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Ultrasensitive immunoassay of proteins based on in-situ enzymatic formation of quantum dots and microliter-droplet anodic stripping voltammetry

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ABSTRACT

We report an ultrasensitive sandwich-type electrochemical immunoassay of proteins, on the basis of in situ enzymatic formation of CdS quantum dots (QDs), simultaneous chemical dissolution of CdS and cathodic preconcentration of Cd, and microliter-droplet anodic stripping voltammetry (ASV) detection directly on the immunoelectrode. The antibody 2 labeled with alkaline phosphatase (ALP) and Au nanoframes (Ab_2-ALP-AuNFs) can catalyze hydrolysis of ascorbic acid 2-phosphate to form ascorbic acid and inorganic phosphate, and the latter can stabilize the formation of CdS QDs on the immunoelectrode through Cd^{2+}-S^{2-} reaction. A beforehand "potential control" and then an injection of 7 μL 0.1 M aqueous HNO_3 lead to dissolution of the CdS QDs for simultaneous electrodeposition of metallic Cd, and ASV directly on the immunoelectrode is then conducted to quantify the antigen analyte. The use of AuNFs can increase the enzyme load and activity for improved signaling. Under optimized conditions, this method is used for ultrasensitive analysis of human immunoglobulin G (IgG) and human cardiopathy biomarker cardiac troponin I (cTnI), giving limits of detection (LODs, $S/N = 3$) of 1.2 fg mL^{-1} for IgG and of 1.1 fg mL^{-1} for cTnI (equivalent to 173 molecules in the 6 μL sample employed for cTnI analysis). The LOD of IgG is ca. 3 orders of magnitude lower than that of the fluorescent method by experimentally recording the emission spectrum of the CdS QDs at $\lambda_{ex} = 290$ nm and $\lambda_{em} = 500$ nm.

1. Introduction

Many biosensing methods involve decoration of performed metal nanomaterials with biorecognition elements [1, 2]. Biocatalytic growth of metal nanomaterials is a smart protocol for the design of sensitive bioassays, which is usually based on the reduction of metal ions by a product of enzymatic reaction, followed by the deposition of generated metal atoms on the surface of seeding nanomaterial [3, 4]. Enzymes have been widely used as labels in immunoassays with improved sensitivity, because a single enzyme molecule can catalytically transform many molecules of its substrate into a product at an extremely fast speed. Alkaline phosphatase (ALP) is widely used as a labeling enzyme because of its high turnover number, high stability, low cost, and broad substrate specificity [3–7]. Hydrolysis products of ALP substrates, such as ascorbic acid 2-phosphate (AAP), 3-indoxyl phosphate, and *p*-aminophenyl phosphate, are known to be versatile reducing agents, and they can reduce silver cations to produce metallic silver [3–5, 8]. This character has been used for immunosensing [3, 4] and DNA hybridization assays [5, 8] by the anodic stripping voltammetry (ASV) analysis of the deposited silver. Meanwhile, quantum dots (QDs) offer an attractive alternative to metal nanoparticles as electrochemical labels. The most important advantages of QDs are the high sensitivity and the possibility to perform multi-analyte assays in a single run using different QDs [9, 10]. The signal amplification strategy of the selective staining of nanocrystalline cadmium sulfide (CdS) on ZnO nanocrystals has been proven to be highly efficient [11]. However, to the best of our knowledge, combining the power of enzymatic amplification with the advantages of QDs is not exploited for ultrasensitive nanoelectroanalysis to date.

Gold nanostructures provide a versatile and multifaceted platform for a broad range of biomedical applications [12, 13]. Recent studies have validated their application potential in a number of areas including cancer diagnosis and therapy. Gold nanostructures can serve as labels for the detection of trace biomarkers [14–16]. For example, Xia et al. reported a significant family of plasmonic metal nanostructures with controlled interior nanogaps, including nanoframes (NFs) [17, 18] and nanocages [19, 20], which are hollow nanocrystals enclosed by porous side faces or edges. Obviously, the NFs may offer abundant sites

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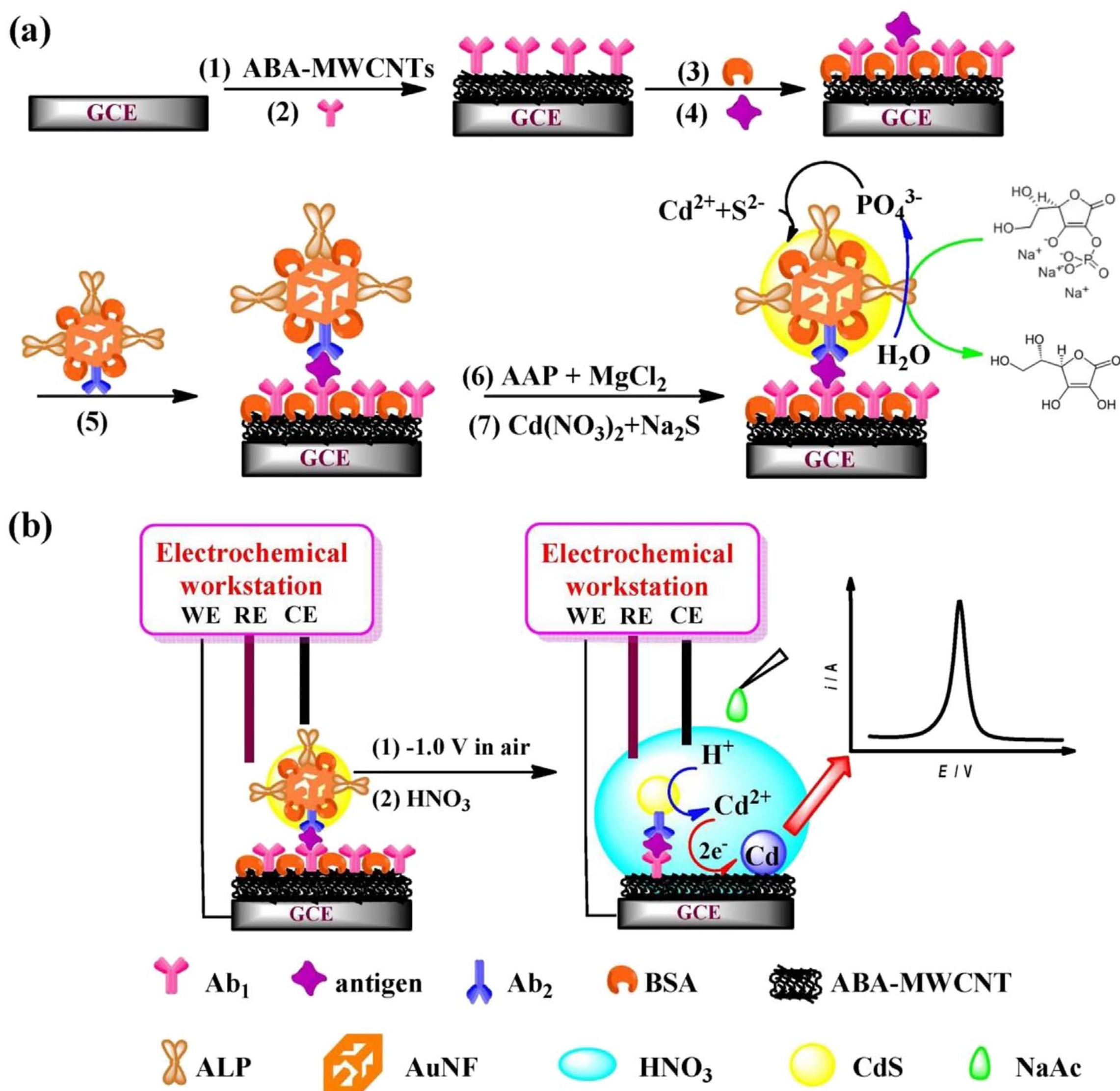

Scheme 1. Illustration of immunoelectrode preparation (a) and key electrochemical steps (b) of our MLAI method.

both at the outer and inner surfaces to bind more biomacromolecules and retain the bioactivity, as reported for polymer-based bionanocomposites [21].

Herein, we report an ultrasensitive metal-labeled amperometric immunoassay (MLAI) of proteins on the basis of catalytic enzyme biolabel and signaling QDs. As shown in Scheme 1, the second antibody (Ab_2) labeled with ALP and Au NFs (Ab_2-ALP-AuNFs) is immunologically captured onto a glassy carbon electrode (GCE) to form a sandwich-type immuno-interface. Afterwards, the AAP substrate undergoes enzymatic cleavage by ALP, producing ascorbic acid and phosphate ions. Upon the addition of Cd^{2+} and S^{2-} ions to the reaction mixture, the CdS QDs are formed by phosphate stabilization [6], which can adsorb on the immuno-electrode. In-situ ASV on the immunoelectrode was used to quantify the antigen analyte, as detailed later. Our MLAI method shows limits of detection (LODs, $S/N = 3$) of 1.2 fg mL^{-1} for IgG and 1.1 fg mL^{-1} for cTnI (i.e., 173 molecules in the 6 μL samples taken for cTnI protein) under optimized conditions, which is a large improvement versus the control fluorescent method and many reported methods (Table S1).

2. Experimental

2.1. Instrumentation and chemicals

All electrochemical experiments were performed on a CHI660C electrochemical workstation and a three-electrode electrolytic cell was used. A disk GCE with 3.0 mm diameter and a platinum wire of 0.1 mm diameter (CH Instruments, Inc.) were used as the working electrode (WE) and the counter electrode (CE), respectively. A KCl-saturated calomel electrode (SCE) of a Luggin capillary filled with saturated KNO_3 served as the reference electrode (RE). All potentials are cited versus SCE. A computer-interfaced HP4395A impedance analyzer was employed in the quartz crystal microbalance (QCM) experiments [22]. AT-cut 9 MHz piezoelectric quartz crystals with 12.5-mm wafer diameter (Model JA5, Beijing Chenjing Electronics Co., LTD, China) were used. The Au electrode of 6.0-mm diameter (key-hole configuration, area = 0.29 ± 0.01 cm^2) on one side of the quartz crystal was exposed to the solution and served as the working electrode, while that on the other side faced air by silica adhesive sealing. The UV–Vis spectra were recorded on a UV-2450 spectrophotometer (Shimazu Co., Japan). Immunoassays based on formation of fluorescent CdS QDs were performed in black flat-well (330 μL) NUNC 96-well microtiter plates. The

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