Sensors and Actuators B 251 (2017) 836–841

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Colorimetric detection of tyrosinase during the synthesis of kojic acid/silver nanoparticles under illumination

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ARTICLE INFO

Article history:
Received 5 January 2017
Received in revised form 20 May 2017
Accepted 23 May 2017
Available online 25 May 2017

Keywords:
Tyrosinase
AgNPs
Kojic acid
Light
Green synthesis

ABSTRACT

We report a green, reliable and single-step colorimetric assay for tyrosinase (Tyr) using the silver nanoparticles (AgNPs) as the signal readout. With the aid of light as the catalyst, kojic acid can rapidly reduce Ag^+ ions and stablize the produced AgNPs. While uopn addition of Tyr during the synthesis of AgNPs, Tyr can effectively interrupt the formation of AgNPs due to its preferential combination with kojic acid, eventually leading to color fading and signal decrease of AgNPs solution. The presented method shows a good linear response toward Tyr over the range 0.5–4 u/mL with low detection limit of 0.117 u/mL. Common coexisting substances including metal ions, amino acids and biomacromolecules exert no effect on the determination of Tyr. This colorimetric sensor was successfully employed to quantify Tyr in human serum samples.

1. Introduction

Tyrosinase (Tyr), a typical polyphenol oxidase, is a copper-containing enzyme that controls the production of melanin by catalyzing the oxidation of phenolic substrates into respective quinones. This enzyme is widely distributed in all kinds of organisms, such as fungi, plants and animal tissues [1,2] and has been considered as an important biomarker of melanoma cancer because of its overexpression in melanoma cancer cells [3,4]. In addition, an abnormal level of tyrosinase may cause serious skin diseases such as vitiligo and neurological syndromes like Parkinson's disease [5,6]. It is also an influencing factor in the appearance of human beings and the nutritional value of fruits and vegetables [7,8]. Therefore, tyrosinase assay possesses vast importance for both fundamental research and practical applications in clinical diagnosis, cosmetic, and food industry.

Despite being quantitative, the traditional colorimetric assay is limited by its low sensitivity [9,10]. Recently, several other methodologies have been developed for the detection of Tyr, including electrochemical [11–13], spectrophotometric [14–17] and fluorescent [18–24] methods. Among these methods, noble metal nanoparticles, like gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs), have been used as promising probes due to their simplicity, high sensitivity and biocompatibility [14–16]. To date, noble metal nanoparticles based colorimetric assays mainly rely on apparent color changes of the monodispersed or aggregated state of Au or AgNPs owing to their high extinction coefficients and localized surface plasmon resonance (LSPR) effect [25,26]. Although this feature enables the detection of target-induced molecular events, external factors in complicated application environments (e.g., the impurities, extreme temperature, or high ionic strength) may induce undesirable aggregation of Au or AgNPs, leading to unreliable detection possibility [27]. Furthermore, these colorimetric assays generally require three steps: synthesis of nanoparticles, modification of nanoparticles, and detection of targets. The first two steps usually involve toxic reagents and/or complex chemical procedures, which limits their applicability [14,28,29]. It is thus highly desirable to establish a more facile and rapid assay for reliable detection of Tyr [30–32].

Herein, we developed a simple, rapid, and label-free colorimetric assay for the determination of Tyr based on the specific binding between Tyr and kojic acid which prevented the formation of AgNPs. As illustrated in Scheme 1, under illumination, the AgNPs were readily prepared by using kojic acid (KA) as both reducing and capping agents in aqueous solution. While in the presence of Tyr, KA would strongly bind with it by chelating the copper ions normally present in the active site of tyrosinase [33], consequently resulting in an obvious inhibition effect on the formation of AgNPs. According to our method, the synthesis, modification of AgNPs and the detection of Tyr can be accomplished in a single step, which is more facile and rapid than traditional three-step methods. This kind of signal-generated method via the involvement of the target dur-

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http://dx.doi.org/10.1016/j.snb.2017.05.129
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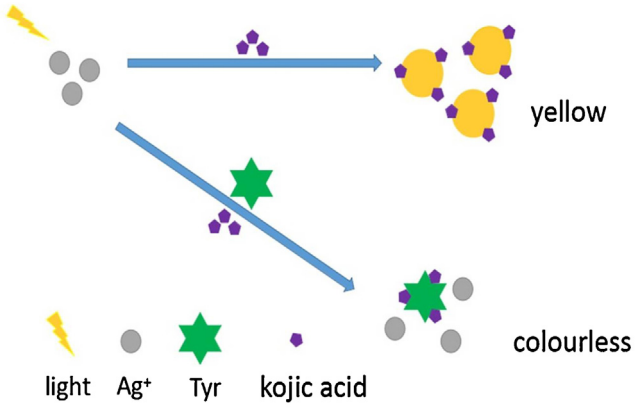

Scheme 1. Schematic representation for colorimetric detection of Tyr during the synthesis of kojic acid/Ag nanoparticles under illumination.

ing the generation of AgNPs is also much more reliable and specific than the aggregated colorimetric signal method. In our assay, the solution color gradually faded from bright yellow to light yellow and even to colorless, which was easily recognized by the naked eye. Moreover, light-driven green synthesis of AgNPs with KA as the reducing agent makes this method economical and environmentally benign [34–37]. Thanks to light irradiation, AgNPs can be produced much faster than under normal conditions. Besides, compared with previous reports about synthesis of AgNPs through light irradiation strategy [34–36], the preparation of synthetic reducing agents or natural extracts by tedious pretreatment is not included in our method. Both of them would further save some time and simplify operation procedures.

2. Experimental

2.1. Materials

All reagents and solvents were at least analytical grade and used directly without further purification. $AgNO_3$, NaOH, kojic acid, $NaNO_3$, KNO_3, $Mg(NO_3)_2$, $Ca(NO_3)_2$, glucose, Bovine Serum Albumin (BSA), glycine (Gly), alanine (Ala), glucose oxidase (GOD), phenylalanine (Phe), leucine(Leu) and isoleucine(Ile) were purchased from Shanghai Qingxi Technology Co., Ltd. (Shanghai, China). Mushroom tyrosinase (EC 1.14.18.1) was purchased from Sigma (St. Louis, MO, USA). Milli-Q-purified distilled water was used to prepare all the solution in this study. All glassware was cleaned thoroughly with freshly prepared aqua regia (3:1 (v/v) HCl/HNO_3) and rinsed thoroughly with doubly distilled water prior to use. All experiments were operated at room temperature.

2.2. Instruments

UV–vis absorption spectra were examined on a UV-2550 spectrophotometer (Shimadzu, Kyoto, Japan) using a 1.0-cm quartz cell at room temperature. Fourier transform infrared (FT-IR) spectra were measured with KBr pellets on a Nicolet 5700 FTIR Spectrometer (Nicolet, USA). Transmission electron microscopy (TEM) was recorded by a JEM-2100 transmission electron microscope (JEOL Ltd. Japan). The data of dynamic light scattering (DLS) were obtained on NPA152 Nanoparticle size analyzer (Microtrac Inc., USA). The Simulated light source is a LED folding lamp (AS-CT104, Aisan-led Inc., China). Its power and luminous flux are 3 W and 240 LM, respectively. The light wavelength range is between 400 nm and 800 nm.

2.3. Colorimetric detection of tyrosinase

First, 20 μL of kojic acid (4 mM) and corresponding concentrations of tyrosinase were mixed in a 5 mL centrifugal tube. Then, 30 μL of NaOH (0.1 M) and 40 μL of $AgNO_3$ (8 mM) were added to the tube in sequences. Finally, the solution was added to 3910 μL ultrapure water. The mixture was incubated at room temperature for 40 min under the light before UV–vis measurents and photograph-taken. Here a LED folding lamp mentioned above was used as the light source.

Experimental conditions including the concentration of NaOH (0–1 mM), kojic acid (0–60 μM), $AgNO_3$ (0–120 μM), and reaction time were studied with UV–vis absorption spectra. The spiked-recovery detection of tyrosinase in the human serum sample was manipulated according to the same procedure.

Human serum was obtained from Nanchang University Hospital from healthy donors. Human serum (0.5 mL) was placed in a centrifuge tube and acetonitrile (2.0 mL) was added to precipitate proteins. After vortex-mixing, the sample was centrifuged at 12, 000 rpm for 15 min, and the supernatant was transferred into a 25 mL volumetric flask and diluted to the mark with deionized water.

3. Results and discussion

3.1. Establishment of colorimetric assay for Tyr

The hydroxyl groups of KA were reported to show moderate reducing ability[38]. In our experiment, KA was used to reduce Ag^+ ions under illumination. The formed KA/AgNPs in bright yellow color (Fig. 1A-c) displayed a strong SPR absorption band at 410 nm (Fig. 1B-c), indicative of the characteristics of monodispersed AgNPs[30]. This is mainly because KA serves as the capping agent on the surface of AgNPs to protect them from aggregation. During the reaction process, the hydroxyl groups lost electrons to form quinone structure, which would be attached onto the AgNPs surface and stabilize the monodispersed AgNPs. FTIR measurement further testified the modification of AgNPs with KA (Fig. S1). The intense absorption peaks at ca. 3450, 2917, and 1652 cm^{-1} were ascribed to the —OH, —CH—, and C=O groups of KA, respectively[39]. These characteristic peaks could also be discerned in the pure KA sample. It should be noted that compared with KA, the band of the —OH group of KA/AgNPs blueshifted to some extent, probably due to the combination between the —OH group of KA and the Ag atoms.

It was also demonstrated that illumination treatment was an important factor which had significant influence on the synthesis of AgNPs. As shown in Fig. 1A-a and B-a, a similar control solution kept in the dark for the same time did not show any color or spectral change. And even after 5 h, the solution exhibited very slight yellow color together with subtle increase of the absorbance at 410 nm (Fig. 1A-b and B-b). These results indicated that light plays an indispensable role in accelerating the reaction rate during the synthesis of KA/AgNPs. As reported previously, light may facilitate efficient and fast electron transfer for the reduction of Ag^+ ions to Ag(0) state during the formation of AgNPs [34–37].

While upon addition of Tyr during the synthesis of KA/AgNPs, and the solution color finally faded away (Fig. 1A-d), accompanied by sharp decrease of the corresponding absorption peak (Fig. 1B-d). Such spectral and color changes of KA/AgNPs upon addition of Tyr could be explained well by the coordination interaction between copper ions in the active site of Tyr and the ketone group and hydroxyl group of KA [33]. The formation of the adduct composed of Tyr and KA decreased the amount of free KA to reduce Ag^+ ions, eventually producing a small quantity of pale-colored AgNPs. We investigated UV–vis absorption spectra of kojic acid after the addi-