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Growth-sensitive gold nanoshells precursor nanocomposites for the detection of L-DOPA and tyrosinase activity

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ABSTRACT

In the present work, we have developed a novel nanocomposite-based method for the detection of L-DOPA and tyrosinase (TR) activity. This was accomplished by growth-sensitive gold nanoshells (GNSs) precursor nanocomposites (SiO_2/GNPs II) and the formation process of GNSs. L-DOPA can reduce $AuCl_4^-$ to Au^0, depositing on the surface of SiO_2/GNPs II and mediating the enlargement of gold nanoparticles (GNPs). Here, the preadsorbed GNPs on SiO_2/GNPs II serve as nucleation sites for Au^0 deposition. As the concentration of L-DOPA increases, the surface coverage of resultant gold on silica cores increases accordingly until continuous GNSs are formed. In this growth procedure, the spectra changes in wavelength correlate well with the concentration of L-DOPA, which indicates that this nanocomposite is a good nanoprobe for detecting L-DOPA. Because TR can catalyze the hydroxylation of L-tyrosine to form L-DOPA, this approach can also be employed to analyze the activity of TR, which possesses vast clinical and food industrial importance.

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1. Introduction

L-DOPA, the precursor of dopamine, is an important neurotransmitter which is commonly used for the treatment of neural disorders such as Parkinson's syndrome (Nutt et al., 1984; Olanow and Tatton, 1999; Antonini et al., 2009). After administered orally, L-DOPA is rapidly absorbed through the bowel, then widely distributed in the tissues and converted to dopamine by dopa-decarboxylase (Muzzi et al., 2008; Fuentes et al., 2009). Nevertheless, some side effects such as nausea, vomiting and cardiac arrhythmias can appear if L-DOPA is taken at high dosages (Kim et al., 2008). In vitro, L-DOPA is a powerful toxin to the culture of neurons, and may be also toxic in vivo according to some animal studies (Daneshgar et al., 2009). Therefore, to achieve better curative effect and lower toxicity, the development of a method for the accurate and specific measurement of L-DOPA in different sample matrices is of substantial significance.

A variety of electrochemical and optical methods coupled with numerous techniques have been reported for the determination of L-DOPA in recent years, such as high-performance liquid chromatography, capillary electrophoresis, mass spectrometry, flow-batch techniques, the fast Fourier transform square-wave voltammetry, etc. (Swanek et al., 1996; Raj et al., 2002; Moini et al., 2003; Chen et al., 2003; Gu et al., 2008). However, owing to the low concentration in biological samples and the tendency to be oxidized, analysis of L-DOPA presents plenty of difficulties to researchers. Besides, these conventional approaches or techniques are always expensive, labor-intensive and time consuming. Some of them may produce chemical waste as well. Thus, the development of new and practical methods for the detection of L-DOPA still remains a great challenge.

During the past few years, the unique optical properties and chemical stability of noble metal nanoparticles have made them ideal probes for studying biological systems (Talley et al., 2004). Their efficient localized surface plasmon resonance (LSPR) phenomenon qualifies them attractive blocks for signal transducers and/or signal amplifiers in biomolecular sensing (Nehl et al., 2004; Xiao et al., 2004; Shi et al., 2005; Zayats et al., 2005; Khlebtsov, 2008). Among these particles, gold nanoshells (GNSs) are extraordinarily attractive because of their highly tunable plasmon resonances. They consist of a spherical silica core covered with a thin gold shell, which ensures both convenient surface bioconjugation with molecular probes and remarkable plasmon-derived optical properties (Khlebtsov et al., 2008). By simply varying the ratio of the inner to outer diameter of the shell, the plasmon resonance frequency of GNSs can easily be tuned from the visible into the near-infrared region where optical transmission through tissue is optimal (Prodan et al., 2003; Wu et al., 2008; Hu et al., 2008). With such fascinating characteristics as well as high biocompatibility, GNSs have been proved to be an efficient platform for analytical biosensing (Hirsch et al., 2003; Wang et al., 2008).

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Noticeably, Willner's recent studies have revealed that L-DOPA could mediate the generation and growth of gold nanoparticles (GNPs) (Baron et al., 2005), whose plasmon absorption intensity enabled the quantitative determination of L-DOPA. In this paper, we describe a novel and facile spectrophotometric strategy to detect L-DOPA not by functionalizing continuous GNSs with biospecific molecules traditionally but by the biocatalyzed growth of GNSs based on growth-sensitive GNSs precursor nanocomposites (SiO_2/GNPs II), where L-DOPA acts as a reducing agent for the reduction of $AuCl_4^-$ to Au^0. During this process, concomitant with the increase of surface coverage on silica cores of resultant gold, the LSPR absorption peak of GNSs manifested an obvious red shift in wavelength, which was quantitatively correlated with the concentration of L-DOPA. Moreover, tyrosinase (TR) can biocatalyze the hydroxylation of L-tyrosine to form L-DOPA, this growth process can be utilized for the analysis of TR activity as well, which possesses great clinical and food industrial importance.

2. Experimental

2.1. Materials and reagents

Ultrapure water from Milli-Q (Millipore, America, resistivity >18 MΩ) source was used throughout the experiments. L-Tyrosine and TR (from mushroom, 1.14.18.1, stored at 4 °C) were obtained from Bio Basic Inc. and Worthington Biochemical Corporation respectively. L-DOPA was purchased from Nanjing Kai Yang Biotechnology Co., Ltd., China. 3-Aminopropyltriethoxysilane (APTES, 97%), from Sigma, was stored at 4 °C. Monodispersed silica colloids (∼110 nm in diameter) were obtained from Nissan Chemical Ind., Ltd., Japan. Hydrogen tetrachloroaurate trihydrate ($HAuCl_4{\cdot}3H_2O$, 99.9%), potassium carbonate (K_2CO_3, 99%), sodium borohydride ($NaBH_4$, 96%), hydrogen peroxide (H_2O_2, 30%), monosodium dihydrogen phosphate (NaH_2PO_4), and disodium hydrogen phosphate (Na_2HPO_4) were all purchased from Nanjing Sunshine Biotechnology Ltd., China. All reagents were used as received without further purification.

2.2. Fabrication of APTES-functionalized silica colloids (SiO_2-APTES)

The functionalizations of silica colloids were carried out according to the literature procedures by our previous paper (Wang et al., 2007). First, silica colloids were purified by centrifuging and redispersing in ethanol at least five times. And then 0.113 mL of APTES was added to 48.5 mL of the vigorously stirred silica colloids suspension (0.02 g/mL in ethanol). After the mixture was left to react at 70 °C for 3 h, APTES groups are then covalently bonded onto the surface of these colloids. The products were purified by repeated centrifugations and redispersed into ethanol for five times.

2.3. Synthesis of GNPs-attached silica colloids (SiO_2/GNPs I)

Aqueous solution of GNPs (∼5 nm in diameter) was prepared by the reduction of $HAuCl_4{\cdot}3H_2O$ with $NaBH_4$ as reported in our previous work (Ding et al., 2006). The obtained solution was stored at 4 °C until it had aged for at least 1 week, 0.325 mL of APTES-functionalized silica colloids (0.073 g/mL in ethanol) were then added dropwise to it under vigorous stirring to form SiO_2/GNPs I via electrostatic absorption. SiO_2/GNPs I were separated by centrifugation, washed for three times and finally ultrasonically diluted with ultrapure water until the absorbance value at 520 nm of absorption spectra of the suspension reached 0.5 ± 0.005.

2.4. H_2O_2-mediated growth of GNSs

0.01 M of phosphate buffer solution (PBS, pH 7.4) was previously prepared. K_2CO_3/$HAuCl_4$ solution (K–H) was prepared by mixing 100 mg K_2CO_3 in 400 mL of 0.01 M PBS with 6 mL of 25 mM aqueous chloroauric acid stock solution under continuous stirring for 30 min and then aging in the dark at 4 °C for latter use (Brinson et al., 2008). Reaction solution included 3 mL of K–H, 3 mL of 0.01 M PBS, 0.5 mL of SiO_2/GNPs I and different concentrations of H_2O_2 with a reaction time of 10 min. All measurements were performed once at ambient temperature.

2.5. Preparation of growth-sensitive GNSs precursor nanocomposites (SiO_2/GNPs II)

Under continuous stirring, 10 mL of the suspension containing SiO_2/GNPs I was simply added to 120 mL of diluted K–H consisting of a 1:3 (v/v) mixture of K–H and PBS, followed by the addition of 66 μL (0.1 M) of H_2O_2, resulting in some enlargement of GNPs on the SiO_2/GNPs I surface. 20 min later, the suspension was centrifugated and washed for three times with ultrapure water. SiO_2/GNPs II were obtained by the additional dilution with ultrapure water until the absorbance value at 590 nm of absorption spectra of the suspension reached 1 ± 0.005.

2.6. Biocatalyzed growth of GNSs by L-DOPA and its application in the detection of TR activity

Growth solution of GNSs for the detection of L-DOPA and TR activity contained 6 mL of K–H, 0.33 mL of SiO_2/GNPs II and different concentrations of L-DOPA or different amounts of L-tyrosine and TR in 0.01 M PBS in a final volume of 7 mL. All measurements were performed at least in triplicate at ambient temperature and values were averaged. Results are given as means ± standard deviation (S.D.). Absorption spectra were recorded using a Shimadzu UV3150 UV-vis-NIR spectrophotometer. The sequential growth of GNSs was characterized by transmission electron microscope (TEM), JEM 2100EX at 200 kV. The samples were prepared by placing a drop of the respective solution on a carbon-coated copper grid and subsequent air-drying.

3. Results and discussion

3.1. Preparation of growth-sensitive GNSs precursor nanocomposites (SiO_2/GNPs II)

Scheme 1 depicts a schematic illustration of the fabrication of SiO_2/GNPs I, SiO_2/GNPs II and biocatalytic growth of GNSs induced by L-DOPA, which is an active reducing agent for the reduction of $AuCl_4^-$ to Au^0. As described above, we then used attached GNPs on the surface of SiO_2/GNPs II as the nucleation sites to template the growth of a gold shell layer. Concomitant with the increasing coverage of gold on the SiO_2/GNPs II surface, the LSPR absorption peak of GNSs exhibits a pronounced red shift in wavelength, which quantitatively correlates with the concentration of L-DOPA. Actually, conventional spectrophotometry for measuring L-DOPA is mainly using absorbance change of its oxidized product (dopaquinone) at ∼470 nm as the optical signature (Nagaraja et al., 1998; Coello et al., 2000; Nagaraja et al., 2001; Kankkunen et al., 2002), but the sensitivity is very limited. Here, our method is based on the morphology change during the L-DOPA-induced growth of GNSs and detects the corresponding wavelength shift. Compared with variation of absorption value, the variation of wavelength is more sensitive and more direct for visual discrimination, which has more potential application in visual colorimetric detection.

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