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Direct application of gold nanoparticles to one-pot electrochemical biosensors

Guifang Chen [a], Hui Tong [a], Tao Gao [b], Yangyang Chen [a], Genxi Li [a,b,*]

[a] Laboratory of Biosensing Technology, School of Life Sciences, Shanghai University, Shanghai 200444, PR China
[b] State Key Laboratory of Pharmaceutical Biotechnology, Department of Biochemistry, Nanjing University, Nanjing 210093, PR China

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

* Corresponding author at: State Key Laboratory of Pharmaceutical Biotechnology, Department of Biochemistry, Nanjing University, Nanjing 210093, PR China. Tel.: +86 25 83593596; fax: +86 25 83592510.
E-mail address: genxili@nju.edu.cn (G. Li).

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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.

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CHI660D electrochemical workstation (Chenhua, China) was employed for all the electrochemical measurements.

2.2. Preparation of colloidal gold nanoparticles

Colloidal AuNPs with average diameter of 13 nm are synthesized using a citrate reduction method. Briefly, trisodium citrate (10 mL, 38.8 mM) was added to a boiling solution of $HAuCl_4$ (100 mL, 1 mM) under reflux and rapid stirring. After further 30 min of boiling, the color of the solution changed from pale yellow to burgundy. Then, the solution was slowly cooled down to room temperature and filtered through a 0.8 μm membrane. Thus, citrate-stabilized colloidal AuNPs were prepared, and were kept at 4 °C while not in use.

2.3. Preparation of glassy carbon electrode

The surface of a substrate glassy carbon electrode (GCE) was firstly polished on an abrasive paper to remove impurities. Then, it was polished successively with 1.0, 0.3, and 0.05 μm alumina slurries on silk to mirror smoothness, followed by thoroughly ultrasonicated in ethanol and doubly distilled water for about 5 min, respectively. After drying with nitrogen, the electrode is ready for use.

2.4. Fabrication of colloidal AuNPs-based electrochemical detection system

2 mL of freshly prepared colloidal AuNPs was diluted with equal volume of doubly distilled water to work as electrolyte. For the detection of glucose, different concentrations of glucose were added into the electrolyte and incubated at 37 °C for 3 h. Then, HRP (10 μL, 10 mg mL^{-1}) and thionine (10 μL, 2 mM) were added into the electrolyte, and cyclic voltammetric scanning (potential range: 0 V to −0.25 V, scan rate: 5 mV s^{-1}) was conducted. Electrochemical impedance spectroscopy (EIS) was also adopted to characterize the conductivity of the electrode surface (frequency range: 0.01–100 kHz, amplitude of signal: 5 mV). $[Fe(CN)_6]^{4-/3-}$ (5 mM) working as the electrochemical probe is added into the electrolyte during the EIS measurements. To regenerate the detection system, the electrode was totally rinsed by doubly distilled water, and the electrolyte was replaced by a new one. Then, it was ready for the next measurement.

To detect single-nucleotide polymorphism of a target DNA, the diluted AuNPs working as the electrolyte was added with the probe DNA and incubated together at 37 °C for 30 min. Then, target DNA or mismatched variants was added into the electrolyte and incubated for another 30 min. The following steps were the same as that in the detection of glucose with the only difference that the concentration of glucose was fixed at 2 mM.

3. Results and discussion

3.1. Principle of the colloidal AuNPs-based electrochemical detection system

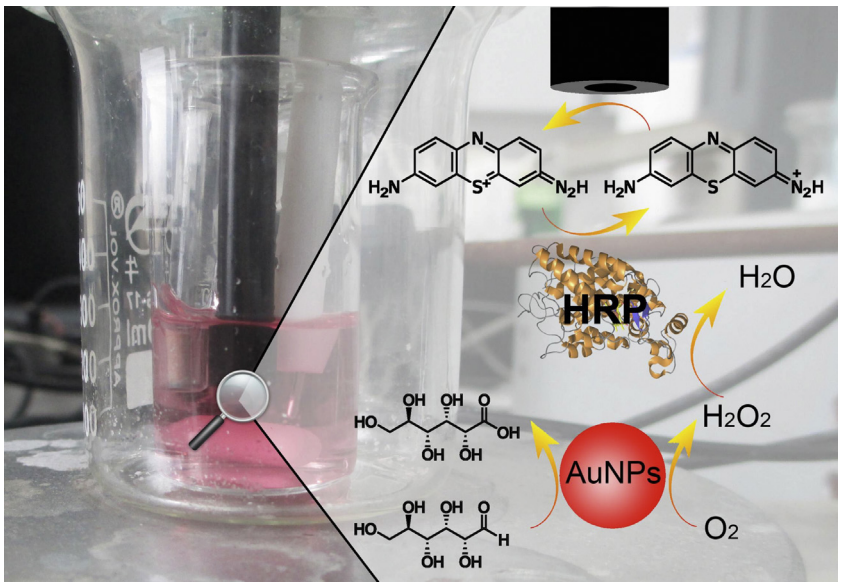
It has been reported that colloidal AuNPs exhibit catalytic activity toward the oxidation of glucose and some other hydroxyl/carbonyl-containing molecules to produce carboxylates and hydrogen peroxide [29]. This property has been successfully adopted for the colorimetric detection of glucose [28]. We have also developed a chemical approach to accurately characterize the coverage rate of AuNPs by using this catalytic property [30]. Here, the AuNPs-catalyzed reaction is combined with a HRP-catalyzed reaction as well as an electrocatalytic reaction to compose cascade reactions, which can be described by Eqs. (1)–(3):

$$\text{Glucose} + O_2 \xrightarrow{\text{AuNPs}} \text{Gluconic acid} + H_2O \quad (1)$$

$$H_2O_2 + \text{thionine(Red)} \xrightarrow{\text{HRP}} H_2O + \text{thionine(Ox)} \quad (2)$$

$$\text{thionine(Ox)} + 2e^- + 2H^+ \xrightarrow{\text{electrode}} \text{thionine(Red)} \quad (3)$$

The “Ox” indicates the oxidative states and “Red” indicates the reductive states. The electrochemical workstation provides two electrons in Eq. (3), representing the catalytic wave. Schematic presentation of the reactions has also been shown in Scheme 1. With the increase of the concentration of glucose, more thionine(Ox) can be produced after Eq. (2), and then reduced on the surface of an electrode to produce intense electrochemical signals. Consequently,



Scheme 1. Schematic illustration of the colloidal AuNPs-based electrochemical detection system.

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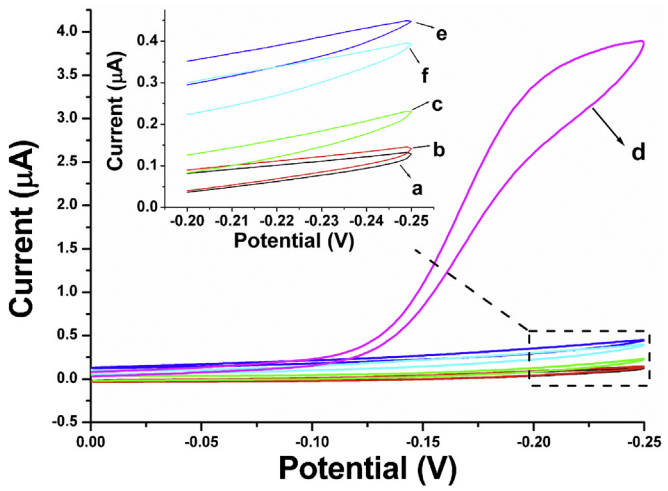

Fig. 1. Cyclic voltammograms obtained in colloidal AuNPs (1.8 nM AuNPs, 1.8 mM trisodium citrate, pH 6.0) only (a), or added with 2 mM glucose (b), 25 μg mL^{-1} HRP (c), and 5 μM thionine (d) in sequence. Curve (e) is obtained in colloidal AuNPs with HRP and thionine, but in the absence of glucose; Curve (f) is obtained in a solution containing thionine only. Scan rate: 5 mV s^{-1}.

a positive relationship between the concentration of glucose and the intensity of electrochemical signals is established. It is also noted that the cascade reactions all take place in the electrolyte (i.e., the AuNPs colloid) that may greatly facilitate the one-pot detention.

It has been reported that ssDNA may be adsorbed onto the surface of AuNPs through the electrostatic interaction between the positively charged bases of ssDNA and the negatively charged surface of AuNPs [31]. We have also proven that the adsorption may block the catalysis of AuNPs, since that the catalysis depends strongly on the exposed surface of AuNPs [30]. When a complementary sequence is presented to hybridize with the ssDNA to form dsDNA, which has been proven to be not able to adsorb onto AuNPs, the surface of AuNPs is exposed again, and the block effect may be removed. Otherwise, if a mismatched or unmatched sequence is instead presented, the hybridization may be restricted. Thus, the two single-stranded oligonucleotides may both adsorb on the surface of AuNPs, intensifying the block effect. Therefore, taking advantage of the electrochemical signals produced from cascade reactions, we can also extend the strategy proposed in this work to detect the polymorphism of oligonucleotides.

3.2. Colloidal AuNPs-based electrochemical detection of glucose

Firstly, we have done experiments to confirm that remarkable electrochemical signals can be obtained through the cascade reactions. As shown in Fig. 1, the components of the cascade reactions are added in a sequence: AuNPs (without deoxidization), glucose, HRP, and thionine(Red). Electrochemical measurements are carried out each time after a component is added. It is observed that only in the presence of all the components, a very large reduction peak with the shape of a catalytic wave can be observed (curve d). It is noticed that on addition of HRP, there is a slight increase of the current (curve c). This can be ascribed to the low efficient HRP-catalyzed reduction of H_2O_2 without the help of the electron mediator thionine [32]. On the other hand, if glucose is absent from the whole cascade reactions, a tiny response is also obtained (curve e), which has been confirmed to be the direct redox response of thionine (curve f). Since the concentration of thionine is fixed during the test, and the current response of thionine itself is much lower than that of the catalytic wave; this background current will hardly affect the detection.

Taking advantage of the catalytic signals, we have then studied the possibility for sensitive detection of glucose by using this novel electrochemical system. Different concentrations of glucose are adopted, while all other components of the cascade reactions are settled during the detection. As is shown in Fig. 2A, the catalytic peak currents of the cyclic voltammogram rise apparently along with the increase of the concentrations of glucose. The results are reasonable and expected, since more glucose may drive more electrons to be involved to produce larger currents. Fig. 2B shows the relationship between the net peak currents at −0.25 V and the concentrations of glucose. The current value in the case of no glucose has been subtracted to obtain net currents. As is shown in Fig. 2B, the current rises sharply in a concentration range from 1 μM to 1 mM, and reaches a plateau at 5 mM. In a range 1–100 μM, the data points may also be linear fitted by an equation: $Y = 0.3633 + 0.00558X$ ($R^2 = 0.98$). Thus, sensitive detection of glucose with a detection limit of 1 μM is achieved.

Since the electrode here is unmodified, the detection system can be regenerated very easily just by rinsing the electrode after measurements. Fig. 3A shows the results of successive detection of glucose in 5 cycles. In each cycle, a net current at −0.25 V from the cyclic voltammogram is obtained for the detection of 2 mM glucose, after which the electrode is totally rinsed and moved to a

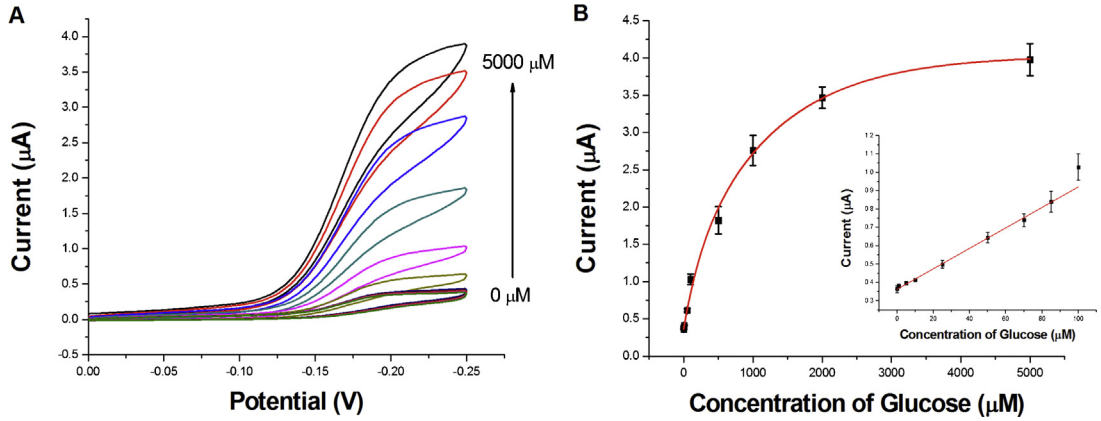

Fig. 2. (A) Cyclic voltammograms obtained upon analyzing different concentrations of glucose. Bottom to up: the concentration is 0, 1, 5, 10, 50, 100, 500, 1000, 2000 and 5000 μM, respectively. The electrolyte contains 1.8 nM AuNPs, 1.8 mM trisodium citrate, 25 μg mL^{-1} HRP, and 5 μM thionine, pH 6.0. Scan rate: 5 mV s^{-1}. (B) Relationship between the peak currents at −0.25 V and the concentrations of glucose. The inset shows a linear relationship in a concentration range from 0 μM to 100 μM. The error bars represent the standard deviation of at least three repetitive measurements.

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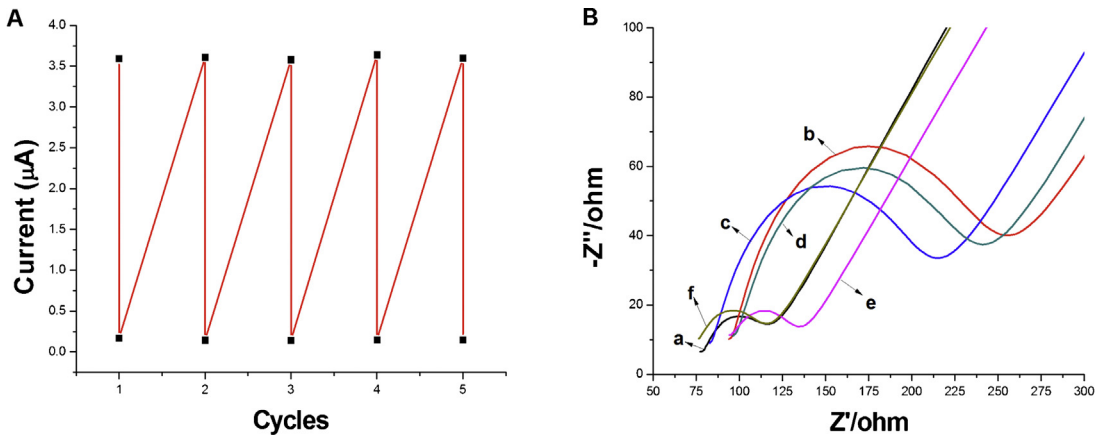

Fig. 3. (A) The reproducibility of the detection system in 5 cycles. The upper dots represent the currents response at −0.25 V for the detection of 2 mM glucose (other conditions are same as that in Fig. 2A), while the bottom dots represent the regeneration currents in a regular phosphate buffer solution (5 mM, pH 7.4, containing no other substrates). (B) Electrochemical impedance of the electrode in AuNPs only (a), AuNPs together with glucose (b) and incubated at 37 °C for 3 h (c). HRP (d) and thionine (e) are further added into the (c) system, in sequence. Curve (f) represents the result of the electrode after totally rinsed. $[Fe(CN)_6]^{4-/3-}$ (5 mM) works as the electrochemical probe. Frequency range: 0.01–100 kHz, amplitude of signal: 5 mV.

regular electrolyte (phosphate buffer solution, 5 mM, pH 7.4, containing no other substrates) to check whether there is any residual signal. As is shown in Fig. 3A, the current response (the upper dots) for the detection of glucose can be well reproduced in 5 cycles without apparent decay. In the meantime, in the case of the regular electrolyte containing no substrates, no current response (the bottom dots) is observed. The results suggest that the acquisition of the detection signal is a diffusion-controlled process. The electrode does not adsorb the redox active molecules after the measurements, which is quite positive for the quick regeneration of the detection system. Further study by using EIS to characterize the surface adsorption has also been conducted. Electrochemical probe $[Fe(CN)_6]^{4-/3-}$ is added into the colloidal AuNPs directly during the detection. As is shown in Fig. 3B, in the case of colloidal AuNPs only, the electrode exhibits a tiny semicircle domain (curve a), which implies fast electron transfer between the probe and the clean electrode. On addition of glucose and HRP, an increase of the electron transfer resistance is observed (curves b–d). Nevertheless, after being rinsed, the electrode shows a fast electron transfer again (curve f). The result confirms that the electrode can be easily regenerated without any contamination during the measurements.

3.3. Colloidal AuNPs-based electrochemical detection of single-nucleotide polymorphism

We have previously proven that ssDNA may block the catalysis of AuNPs through an electrostatic interaction [30]. Here, by integrating the catalysis of AuNPs with the sensitive electrochemical detection system, we show that detection of single-nucleotide polymorphism can be achieved. In our experiments, the AuNPs are firstly allowed to interact with different concentration of the single-stranded probe DNA before the cascade reactions are launched. As is shown in Fig. 4, after the interaction of AuNPs with the probe DNA, the catalytic currents of the detection system decrease apparently. The higher the concentration of the probe DNA is, the lower the current will be. A linear range from 0 to 25 nM

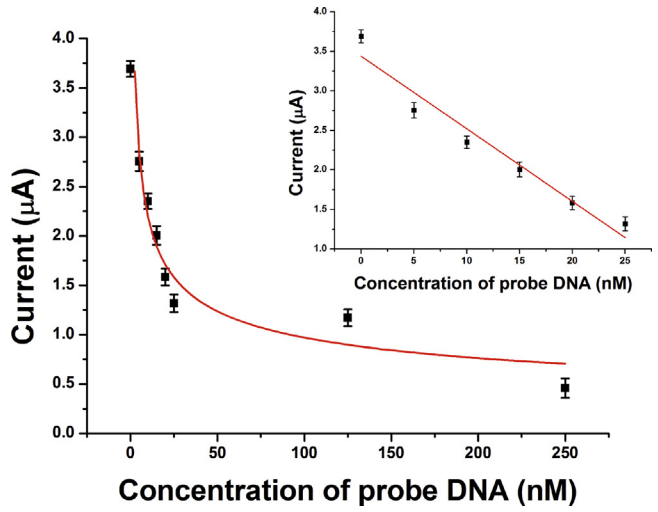

Fig. 4. The calibration curve of the cyclic voltammetric peak currents against the concentrations of the probe DNA (currents response at −0.25 V from the cyclic voltammograms, potential range: 0 V to −0.25 V, scan rate: 5 mV s^{-1}). The electrolyte contains 1.8 nM AuNPs, 1.8 mM trisodium citrate, 25 μg mL^{-1} HRP, 5 μM thionine, and 2 mM glucose, pH 6.0. The inset shows a linear relationship between the peak currents and the concentrations of the probe DNA from 0 nM to 25 nM. The error bars represent the standard deviation of at least three repetitive measurements.

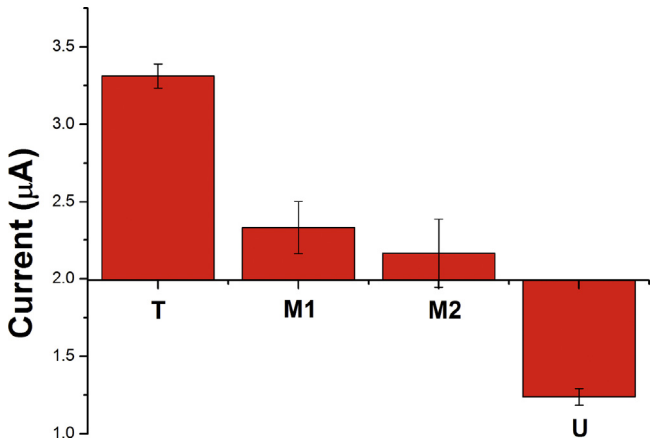

Fig. 5. Specificity of the proposed biosensor toward the discrimination of target DNA (T), 1-base mismatched DNA (M1), 2-base mismatched DNA (M2) and unmatched variant (U). The concentration of each variant is fixed at 15 nM. Because without these variants, the current response of 15 nM probe DNA only is 1.95 μA, this current value is set as a benchmark. The error bars represent the standard deviation of at least three repetitive measurements. Other conditions are same as that in Fig. 4.

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is also obtained (Fig. 4 inset). We next settle the concentration of the probe DNA at 15 nM, a concentration in the most sensitive range, to detect the target DNA. With the addition of the target DNA, the current increases, suggesting that the cascade reactions become more active (data not shown). Since the target DNA may river the AuNPs to hybridize with the probe DNA, the block effect of the probe DNA on AuNPs may be gradually removed along with the increasing of the concentration of the target DNA. A linear relationship between the current and the concentration of the target DNA is also obtained with a linear equation $Y = 2.0445 + 0.0897X$ ($R^2 = 0.99$). This result may suggest the potential application for DNA detection. Otherwise, Fig. 5 shows that the current barely increases, if different mismatched variants instead of the target DNA are added in the test solution. In the case of 1 or 2-base mismatched variants, the growth is only ca. 28% and 16%, respectively, of that for the target DNA. For a totally unmatched variant, the current decreases further. The reason is that the unmatched variant cannot hybridize with the probe DNA, but on the contrary, adsorbs onto the surface of AuNPs, thus blocking the catalysis. So, this system is very sensitive to the changes of the sequence of the target DNA, and consequently can be applied for the detection of single-nucleotide polymorphism. This detection system can be also regenerated simply by rinsing the electrode and replacing the electrolyte. Thus, successive detection with high efficiency may be achieved.

4. Conclusion

In summary, we have developed a novel colloidal AuNPs-based electrochemical detection system. Unlike the conventional strategies, in which AuNPs are immobilized on the surface of electrodes, colloidal AuNPs are prepared and adopted directly as the electrolyte. Therefore, on the one hand, it is not necessary to modify the working electrode, thus regeneration of the electrode is very easy and simple. On the other hand, cascade reactions initiated by the catalysis of AuNPs and ended by electrocatalysis have been established in this system to produce electrochemically catalytic signals. So, sensitive detection of glucose as an example is thereby achieved with a detection limit down to 1 μM, which is much lower than that by using a colorimetric method. This colloidal AuNPs-based electrochemical detection system has also been expanded for the detection of single-nucleotide polymorphism. Clear distinction of target DNA and 1-base mismatched variant can be achieved by using the sensitive electrochemical signals. This one-pot detection system also has the potential to be applied for the fabrication of other kinds of biosensors, e.g., aptasensors.

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