Colloids and Surfaces B: Biointerfaces 125 (2015) 90–95

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

A sensitive biosensor for the fluorescence detection of the acetylcholinesterase reaction system based on carbon dots

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ARTICLE INFO

Article history:
Received 9 September 2014
Received in revised form 28 October 2014
Accepted 5 November 2014
Available online 22 November 2014

Keywords:
Carbon dots
Fluorescence
Biosensor
Hydrogen peroxide
Acetylcholinesterase

ABSTRACT

The carbon dots (C-dots) with high fluorescence quantum yield were prepared using hydrothermal method. C-dots have been adopted as probes for the fluorescence turn-off detection of H_2O_2 based on the special sensibility for the hydroxyl radical. And then the biosensors for the detection of substrate and enzymes activities were established in the acetylcholinesterase reaction system, which were related to the production of H_2O_2. Specifically, the proposed fluorescent biosensor was successfully applied to detect the concentration of choline (in the range from 0.025 to 50 μM) and acetylcholine (in the range from 0.050 to 50 μM), and the activity of choline oxidase (in the range from 1 to 75 U/L) and acetylcholinesterase (1 to 80 U/L). These results showed a sensitive, universal, nontoxic and eco-friendly detecting technique has been developed.

1. Introduction

Fluorescence detection method has simple, convenient, rapid and real-time detection and other advantages. A lot of researches focus on quantum dots (QDs) biosensors in recent years due to their novel properties [1–3]. However, the biological toxicity of QDs limits their practical application in clinical analysis. There has been growing interest in the development of nontoxic, biocompatible, and highly fluorescent nanoparticles for biological applications. Carbon dots (C-dots) are one of the most promising class of fluorescent nanoparticles, whose excellent low-toxicity and eco-friendly characteristics are similar to other popular carbon nanomaterials, such as the fullerene, the carbon nanotube and graphene [4,5]. Moreover, the preparation of C-dots does not need for tedious, stringent, and costly preparation steps and it is easy to prepare in large-scale [4]. Because of these inherent advantages, significant progress has been achieved in biological labeling, bioimaging and related biomedical applications using C-dots as optical labels [6], since its first discovery in 2006 [7]. Furthermore, C-dots can be designed for sensors, such as sensors for nitrite [8], phosphate [9], glutathione [10], α-fetoprotein [11], glucose and metal ions [12–17], and so on. However, the detection of biomolecules using C-dots is still rather scarce. The design of novel biomolecules biosensor using C-dots as fluorescent probes are highly required.

Acetylcholinesterase (AChE) is an enzyme regulating acetylcholine (ACh) neurotransmitter that can catalytically break down ACh at cholinergic synapses, resulting in the termination of synaptic transmission. The decrease of AChE makes individuals prone to various nerve disorders including Alzheimer's and Parkinson's disease. Organophosphorus pesticides (OPs), and nerve gases such as sarin may cause health threats to humans and animals, which stems from the inhibition of AChE [18,19]. The substances in AChE reaction system are also significant in clinical analysis. ACh is an essential messenger involved in neurotransmission in both the peripheral and central nervous systems. The decrease of this compound makes individuals prone to various nerve disorders including Parkinson's disease, Alzheimer's disease and multiple sclerosis. Choline is frequently added in food as a nutrient for humans. Hence, the quantitative determination of the ACh, choline and activity of AChE is significant in clinical analysis, such as human serum, brain extracts, amniotic fluid and pharmaceutical products [20–23].

Herein, we investigate a fluorescent biosensor to sensitive quantitative analysis of the AChE reaction system based on the C-dots. The C-dots used in the fluorescent detection is restricted because

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http://dx.doi.org/10.1016/j.colsurfb.2014.11.007
0927-7765/

the fluorescence intensity of C-dots is less susceptible to many active substance, such as H_2O_2. We first prove that the fluorescence intensity of C-dots is quenched by hydroxyl radical. Based on the powerful hydroxyl radical production in Fenton reaction, a biosensor adopting C-dots as probes for the fluorescence turn-off detection of H_2O_2 is fabricated, which is of great importance in environmental, clinical and pharmaceutical fields [24,25]. Following that, the biosensor is also constructed for the detection of the choline, ACh, and activity of choline oxidase (ChOx) and AChE, due to these enzyme reactions are related with H_2O_2.

2. Materials and methods

2.1. Materials

Gelatin (average molecular weight 100,000), choline, ACh and AChE (EC 3.1.1.7, from Electrophorus electricus, electric eel., 425.94 units/mg) were purchased from Sigma–Aldrich. ChOx (EC 1.1.3.17, from Alcaligenes Sp. Lyophilized powder, 13 units/mg) was obtained from J&K Scientific Ltd. Ammonium iron (II) sulfate hexahydrate, disodium hydrogen phosphate, and sodium dihydrogen phosphate were purchased from Xilong Chemical Co. Ltd. All reagents used were of analytical grade and used without further purification. All solutions were prepared using ultrapure water and the ultrapure water (0.22 μm) was produced using a Millipore-Q water system.

2.2. Synthesis of water-soluble C-dots using hydrothermal method

Briefly, 1.1 g gelatin were dissolved in 20 mL water and transferred into a 50 mL Teflon-lined stainless steel autoclave. After that, the autoclave was kept in 240 °C for 24 h. The product can be used after filtered with cylinder filtration membrane filter (0.22 μm). The concentration of the C-dots was 8 mM. Transmission electron microscopy (TEM) (Model JEM-2100 and JEM-2100F, JEOL) was used to characterize the surface morphology of the as-prepared C-dots.

2.3. Electron spin resonance (ESR)

120 μL samples were prepared by mixing 100 μL of Phosphate Buffer (PB) solution containing different concentration of H_2O_2 with 20 μL of 0.2 M dimethyl pyridine N-oxide (DMPO) in a 1 mL plastic tube. The prepared sample was transferred to a quartz capillary tube and placed in the ESR cavity. DMPO was used to trap the hydroxyl radical (•OH) radicals to form the DMPO/•OH spin adduct. Each sample was UV-irradiated at 254 nm for 2 min, and spectra were recorded afterwards. The ESR spectra were obtained on a Bruker ESP 300E.

2.4. Detection of the concentration of hydrogen peroxide

2.5 μL C-dots was diluted into 1 mL PB solution with different concentration of hydrogen peroxide. After that, the solution was adjusted to pH 3.0 with 1 M HCl and measured the fluorescence intensity, which was defined as the fluorescence intensity of the starting point (F_0). After the ammonium iron (II) sulfate hexahydrate solution was added to the mixture obtained, the fluorescence intensity was measured at different time (F_t).

2.5. Detection of the concentration of choline

ChOx was added into PB solution with different concentration of choline and the mixture obtained was kept for a period of time before adjusting the solution pH to 3.0 with 1 M HCl. 2.5 μL C-dots were added to the obtained mixture and measured the fluorescence intensity (F_0). What calls for special attention was the volume of the mixture is 1 mL. After the ammonium iron (II) sulfate hexahydrate solution (0.34 mM) was added to the mixture obtained, the fluorescence intensity was measured at 10 min (F_t).

2.6. Detection of the activity of ChOx

The different amount of enzyme was added into PB solution of choline (0.2 mM) and the mixture obtained was kept for 5 min before adjusting the solution pH to 3.0 with 1 M HCl. 2.5 μL C-dots were added to the obtained mixture and measured the fluorescence intensity (F_0). What calls for special attention was the volume of the mixture is 1 mL. After the ammonium iron (II) sulfate hexahydrate solution (0.34 mM) was added to the mixture obtained, the fluorescence intensity was measured at 10 min (F_t).

2.7. Detection of the concentration of ACh

AChE was added into PB solution with different concentration of ACh and ChOx, and the mixture obtained was kept for a period of time before adjusting the solution pH to 3.0 with 1 M HCl. 2.5 μL C-dots were added to the obtained mixture and measured the fluorescence intensity (F_0). What calls for special attention was the volume of the mixture is 1 mL. After the ammonium iron (II) sulfate hexahydrate solution (0.34 mM) was added to the mixture obtained, the fluorescence intensity was measured at 10 min (F_t).

2.8. Detection of the activity of AChE

The different amount of AChE was added into PB solution of acetylcholine (0.2 mM) and ChOx and the mixture obtained was kept for 5 min before adjusting the solution pH to 3.0 with 1 M HCl. 2.5 μL C-dots were added to the obtained mixture and measured the fluorescence intensity (F_0). What calls for special attention was the volume of the mixture is 1 mL. After the ammonium iron (II) sulfate hexahydrate solution (0.34 mM) was added to the mixture obtained, the fluorescence intensity was measured at 10 min (F_t).

3. Results and discussion

3.1. Characterization of C-dots

Fig. 1A shows a TEM image of the as-prepared C-dots, showing that the products consist of small particles which are well separated from each other. The diameter of the C-dots is about 4–10 nm. The inset in Fig. 1A shows the obvious lattice structure confirming the formation of C-dots. To evaluate the optical properties of C-dots, the emission and excitation spectra (Fig. 1B) were investigated. The fluorescence excitation spectrum shows a peak centered at 330 nm upon emission at 410 nm. The inset in Fig. 1B shows that the as-prepared C-dots solution is transparent brown in color under visible light, while it emits a blue fluorescence under UV light (254 nm). The fluorescence quantum yield for the as-prepared C-dots is determined by calibrating against quinine sulphate in 0.1 M H_2SO_4 solution (Fig. S1). The fluorescence quantum yield of quinine sulfate in 0.1 M H_2SO_4 solution is 54% and the fluorescence quantum yield of C-dots is 23%.

3.2. The special sensibility of C-dots for the hydroxyl radical

Further research shows the prepared C-dots are insensitive to H_2O_2. There is no apparent change of the fluorescence intensity of C-dots storing in H_2O_2 solution. However, the fluorescence intensity of C-dots decreases apparently, when the solution containing H_2O_2 is exposed to UV light. Fig. 2A shows the change of