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Novel synthesis of gold nanoclusters templated with L-tyrosine for selective analyzing tyrosinase

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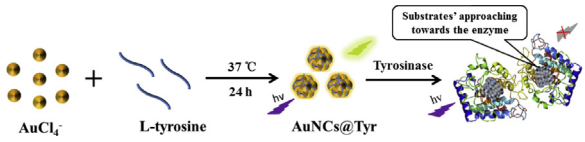
HIGHLIGHTS

- A novel, one-pot strategy for synthesizing fluorescent AuNCs@Tyr was proposed.
- A selective and cost-effective assay for TR activity has been well established.
- This AuNCs@Tyr here may broaden avenues for detecting TR in clinical applications.

GRAPHICAL ABSTRACT

One-pot and novel synthesized fluorescent gold nanoclusters templated with L-tyrosine (AuNCs@Tyr) were employed for investigating tyrosinase activity on the basis of aggregations of AuNCs@Tyr on its active sites during the catalysis reactions, thus leading to the fluorescence quenching of AuNCs@Tyr.



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ABSTRACT

L-Tyrosine (Tyr), playing roles as both a reducing reagent and a protecting ligand, has been first employed for synthesizing fluorescent gold nanoclusters (AuNCs@Tyr) via a novel one-pot strategy. The as-prepared AuNCs@Tyr exhibited a fluorescence emission at 470 nm with a quantum yield of approximately 2.5%. Subsequently, the AuNCs@Tyr described here was applied for detections of tyrosinase (TR) activity, which was based on the mechanism of aggregations of AuNCs@Tyr occurring on the active sites of TR since TR was introduced, thus leading to the fluorescence quenching of AuNCs@Tyr. Accordingly, TR was analyzed in a linear range of 0.5–200 u mL^{-1} with a detection limit of 0.08 u mL^{-1} at a signal-to-noise ratio of 3. Significantly, TR has been considered as a critical marker for melanoma owing to its specifically expressing in melanoma cells. Therefore, this analytical method towards investigating TR activity may broaden avenues for meaningfully clinical applications.

1. Introduction

Noble metal nanoclusters (NCs), only consisting of several to tens of atoms, existed in sizes near by Fermi wavelength, and showed properties regulated by their subnanometer dimensions [1–4]. Hence, various methods have been developed for the synthesis of fluorescent AuNCs by using kinds of reducing reagents [5–11]. Usually, AuNCs were synthesized by two major ways. One way is based on the template-assisted synthesis with polymers [12] and biomolecules (e.g., proteins [7,13,14] and DNA [15,16] commonly as templates). Meanwhile, another way was build up on the basis of monolayer protection in the presence of molecules with thiol ligands [17–20]. As a new type of fluorescent material, AuNCs exist in ultra-small size with low toxicity compared with quantum dots. Besides, unique characteristics of AuNCs have recently attracted numerous attentions, potentiating it as a satisfactory candidate for biosensing, catalysis, and imaging [1,4,21,22].

Tyrosinase (TR), as a copper-containing enzyme widely existing in plants, animal tissues and fungi [23], mainly functions as catalyzing the hydroxylation of phenolic substrates to catechol derivatives, and further oxidizing the catechol derivatives as orthoquinone products. Additionally, these reactions have been

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proved as key points during the biosynthetic pathway of melanin and other natural pigments [24,25]. Importantly, TR has been recognized as a specific marker for these cells due to its specifically expressing in melanocytes and melanoma cells [26]. Thus, exploring ways for detecting TR activity showed critical value towards clinical objectives. For this purpose, diverse methods have been developed. However, low sensitivity of traditional analyses for TR has exhibited obvious limitation [27]. Interestingly, new strategies have been reported. For instance, gold nanoparticles have been applied to quantifying TR as well as improving not only the detection feasibility but also the sensitivity [28]. Again, several fluorescent probes for assaying TR have also been well built up including cyanine dyes, conjugated polymers and quantum dots [29]. Among these methods, innovative nanomaterials mainly including metal nanoparticles and semiconductor quantum dots have been generally employed for serving as probes to determine TR activity, and these ideas have indeed showed attractive prospect. Nevertheless, these promising methods are still lacking. Therefore, it is necessary to develop sensitive and selective strategies to broaden ways for more effectively monitoring TR activity.

In this contribution, novel fluorescent gold nanoclusters stabilized with L-tyrosine were first successfully synthesized, while Tyr served as both a reducing reagent and a protecting ligand. Next, high resolution transmission electron microscopy (HR-TEM), UV–vis absorption and fluorescence spectroscopy were introduced to characterize the properties of AuNCs@Tyr prepared here. Moreover, the current AuNCs@Tyr was applied for sensitive and selective detections of tyrosinase. The sensing principle was based on TR induced fluorescence quenching of AuNCs@Tyr, due to the aggregations of AuNCs@Tyr on active sites of TR during the catalysis reactions (Fig. 1), which has been further confirmed by the HR-TEM. Herein, a novel, simple, selective and cost-effective fluorescent probe has been established, suggesting its potential to broaden avenues for sensing TR.

2. Experimental

2.1. Materials and reagents

Hydrogen tetrachloroaurate trihydrate ($HAuCl_4$), L-tyrosine (Tyr), tyrosinase (TR) were purchased from Sigma–Aldrich (Milwaukee, WI). Bovine serum albumin (BSA), urease, subtilisin, ExoIII, glucose oxidase and all the metal ions were obtained from Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). Hydrochloric acid (HCl), disodium hydrogen phosphate (Na_2HPO_4) and sodium dihydrogen phosphate (NaH_2PO_4) were purchased from Dingguo Changsheng Biotechnology Co., Ltd. (Beijing, China). Ultrapure water, 18.25 MΩ cm, produced by an Aquapro AWL-0520-P ultrapure water system (Chongqing, China), was employed for all the following experiments.

2.2. Apparatus

All fluorescence measurements were performed on a Hitachi F-7000 fluorescence spectrophotometer (Tokyo, Japan) with excitation slit set at 5 nm band pass and emission at 5 nm band pass in 1 cm × 1 cm quartz cells. In addition, UV–vis spectra were recorded by a Shimadzu UV-1750 spectrophotometer (Tokyo, Japan). The high resolution transmission electron microscopy (HR-TEM) images were obtained by using a TECNAI G2 F20 microscope (FEI, America) at 200 KV. Images were taken with an Olympus E-510 digital camera (Tokyo, Japan). The quantum yields were obtained by using Absolute PL quantum yield spectrometer C11347 (Hamamatsu, Japan). A Fangzhong pHS-3C digital pH meter (Chengdu, China) was used to measure pH values of the aqueous solutions and a vortex mixer QL-901 (Haimen, China) was applied to blend the solution. The thermostatic water bath (DF-101s) was purchased from Gongyi Experimental Instruments Factory (Henan, China).

2.3. Synthesis of AuNCs@Tyr

All glassware were thoroughly cleaned with freshly prepared aqua regia (HNO_3/HCl, 1:3) and rinsed with ultrapure water prior to use. The AuNCs@Tyr was prepared by the following procedures. L-tyrosine was dissolved in hydrochloric acid solution (0.01 M) at the very beginning. Then, an aqueous solution of $HAuCl_4$ (1.0 mL, 10 mM) was mixed with tyrosine solution (3.0 mL, 6 mM) and stirred vigorously at 37 °C for 24 h. Finally, the products were centrifuged (3000 rpm, 3 min), and the supernatant was subjected to 1000 MWCO of dialysis membrane for purification before further characterization and applications.

2.4. Detection of tyrosinase

Towards the purpose of detecting TR, stock solutions of TR were prepared respectively. Then, 50 μL of phosphate buffer solution (0.2 M, pH 6.4) was mixed with 20 μL of AuNCs@Tyr initially, and 430 μL of various concentrations of TR were added. After incubation for 4 h at 37 °C, the fluorescence intensity of these mixtures were measured upon being excited at 385 nm. To evaluate the interference of biological metal ions, since TR was well known as a metal-containing enzyme, a variety of metal ions (e.g., Ca^{2+}, Ba^{2+}, Fe^{3+}, Ni^{2+}, Pb^{2+}, Mn^{2+}, Co^{2+}, and K^{+}) were introduced with the identical concentration of 250 μM. Furthermore, the selectivity of this assay has been evaluated in the presence of other biological protein (BSA, 1 mg mL^{-1}) and enzymes (e.g., urease, subtilisin, ExoIII, and glucose oxidase, 50 u mL^{-1}).

2.5. Calculation of detection limit

As the lowest analyte concentration, limit of detection (LOD) reflects the sensitivity of analysis methods. To obtain LOD, the

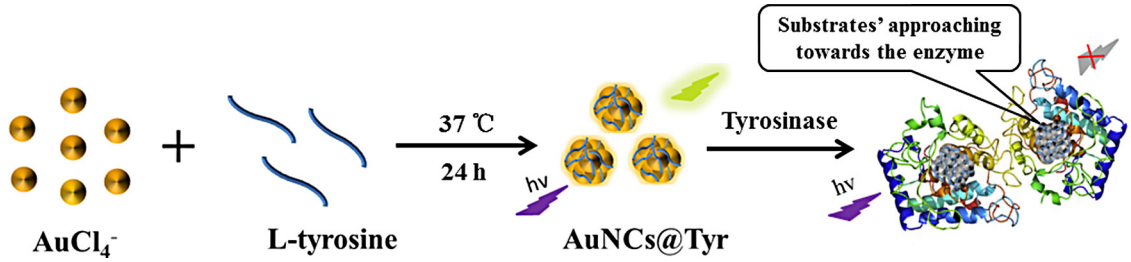

Fig. 1. Schematic illustration of the synthesis of AuNCs@Tyr and the fluorescence quenching mechanism of AuNCs@Tyr by tyrosinase.

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