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Nanogold-penetrated poly(amidoamine) dendrimer for enzyme-free electrochemical immunoassay of cardiac biomarker using cathodic stripping voltammetric method

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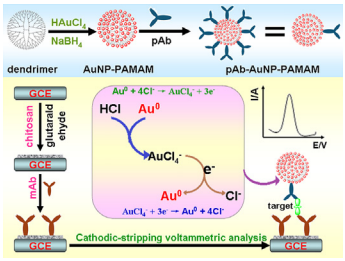
HIGHLIGHTS

- We designed a new voltammetric immunoassay for detection of cardiac biomarker.
- The signal was amplified by nanogold-penetrated poly(amidoamine) dendrimers.
- The encapsulated nanogold particles were used as the signal-generation tag.

GRAPHICAL ABSTRACT



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ABSTRACT

Methods based on immunoassays have been developed for cardiac biomarkers, but most involve the low sensitivity and are unsuitable for early disease diagnosis. Herein we design an electrochemical immunoassay for sensitive detection of myoglobin (a cardiac biomarker for acute myocardial infarction) by using nanogold-penetrated poly(amidoamine) dendrimer (AuNP-PAMAM) for signal amplification without the need of natural enzymes. The assay was carried out on the monoclonal mouse anti-myoglobin (capture) antibody-anchored glassy carbon electrode using polyclonal rabbit anti-myoglobin (detection) antibody-labeled AuNP-PAMAM as the signal tag. In the presence of target myoglobin, the sandwiched immunocomplex could be formed between capture antibody and detection antibody. Accompanying AuNP-PAMAM, the carried gold nanoparticles could be directly determined *via* stripping voltammetric method under acidic conditions. Under optimal conditions, the detectable electrochemical signal increased with the increasing target myoglobin in the sample within a dynamic working range from 0.01 to 500 ng mL^{-1} with a detection limit of 3.8 pg mL^{-1}. The electrochemical immunoassay also exhibited high specificity and good precision toward target myoglobin. Importantly, our strategy could be applied for quantitative monitoring of myoglobin in human serum specimens, giving well matched results with those obtained from commercialized enzyme-linked immunosorbent assay (ELISA) method.

1. Introduction

Knowledge about disease-related proteins (biomarkers) has tremendously increased and provided the great opportunities for

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improving the management of patients by early diagnosis and efficacy of treatment [1,2]. Cardiovascular diseases are the top-ranked causes of death worldwide [3]. Myoglobin (a potential marker for heart attack in patients with chest pain) is a relatively small protein molecule that is released to the blood within 60 min after acute myocardial infarction, and reached a peak within 4–12 h, and then rapidly eliminated [4,5]. The as-released myoglobin is filtered by the kidneys but is toxic to the renal tubular epithelium and so many cause acute the renal failure [6]. A major advantage of using myoglobin as the cardiac marker is that is early released from damaged cells than other cardiac markers, allowing early monitoring of acute myocardial infarction [7,8]. Therefore, sensitive and accurate detection of myoglobin would be advantageous in clinical diagnosis for acute myocardial infarction (AMI).

Immunoassay, based on specific antigen–antibody reaction, has been the predominant analytical technique for quantitative detection of protein biomarkers [9,10]. Recently, different immunoassays on the basis of different signal-transduction principles have developed for determination of protein biomarkers, *e.g.*, spectroscopy, electrochemistry, chromatography and colorimetric analysis [11–15]. Among these methods, electrochemical detection holds the great potential as the next-generation assay scheme due to its high sensitivity, simple instrumentation and low cost [16,17]. From the experienced point of view, there are two key aspects for the development of the electrochemical immunoassays with good analytical properties. The first concern of interest is to design a highly efficient signal-generation tag for the signal amplification to attain a low limit of detection and quantification [18]. The secondary issue is to employ a high-sensitivity detection mode [19]. The rapidly emerging research field of nanotechnology, and the processes used to generate, manipulate and deploy nanomaterials, provides excitingly new possibilities for advanced development of the new analytical tools and instrumentation [20,21]. Zhang et al. developed a sensitive immunoassay for IgG detection by anodic stripping voltammetric assay toward CdS quantum dots under acidic conditions [22]. Owing to highly effective "built-in" preconcentration step (because the substrate is electroplated on the working electrode during a deposition step and oxidized from the electrode during the stripping step), stripping voltammetric detection offers remarkably low detection limits. Favorably, gold nanostructure with the inherent merits (*e.g.*, easy preparation and good electronic activity) has become an ideal candidate for stripping voltammetric detection. Khunrattanaporn et al. reported a stripping voltammetric label with a hollow polyelectrolyte shell containing approx. 1.0×10^{11} Au atoms in the form of well dispersed Au nanoparticles for highly sensitive detection of genomic DNA [23]. Pumera et al. designed a new electrochemical sensor for the detection of DNA hybridization by stripping voltammetric monitoring of Au_{67} nanoparticle tag [24]. Despite some advances in this field, there is still the request for exploring new protocols to improve the sensitivity of the electrochemical immunoassays.

Dendrimers are repetitively branched molecules. Application of the dendrimers typically involve in conjugating other chemical species to the surface that can function with the detection agents, affinity ligands, targeting components, radioligands, imaging agents or pharmaceutically active compounds [25–27]. Also, dendrimers are usually utilized for synthesis of monodisperse metallic nanoparticles [28,29]. Poly(amidoamine) (PAMAM) dendrimers provide a strong potential for the synthesis of dendrimer-encapsulated nanoparticles since one dendrimer has hundreds of possible sites to couple with active species [30]. By combining the unique properties of gold nanostructures with highly sensitive stripping voltammetric method, our motivation of this work is to design a new immunoassay for sensitive screening of cardiac myoglobin by using nanogold-penetrated PAMAM dendrimer (AuNP-PAMAM) as the signal-generation tag. With a sandwich-type assay format, the immunoreaction occurs between monoclonal mouse anti-myoglobin antibody-modified electrode and polyclonal rabbit anti-myoglobin antibody-labeled AuNP-PAMAM. Subsequently, the stripping voltammetric detection of gold released from AuNP-PAMAM is conducted. The electrochemical signal indirectly relies on the concentration of myoglobin in the sample.

2. Experimental

2.1. Reagents and chemicals

Monoclonal mouse anti-human myoglobin capture antibody (designated as mAb, 0.29 mg mL^{-1} in PBS containing 0.09% sodium azide, w/v), polyclonal rabbit anti-human myoglobin detection antibody (designated as pAb, application: 1:500–1:1000), myoglobin (Mb) standards and human myoglobin ELISA kit were purchased from Abcam (Shanghai, China). Chitosan and glutaraldehyde were obtained from Alfa. Bovine serum albumin (BSA) and $HAuCl_4 \cdot 4H_2O$ were achieved from Sinopharm Chem. Inc. (Shanghai, China). Poly(amidoamine) dendrimer (PAMAM, generation 5) was acquired from Sigma–Aldrich. All other chemicals were of analytical grade. Ultrapure water obtained from a Millipore water purification unit was used in all runs (18.2 MΩ cm^{-1}, Milli-Q, Millipore). Phosphate buffer solution (PBS buffer, pH 7.4) contained 10 mM phosphate-buffered saline, 0.137 M NaCl and 0.03 M KCl. The washing buffer was prepared with 0.02 M Tris–HCl buffer containing 0.1% Tween-20, 0.5% BSA and 0.15 M NaCl.

2.2. Preparation of nanogold-penetrated PAMAM dendrimer

Nanogold-penetrated PAMAM dendrimer (designated as AuNP-PAMAM) was prepared *via* the *in-situ* reduction of Au(III) attached to the dendrimer with $NaBH_4$ [31,32]. Briefly, 1.0 mL of 29.4 mM $HAuCl_4$ aqueous solution was initially added to 1.0 mL of 50 μM PAMAM dendrimer aqueous solution (*Note*: Methanol in the PAMAM sample was removed under vacuum before use). Then, the resulting mixture was stirred vigorously for 4 h at room temperature. During this process, $[AuCl_4]^-$ ions were chelated to PAMAM dendrimer with the help of the tertiary amines. After complexion, these ions were reduced by 0.1 M $NaBH_4$ to form a nanoparticle that was encapsulated within the dendrimer. After that, the mixture was dialyzed with a cellulose dialysis sack having a molecular weight cutoff of 12,000 to remove the impurities including gold nanoparticles alone. Finally, the as-prepared AuNP-PAMAM was dispersed into 1.0 mL distilled water for the bioconjugation of the detection antibody.

2.3. Bioconjugation of AuNP-PAMAM with pAb antibody

Bioconjugation of the as-synthesized AuNP-PAMAM with pAb detection antibody was prepared similar to the literature [33]. Briefly, the mixture containing pAb antibody (100 μL, 1:500 dilution ratio) and the above-prepared AuNP-PAMAM suspension (1.0 mL) was initially shaken gently for 6 h at 4 °C on an end-over-end shaker. Following that, the mixture was centrifuged (8000 *g*) for 10 min at 4 °C to remove the excess antibodies. Finally, the obtained precipitate (designated as pAb-AuNP-PAMAM) was redispersed into 1.0 mL of pH 7.4 PBS containing 1.0% BSA and stored at 4 °C for further use.

2.4. Preparation of the electrochemical immunosensor

Prior to modification, a 1.0 mg mL^{-1} chitosan acetic solution was