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Irregular-shaped platinum nanoparticles as peroxidase mimics for highly efficient colorimetric immunoassay

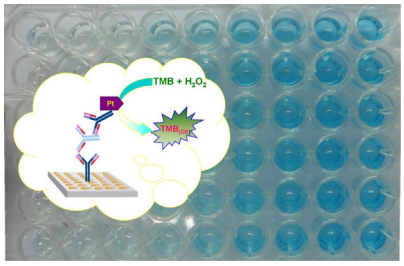
Zhuangqiang Gao, Mingdi Xu, Li Hou, Guonan Chen, Dianping Tang*

Key Laboratory of Analysis and Detection for Food Safety (Ministry of Education and Fujian Province), Department of Chemistry and Chemical Engineering, Fuzhou University, Fuzhou 350108, PR China

HIGHLIGHTS

- We report a new colorimetric immunoassay of rabbit IgG.
- Irregular-shaped platinum nanostructures were used as peroxidase mimics.
- The assay was implemented based on nanocatalysts.

GRAPHICAL ABSTRACT



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ABSTRACT

Enzyme-linked immunosorbent assay (ELISA) methods based on natural enzyme-labeled probes have been applied in the immunoassays, but most have some inevitable limitations (e.g. harsh preparation, purification and storage) and are unsuitable for routine use. Herein we synthesized a new class of irregular-shaped platinum nanoparticles (ISPtNP) with a mean length of 7.0 nm and a narrowing width from 2.0 to 5.0 nm along the longitudinal axes, which were utilized as peroxidase-like mimics for the development of colorimetric immunoassays. Compared with bioactive horseradish peroxidase (HRP), the synthesized ISPtNP exhibited a low K_m value (~0.12 mM) and a high K_{cat} value (~2.27×10^4 s^{-1}) for 3,3′,5,5′-tetramethylbenzidine (TMB) with strong thermal stability and pH tolerance. The catalytic mechanism of the ISPtNP toward TMB/H_2O_2 was for the first time discussed and deliberated in this work. Based on a sandwich-type assay format, two types of colorimetric immunoassay protocols were designed and developed for the detection of rabbit IgG (RIgG, as a model) by using the synthesized ISPtNP and conventional HRP as the labeling of detection antibodies, respectively. Similar detection limits (LODs) of 2.5 ng mL^{-1} vs. 1.0 ng mL^{-1} were obtained toward RIgG with the ISPtNP labeling compared to HRP format. Intra- and inter-assay coefficients of variation were less than 13%. Importantly, the ISPtNP-based assay system could be suitable for use in a mass production of miniaturized lab-on-a-chip devices and open new opportunities for protein diagnostics and biosecurity.

1. Introduction

Enzyme-linked immunosorbent assay (ELISA) is an important analytical method commonly used for diagnoses in medicine and plant pathology, residue analysis in food and environment, and quality control in various industries [1]. Peroxidase, e.g. horseradish peroxidase (HRP), is one of protein-based enzymes that act as catalysts to facilitate a variety of biological processes, which is commonly used in the ELISAs [2,3]. However, the bioactivity of most natural enzymes are easily regulated and controlled by external conditions, e.g. temperature and pH value [4]. Moreover, the preparation, purification and storage of natural enzymes are usually time-consuming, expensive and complicated, especially for enzymes functionalized with other biomolecules [5,6]. These

* Corresponding author. Tel.: +86 591 2286 6125; fax: +86 591 2286 6135.
E-mail address: dianping.tang@fzu.edu.cn (D. Tang).

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inevitable disadvantages limit their widespread application. Hence, searching for a new class of catalysts with high catalytic efficiency and mild reaction conditions would be advantageous.

The rapidly emerging research field of nanotechnology, and the process used to generate, manipulate and deploy nanomaterials, provides excitingly new possibilities for the application in catalysis [7–10]. Recent research has looked to develop innovative and powerful novel biofunctionalized nanometer-sized particles, controlling and tailoring their properties in a very predictable manner to meet the needs of specific application [11,12]. Nowadays, various nanomaterials have been discovered with the peroxidase-like activity, e.g., Fe_3O_4 and Co_3O_4 nanoparticles, V_2O_5 nanowires, gold and platinum nanostructures, graphene oxide, and single-walled carbon nanotubes [13]. These nanomaterials are usually used as replacements of natural enzymes that directly catalyze the oxidation of the corresponding substrates. Wang's group used iron phosphate microflowers as peroxidase mimics and superoxide dismutase mimics for detection of H_2O_2 [14]. Su and co-workers discovered that $ZnFe_2O_4$ magnetic nanoparticles possessed intrinsic peroxidase-like activity, which were used for colorimetric detection of glucose [15]. Dong et al. also demonstrated graphene oxide-Fe_3O_4 magnetic nanocomposites with peroxidase-like activity for colorimetric detection of glucose [16]. Unfortunately, peroxidase-like nanomaterials were usually mixed directly with the detection substrates in these methods. To the best of our knowledge, there are a few reports focusing on biofunctionalized peroxidase-like nanostructures and application in the bioassays. One possible reason is that these nanomaterials were difficultly conjugated with biomolecules due to the limitation of availably binding groups on the surface of nanostructures. Certainly, some biomolecules including fluorogen-labeled biomolecules can be covalently conjugated onto the nanomaterials. One of the major problems associated with covalent binding is the bioactive decrease when the biomolecules are exposed to reactive groups and harsh reaction conditions [17]. Noble metals including Pt and Au nanoparticles are often used for the labeling of biomolecules owing to the strong interaction between proteins and nanoparticles [18–21].

Compared with gold nanoparticles, platinum nanoparticles are especially interesting in catalysis, since platinum is one of the most important materials used in catalytic techniques [22]. Hydrogen, oxygen and other gases become bound to platinum. In the form of nanoparticles, platinum has a substantially higher effectiveness because of the increased specific surface area. At the size of 10 nm, 20% of platinum atoms directly interact with the surroundings of the nanoparticle. Ma's group found that 10-nm cubic Pt nanocrystals could directly catalyze the H_2O_2-mediated oxidation of TMB to produce the colored products with high catalytic activity [23]. Regrettably, pure platinum nanoparticles are usually instable and easily aggregated together in a relatively short storage time. Although recent reports were focused on the synthesis of peroxidase-like hybrid nanomaterials, e.g. Au@Pt nanostructures [24], ferritin-platinum nanoparticles [25], and AgM bimetallic alloy nanostructures (M = Au, Pd, Pt) [26], the preparation methods were complicated because separate metal nanoparticles might be formed during the synthesis. Inspiringly, antigens or antibodies in aqueous solution have a net electrical charge polarity, which is correlated to the isoelectric points of the species and the ionic composition of the solution [17]. When platinum nanoparticles are modified with the antigens or antibodies, the functional nanoparticles may be carried with certain charges on the surface of nanoparticles, thus resulting in mutual repulsion in the solution. So the protein molecules make platinum nanoparticles become stabilized and less coagulated [27]. In this regard, our motivation in this work is to synthesize dispersive platinum nanostructures functionalized with the biomolecules and realize their application in bioassays.

Colloidal methods have proven particularly suitable for producing small nanoparticles with controlled morphologies and excellent catalytic properties [28]. Herein we initially synthesized irregular-shaped platinum nanoparticles (ISPtNP) by using the seed-growth method, which were used as replacements of HRP enzymes to catalyze the H_2O_2-mediated oxidation of TMB. Using goat polyclonal anti-rabbit IgG antibody (anti-RIgG) as a model, the synthesized ISPtNP were labeled onto the anti-RIgG antibodies, which were utilized for colorimetric immunoassay of RIgG in a 96-well polystyrene microtiter plates. The aim of this study is to explore a simple and feasible peroxidase mimic with high catalytic activity for use in bioassays.

2. Experimental

2.1. Reagents and chemicals

Goat polyclonal anti-rabbit IgG antibody (anti-RIgG) and goat polyclonal anti-rabbit IgG antibody conjugated with HRP (HRP-anti-RIgG) were purchased from Jackson ImmunoResearch Laboratories Inc. (USA). Rabbit IgG (RIgG) was purchased from Dingguo Biotechnol. Co., Ltd. (Beijing, China). 3,3′,5,5′-tetramethylbenzidine (TMB), bovine serum albumin (BSA) and hexachloroplatinic (IV) acid hexahydrate ($H_2PtCl_6{\cdot}6H_2O$, 99.9 wt %) were purchased from Sinopharm Chem. Re. Co., Ltd. (Shanghai, China). All other reagents were of analytical grade and were used without further purification. Ultrapure water obtained from a Millipore water purification system (18 MΩ, Milli-Q, Millipore) was used in all runs.

A pH 9.6 coating buffer (1.59 g Na_2CO_3, 2.93 g $NaHCO_3$ and 0.2 g NaN_3) and a pH 7.4 phosphate-buffered saline (PBS, 0.01 M) (2.9 g $Na_2HPO_4{\cdot}12H_2O$, 0.24 g KH_2PO_4, 0.2 g KCl and 8.0 g NaCl) were prepared by adding the corresponding chemicals into 1000 mL distilled water, respectively. The blocking buffer and washing buffer (PBST) were obtained by adding 1.0% BSA (w/v) and 0.05% Tween 20 (v/v) in PBS, respectively.

2.2. Preparation of irregular-shaped platinum nanoparticles (ISPtNP)

Irregular-shaped platinum nanoparticles (designated as ISPtNP) were synthesized according to [29] with slight modification. All glassware used in the following procedure was cleaned in a bath of freshly prepared solution (1:3 HNO_3–HCl), thoroughly rinsed with double-distilled water, and dried prior to use. Initially, 100 mL of H_2PtCl_6 aqueous solution (0.234 mM) was added into a 250-mL round-bottom flask equipped with a condenser, and heated to 100 °C. Following that, 1.25 mL of trisodium citrate (38.8 mM) was introduced quickly into the boiling solution, and continuously stirred for 90 min at 750 rpm until the color of the mixture turned from pale yellow to black (*Note*: The aim of this step is to form small-size platinum nanoparticles for the seed growth). Afterwards, the mixture was cooled down to room temperature (RT), and 0.5 mL of H_2PtCl_6 solution (23.4 mM) and 0.625 mL of trisodium citrate (38.8 mM) were simultaneously added into the resulting mixture. Subsequently, the resultant mixture was heated again to 100 °C, and kept for 90 min under stirring. The obtained ISPtNP were finally confirmed by transmission electron microscopy (TEM; Hitachi H-7650, Japan), and stored at 4 °C in a dark-colored glass bottle ($C_{[Pt]} \approx 0.35$ mM) when not in use.

2.3. Preparation of ISPtNP-anti-RIgG conjugates

The ISPtNP-labeled anti-RIgG antibodies (designated as ISPtNP-anti-RIgG) were prepared consulting to literatures with a little