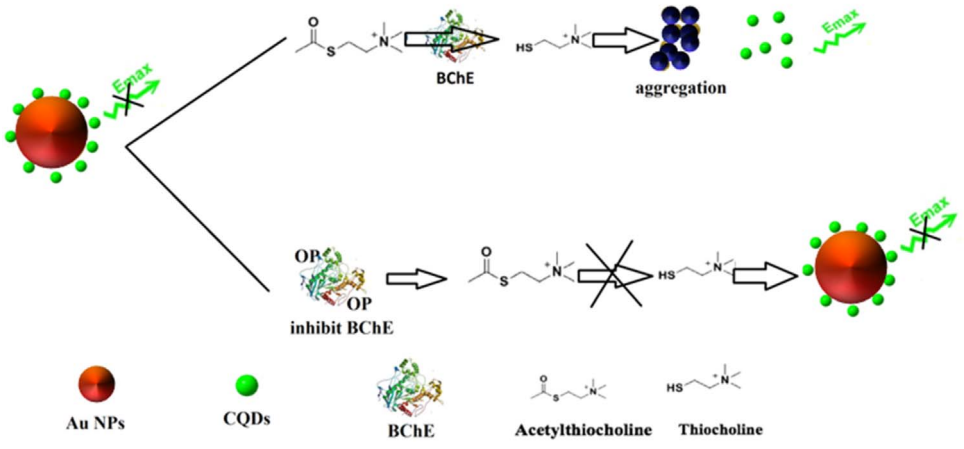
0.2 g of commercial chlorophyll was dissolved in 20 mL of water. Then the mixture was transferred into PTFE autoclaves and heated at 180 °C for 12 h. Dark brown solution was obtained after cooled down to room temperature. And then the solution was purified with a dialysis bag (MWCO ~3.0 kDa) for 1 day. Finally, CQDs were freeze dried to brown solid.

2.3. Preparation of citrate-capped Au NPs

Au NPs were synthesized through the reduction of $HAuCl_4$ by sodium citrate (Grabar et al., 1995). Briefly, trisodium citrate solution (38.8 mmol L^{-1}, 5.0 mL) was added to boiling $HAuCl_4$ (1 mmol L^{-1}, 50 mL) solution under continuous magnetic stirring. After the color of the solution changed from yellow to red wine, the mixture was refluxed for 10 min before cooled down to room temperature. The molar extinction coefficient (ε) of the Au NPs at 520 nm is about 2.7×10^8 mol^{-1} cm^{-1}. The final concentration was calculated to be 4.2 nmol L^{-1} according to Lambert Beer's law (Zhao et al., 2008).

2.4. Experimental procedure for paraoxon determination

Firstly, BChE solution (25 μL, 20 ng mL^{-1}) and various concentrations of paraoxon (25 μL) were incubated at 37 °C (30 min). Then ATC (50 μL, 1 mmol L^{-1}) and tris-HCl buffer (pH=7.5, 10 mmol L^{-1}) were added and incubated for another 15 min. Finally, CQDs (50 μL, 50 mg L^{-1}), Au NPs (150 μL, 4.2 nmol L^{-1}) and water (150 μL) were added. The final concentrations of BChE, ATC, CQDs and Au NPs were 1 ng L^{-1}, 0.1 mmol L^{-1}, 5 mg L^{-1} and 1.26 nmol L^{-1}, respectively. The fluorescence emission spectrums (excited at 440 nm) were recorded. Real sample detection was conducted by investigating the recoveries of different spiking concentrations of paraoxon in tap and river water.



Scheme 1. The principle of FRET-based sensing platform for OPs determination.