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Au(III)-promoted polyaniline gold nanospheres with electrocatalytic recycling of self-produced reactants for signal amplification†

Yuling Cui, Huafeng Chen, Dianping Tang,* Huanghao Yang and Guonan Chen*

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A novel and redox-active nanocatalyst, Au(III)-promoted polyaniline gold nanosphere (GPANG), was designed as the nanolabel for highly efficient electrochemical immunoassay of human IgG by coupling with electrocatalytic recycling of self-produced reactants.

An ultrasensitive and simple method for the detection of disease-related biomolecules is critical for early diagnosis in biomedical researches.[1] Great effort has been made worldwide to design and develop a highly sensitive assay protocol. Electrochemical immunoassay is one of the predominant analytical methods due to its high sensitivity, low cost and low power requirements.[2] To acquire a high sensitivity, the amplification of the electrochemical signal is extremely important. Although the antigen–antibody reaction by itself can cause the change of the electrochemical signal to some extent, it is very limited. Hence, labelling of the secondary antibody is essential. Enzyme labels and nanolabels are usually employed for the amplification of a measurable product as a result of their very fast and highly selective catalytic reactions, which enable high signal amplification in the presence of bound target biomolecules.[3] However, we recently found that (i) since there is, for sterical reasons, usually a 1 : 1 ratio of enzyme and signal antibody used in the conventional ELISA, the obtainable signal amplification is always limited, and (ii) the bioactivity of the labelled enzymes is usually weakened when enzymes are labelled onto the signal antibody or nanostructures.[4] Therefore, exploring a newly amplified strategy for highly sensitive electrochemical immunosensing without the participation of enzymes would be advantageous.

Nanostructures, especially redox-active nanostructures, are usually used as catalysts for electrochemical reactions or organic/inorganic synthesis. Various nanocatalysts, such as Fe_3O_4–MnO_2 hybrid nanocrystals, ZnO–SiO_2 nanocomposites, Pd nanoparticles and gold nanoparticles, have been reported for catalytic organic synthesis and electrocatalytic reaction.[5] Among these nanocatalysts, gold nanoparticles (AuNPs) have been found to play an important role in the catalytic processes including low-temperature CO oxidation, reductive catalysis of chlorinated or nitrogenated hydrocarbons, and organic synthesis.[6] Significantly, AuNPs can catalyze the reduction of *p*-nitrophenol (NP) to *p*-aminophenol (AP) in the presence of $NaBH_4$.[1a,7] Meanwhile, the produced AP molecules can be oxidized to *p*-quinone imine (QI) with the help of electron mediators, which can be reduced again to AP *via* $NaBH_4$. The catalytic recycling of self-produced reactants resulted in the amplification of the electrochemical signal. In these methods, however, AuNPs and electron mediators were often separated in two different phases, which increased the steric hindrance of electron transfer. More importantly, the efficiency of electron communication between AuNPs and electron mediators might be largely decreased. Our motivation in this work is to synthesize a novel class of redox-active nanocatalysts for the catalytic reduction of NP to AP, and the catalytic recycling of AP to QI without the participation of other electron mediators. Conductive polymer nanomaterials represent an attractive family of nanocatalysts due to their unique properties, *e.g.* high conductivity, relatively environmental stability, and simple doping–dedoping chemistry.[8] Polyaniline, with a rather unique structure, contains an alternating arrangement of benzene rings and nitrogen atoms, which can be used as a candidate for the preparation of redox-active nanocatalysts.

Herein, we synthesized a novel, redox-active nanocatalyst, Au(III)-promoted polyaniline gold nanosphere (GPANG), for the highly efficient electrochemical immunosensing of human IgG (HIgG, as a model) by coupling with electrocatalytic recycling of self-produced reactants. The GPANG was prepared through one-pot *in situ* copolymerized deposition of polyaniline on the AuNPs with the help of Au(III) (see ESI†). The as-prepared GPANGs showed excellent adsorption properties for selective attachment of biomolecules and strong catalytic properties. Fig. 1 illustrates GPANG-catalyzed recycling of self-produced reactants for signal amplification of the sandwich-type electrochemical immunosensing. In this configuration, the added NP was initially reduced to AP *via* the catalysis of the doped nanogold labels onto/into the GPANG in the presence of $NaBH_4$, then the generated AP was electrochemically oxidized to QI by the electron mediator (polyaniline) in the GPANG, and then the oxidized QI was reduced back to AP by $NaBH_4$. The self-produced QI reactant was catalytically recycled and thus amplified the signal of the electrochemical response. To verify the successful

Ministry of Education & Fujian Provincial Key Laboratory of Analysis and Detection of Food Safety, Department of Chemistry, Fuzhou University, Fuzhou 350108, P.R. China.
E-mail: dianping.tang@fzu.edu.cn, gnchen@fzu.edu.cn;
Fax: +86 591 22866135; Tel: +86 591 22866125

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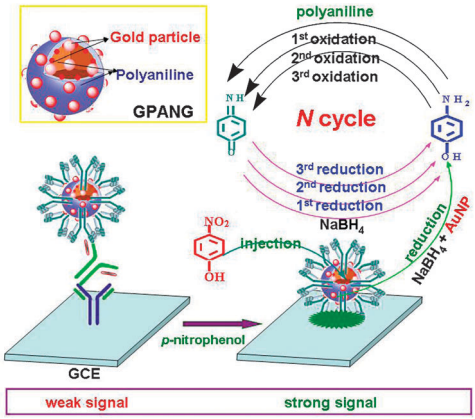

Fig. 1 Schematic illustration of Au(III)-promoted polyaniline gold nanospheres with electrocatalytic recycling of self-produced reactants for signal amplification.

synthesis of the GPANGs, we used wide-angle X-ray diffraction, Raman spectrum, and SEM to characterize the synthesized nanocatalysts, as described in our recent paper.[8c] Meanwhile, a TEM image of the as-synthesized GPANGs is also shown in Fig. S1 (see ESI†), which exhibited a large number of gold nanoparticles on the surface of GPANGs.

An initial question to be answered was whether the designed GPANG nanocatalysts can catalyze the reduction of NP in the presence of sodium borohydride. We used UV-vis absorption spectroscopy to investigate the catalytic process of the nanocatalysts. As indicated in curve 'a' of Fig. 2A, pure *p*-nitrophenol shows a distinct spectral profile with an absorption maximum at 317 nm. When a $NaBH_4$ solution was added into *p*-nitrophenol, a new absorption peak was observed at 402 nm (curve 'b' of Fig. 2A). Moreover, the absorbance at 402 nm was gradually increased upon the addition of $NaBH_4$, while that of 317 nm was gradually decreased (curve 'b–e' of Fig. 2A). The reason might be the formation of the *p*-nitrophenolate ions caused by the addition of $NaBH_4$.[9] When the added *p*-nitrophenol molecules were completely converted to the *p*-nitrophenolate ions, the GPANG nanocatalysts with various concentrations were injected into the mixture. As shown in Fig. 2B, the absorbance at 402 nm was gradually decreased with the increasing GPANG nanocatalysts, while the absorbance at 315 nm was increased. The increased peak derived from the formation of *p*-aminophenol due to the catalytic reduction of *p*-nitrophenol by GPANG nanocatalysts. The gradual development of the peak at 315 nm confirmed the increase in *p*-aminophenol concentration in the reaction mixture. The GPANG nanocatalysts could facilitate electron transfer from BH_4^- to *p*-nitrophenolate and thus acted as catalysts for the purpose. Therefore, the synthesized GPANGs could catalyze the reduction of *p*-nitrophenol to *p*-aminophenol in the presence of $NaBH_4$ (Note: UV-vis absorption spectroscopies using pure gold nanoparticles were monitored, and the results are shown in Fig. S2 of ESI†).

To investigate the electrocatalytic behaviours of the prepared GPANG, polyclonal goat anti-human IgG (Fc specific, Ab_2) antibodies were conjugated onto the nanocatalysts *via* the strong interaction between gold nanoparticles and proteins, which were used for the determination of HIgG (1.0 ng mL^{-1} used in this case) on a monoclonal mouse anti-human IgG (Fab specific) antibody (Ab_1)-modified glassy carbon electrode with a sandwich immunoassay format. The preparation process of the immunosensor was characterized by using electrochemical impedance spectroscopy (see Fig. S3 of ESI†). For comparison, polyaniline nanospheres (PAN, 12 nm in diameter)-labelled Ab_2 and gold nanoparticles (AuNP, 16 nm in diameter)-labelled Ab_2 were also utilized for the detection of HIgG, respectively. Next, cyclic voltammograms of the as-prepared immunosensors were monitored in pH 8.0 $NaBH_4$-PBS with and without NP or AP using various bionanolabels (Fig. 3). As seen from curve 'b' in Fig. 3A, upon addition of 10 mM AP in pH 8.0 $NaBH_4$-PBS, redox peak currents of the AuNP-based immunoassay were not almost changed in comparison with that of curve 'a'. In contrast, the cathodic current was gently increased toward 10 mM NP (curve 'c' in Fig. 3A). The results revealed that AuNP could catalyze the reduction of NP to AP with the help of $NaBH_4$, but could not further catalyze the AP. By the same token, we also found that pure PAN could not reduce the NP to AP, but could catalyze the oxidation of AP to QI (Fig. 3B). More inspiringly, when using the GPANG as nanolabels, the peak currents could be greatly improved (Fig. 3C). Furthermore, addition of NP in pH 8.0 $NaBH_4$-PBS could result in a higher anodic peak current than that of AP (curve 'c' *versus* curve 'b' in Fig. 3C). This is most likely a consequence of the fact that (i) the doped gold nanoparticles in the GPANG could catalyze the reduction of NP to AP in the presence of $NaBH_4$, (ii) the formed AP was oxidized to QI by polyaniline, and (iii) the generated QI was reduced back to AP by $NaBH_4$. Such a catalytic recycling of self-produced reactants could result in the amplification of the electrochemical signal.

By using GPANG-labelled Ab_2 secondary antibodies, the sensitivity and dynamic range of GPANG-based electrochemical immunoassay were evaluated toward HIgG standards in pH 8.0 $NaBH_4$-PBS containing 10 mM NP by using differential pulse

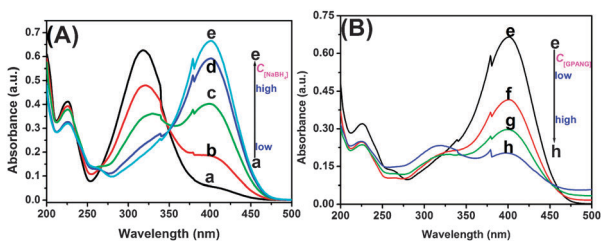

Fig. 2 UV-vis absorption spectra of (a) *p*-nitrophenol, (b–e) *p*-nitrophenol after reaction with various concentrations of $NaBH_4$, and (f–h) the mixture 'e' upon addition of GPANG nanocatalysts with various concentrations.

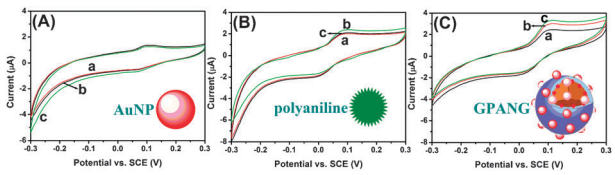

Fig. 3 Cyclic voltammograms of the developed immunoassays toward 1.0 ng mL^{-1} HIgG in (a) pH 8.0 $NaBH_4$-PBS, (b) pH 8.0 $NaBH_4$-PBS + 10 mM AP, and (c) pH 8.0 $NaBH_4$-PBS + 10 mM NP by using various bionanolabels: (A) AuNP-labelled Ab_2 antibodies, (B) PAN-labelled Ab_2 antibodies, and (C) GPANG-labelled Ab_2 antibodies. Scanning rate: 50 mV s^{-1}.

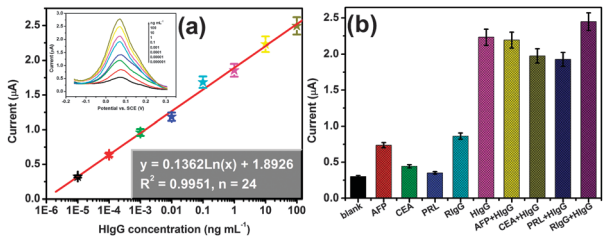

Fig. 4 (a) Calibration plots and (b) specificity of the electrochemical immunoassay toward HIgG standards in pH 8.0 $NaBH_4$-PBS containing 10.0 mM NP (inset: the corresponding DPV curves).

voltammetry (DPV) with a sandwich-type immunoassay format. As shown from the inset of Fig. 4a, DPV peak currents increased with the increasing HIgG concentration. A linear dependence between the peak currents and the logarithm of HIgG was obtained in the range from 0.01 pg mL^{-1} to 100 ng mL^{-1} with a detection limit (LOD) of 1.0 fg mL^{-1} estimated at the $3s_{blank}$ level (n = 11) (Fig. 4a). In addition, we also compared the properties of the electrochemical immunoassay with other HIgG assay methods reported previously. As seen from Table S1 (see ESI†), the wide linear range and a low detection limit of the developed immunoassay were acceptable. Although the system has not yet been optimized for maximum efficiency, the assay sensitivity of using GPANGs as nanocatalysts was 100-fold lower than our most recent report.[1c]

The specificity of the electrochemical immunoassay was monitored by challenging the system with other biomolecules such as alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), prolactin (PRL), and rabbit IgG (RIgG). Significantly higher currents were observed with target HIgG (10 ng mL^{-1} as an example) than those with other biomolecules (10 ng mL^{-1} in this case) (Fig. 4b). These results clearly manifested the high specificity of the electrochemical immunoassay.

The reproducibility and precision of the developed electrochemical immunoassay were evaluated by calculating intra- and inter-assay coefficients of variation (CVs). We repeatedly assayed three different HIgG levels containing 1.0 pg mL^{-1}, 100 pg mL^{-1}, and 10 ng mL^{-1} under the same circumstances. Experimental results revealed that the coefficients of variation (CVs) of the inter-assay were 7.1%, 9.8%, and 9.5% for 1.0 pg mL^{-1}, 100 pg mL^{-1}, and 10 ng mL^{-1} HIgG, respectively, whereas the CVs of the intra-assay were 8.8%, 8.1% and 9.7% towards the above-mentioned analytes. Therefore, the precision and reproducibility of the developed immunosensor was acceptable. In addition, the electrochemical immunosensor exhibited satisfactory stability, and ~90% of the initial peak current was preserved after storage of the immunosensor and nanolabels at 4 °C for 28 days.

To further evaluate the analytical reliability and possible application of the newly developed technique to be applied for the testing of the real sample, it was applied for the analysis of 3 HIgG samples of various concentrations including 0.1 pg mL^{-1}, 1.0 ng mL^{-1} and 50 ng mL^{-1}, which were obtained by spiking HIgG standards into blank fetal calf serum. The assayed results for the above-mentioned 3 HIgG analytes were 0.12 pg mL^{-1}, 0.96 ng mL^{-1} and 47.5 ng mL^{-1}, respectively. The recoveries were 120%, 96% and 95%, respectively. The results revealed that the newly proposed immunoassays are capable of detecting target molecules in real biological samples.

In conclusion, this communication designs a novel sandwich-type electrochemical immunoassay for ultrasensitive determination of human IgG by using redox-active nanocatalysts as labels with electrocatalytic recycling of self-produced reactants. Highlights of this work are to explore a newly multifunctional conductive polymer nanostructure with redox activity for the signal amplification of electrochemical immunoassay by redox recycling of self-produced reactants during organic synthesis (AP ↔ QI). More importantly, the methodology can avoid the use of two working electrodes or multiple enzymes for the amplification of the electrochemical signal. Compared with conventional immuno-PCR assays, the nanocatalyte-based immunoassay is of low-cost, simple, and sensitive.

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