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Direct application of gold nanoparticles to one-pot electrochemical biosensors

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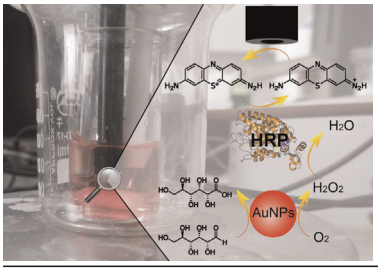
HIGHLIGHTS

- A novel one-pot colloidal AuNPs-based electrochemical biosensor was fabricated.
- AuNPs were first adopted directly as the electrolyte.
- Sensitive detection of glucose and single-nucleotide polymorphism was achieved.
- Without need to modify the electrode, this system can be regenerated easily.

GRAPHICAL ABSTRACT

Gold nanoparticles (AuNPs) have been widely employed for the fabrication of electrochemical biosensors; AuNPs are usually immobilized on the surface of an electrode, so they are difficult to be regenerated, making use of the unfriendly biosensor. In this work, by adopting AuNPs directly as the electrolytes, we have developed a novel AuNPs-based electrochemical detection system. Moreover, the catalytic property of AuNPs is allowed to be associated with the electrochemical reaction to realize cascade reactions. To demonstrate the principle of this design, sensitive detection of glucose as well as single-nucleotide polymorphism has thereby been achieved. This one-pot detection system can be operated and regenerated very easily, since all the components are integrated in the electrolytes of AuNPs, and the unmodified electrode can be reused after being rinsed. This concept by integrating the advantages of sensitive electrochemical detection with the easy-to-operate nanocolloidal system may also promote the development of other kinds of electrochemical biosensors.



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ABSTRACT

Gold nanoparticles (AuNPs) have been widely employed for the fabrication of electrochemical biosensors. In most cases, AuNPs are immobilized on the surface of an electrode, so they are difficult to be regenerated, making the use of the biosensor unfriendly. In this work, by adopting AuNPs directly as the electrolytes, we have developed a novel AuNPs-based electrochemical detection system. In brief, AuNPs-catalyzed oxidation of glucose is combined with a HRP-catalyzed reaction as well as an electrocatalytic reaction to compose cascade reactions in the electrolyte. Thus, the intensity of the electrocatalytic signals has quantitative relation with the concentration of glucose, and favors the sensitive detection of glucose. Furthermore, because the catalysis of AuNPs may be blocked under the interaction with single-stranded DNA and unblocked in the presence of a complementary sequence, detection of DNA and even single-nucleotide polymorphism can thereby been achieved. This one-pot detection system can be operated and regenerated very easily, since all the components are integrated in the electrolytes of AuNPs, and the unmodified electrode can be reused after being rinsed. This concept by integrating the advantages of sensitive electrochemical

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detection with the easy-to-operate nanocolloidal system may also promote the development of other kinds of electrochemical biosensors.

1. Introduction

Gold nanoparticles (AuNPs) have gained the most widespread concern in the areas of research and application of nanomaterial. AuNPs possess good optoelectronic properties, high specific surface area and excellent biocompatibility. These superior characteristics have caused great interest of physicists, materials scientists and biologists [1–3], thus AuNPs have been actively used in diagnostics, therapy, drug delivering, biosensing, etc. [4–7]. Among these applications, development of electrochemical biosensors fabricated with AuNPs has been amazing in recent decade [8,9]. So, detection of a variety of targets such as ions [10,11], micromolecules [12,13], DNA [14,15], proteins [16,17], and cells [18,19] has thereby been successfully achieved by employing AuNPs in electrochemical biosensing systems.

In a typical AuNPs-assisted electrochemical biosensor, AuNPs are immobilized onto the electrode surface and work as building blocks in the interface architecture [20–22]. According to the requirements for the fabrication of different kinds of biosensors, they may play different roles: (1) building a conductive film to improve the conductivity of the interface architecture. For example, Wohltjen and Snow deposited octanethiol-coated AuNPs on the surface of a microelectrode to build a thin electroconductive film (~2 nm) for the fabrication of a fast response (90% response in less than 1 s) biosensor [20]. (2) Working as a signal label. For example, Wang et al. reported the use of colloidal gold tags for electronic detection of DNA hybridization [23]. (3) Working as a carrier for signal amplification. For example, Wang et al. adopted DNA-functionalized AuNPs as the carrier of electrochemical probe $[Ru(NH_3)_5Cl]^{2+}$ for the detection of platelet-derived growth factor [17]. Owing to the large surface area of AuNPs, a large number of electrochemical probes could be carried to produce signal amplification. AuNPs-assisted electrochemical biosensors have also been proven to show many advantages, e.g., high sensitivity and detection of multi-targets [24]. In the meantime, these biosensors also suffer from some drawbacks. For instance, the immobilized AuNPs as well as the whole interface architectures are difficult to be reconstructed, making the biosensor unfriendly.

On the other hand, benefited from the unique surface plasmon resonance property, AuNPs in colloid, i.e., colloidal AuNPs have also drawn great interest [25,26]. Owing to the easy operation, they have been widely used in colorimetric assays [10,12,14,16]. However, it is noticed that in comparison with AuNPs-assisted electrochemical biosensors, the sensitivity of AuNPs-based colorimetric assays is usually not satisfying (Table 1). Immune colloidal gold (ICG) technique, which has been successfully commercialized in paper-based point-of-care diagnostics, still only yields a yes/no result [27].

Inspired by the above mentioned background, we propose that it would be better if the advantage of high sensitivity of AuNPs-assisted electrochemical biosensors can be integrated with the easy operation of colloidal AuNPs. So, we have successfully combined electrochemical technique in this work with colloidal AuNPs to develop a sensitive and re-usable glucose biosensor. It is the first time that citrate-stabilized colloidal AuNPs are employed as the electrolyte directly, while the electrode is unmodified. Taking advantage of cascade reactions initiated by the catalysis of AuNPs and ended by electrocatalysis, sensitivity detection of glucose is achieved. The detection limit is 400 times lower than that by using an AuNPs-based colorimetric assay strategy [28]. Furthermore, since AuNPs do not need to be immobilized onto the surface of electrode, and no complex interface architecture is required, this one-pot detection system is operated and regenerated very easily. In addition to the detection of glucose, this novel colloidal AuNPs-based electrochemical detection system has also been applied for the sensitive and convenient detection of single-nucleotide polymorphism.

2. Material and method

2.1. Regents and apparatus

Single-stranded DNA (ssDNA, Guaranteed Oligos, HPLC-purified) was obtained from Invitrogen, the sequence is shown in Table 2. Horseradish peroxidase (HRP), trisodium citrate, glucose, and thionine were purchased from Sigma. Chloroauric acid ($HAuCl_4$) was purchased from Amresco. All the reagents were of analytical reagent grade. All solutions were prepared with doubly distilled water, which was purified with a Milli-Q purification system (Branstead, USA) to a specific resistance of >18 MΩ cm. A

Table 2
Sequences of adopted oligonucleotides.

Name	Sequence
Probe DNA (P)	5′-GGA AAG TCC CAG-3′
Target DNA (T)	5′ -CTG GGA CTT TCC -3′
1-Base mismatched target (M1)	5′-CTG GG*T* CTT TCC-3′
2-Bases mismatched target (M2)	5′-CTG GG*T A*TT TCC-3′
Unmatched variant (U)	5′-AGA AGA TAA ACA GGT GTG GTT-3′

Table 1
Comparison of the detection range and detection limit of AuNPs-based colorimetric assays and AuNPs-assisted electrochemical biosensors.

Targets	AuNPs-based colorimetric assays	Refs.	AuNPs-assisted electrochemical biosensors	Refs.
Hg^{2+}	0.075–4.0 μM[a] 15 nM[b]	[10]	0.02–1000 nM 0.02 nM	[11]
ATP	4.4–132.7 μM 0.6 μM	[12]	1 nM–10 μM 0.2 nM	[13]
Platelet-derived growth factor	10–100 nM 6 nM	[16]	1 pM–10 nM 0.01 pM	[17]
DNA	– 8 nM	[14]	0.1–100 nM 20 pM	[15]

[a] The detection range.
[b] The detection limit.