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Successively amplified electrochemical immunoassay based on biocatalytic deposition of silver nanoparticles and silver enhancement

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Abstract

A successively signal-amplified electrochemical immunoassay has been reported on the basis of the biocatalytic deposition of silver nanoparticles with their subsequent enlargement by nanoparticle-promoted catalytic precipitation of silver from the silver-enhancer solution. The immunoassay was carried out based on a heterogeneous sandwich procedure using polystyrene microwells to immobilize antibody. After all the processes comprising the formation of immunocomplex, biocatalytic deposition of silver nanoparticles and following silver enhancement were completed, the silver on polystyrene microwells was dissolved and quantified by anodic stripping voltammetry (ASV). The effect of relevant experimental conditions, including the concentration of ascorbic acid 2-phosphate (AA-p) substrate and Ag(I) ions, the biocatalytic deposition time, and of crucial importance, the silver enhancement time, were investigated and optimized. The anodic stripping peak current was proportional to the concentration of human IgG in a dynamic range of 0.1–10 ng ml^{-1} with a detection limit of 0.03 ng ml^{-1}. Scanning electron microscope (SEM) was applied to characterize the silver nanoparticles before and after silver enhancement on the surface of polystyrene microplates. By coupling the highly catalytic effect of enzyme and nanoparticles to successively amplify the analytical signal, the sensitivity of immunoassay was enhanced so dramatically that this approach would be a promising strategy to achieve a lower detection limit for bioassays.

Keywords: Electrochemical immunoassay; Biocatalytic deposition; Silver nanoparticles; Silver enhancement; Anodic stripping voltammetry

1. Introduction

There is an increasing demand for ultrasensitive methods of immunoassay based on the specific recognition of antigen to be detected by corresponding antibody. Among many analytical techniques, electrochemical detections are very attractive for such bioassays due to its unique advantages such as high sensitivity, intrinsic simplicity, field portability and inexpensive instrumentation. As the quantification in immunoassay or DNA assays is generally achieved by measuring the specific activity of a label, enzymes and nanoparticles used as labels play a vital role in the development of signal-amplified electrochemical bioaffinity assays of proteins and nucleic acids (Bakker and Telting-Diaz, 2002; Bakker, 2004; Bakker and Qin, 2006; Wang, 1999; Katz et al., 2004).

Enzyme-linked immunosorbent assay (ELISA) is widely applied for the determination of proteins. Traditionally, the second antibody is conjugated with enzyme that catalyzes a reaction with color change, which can be detected photometrically. However, the low sensitivity of this immunoassay limits its further application in practical, clinical, and environmental analysis. In recent years, new schemes based on coupling the biocatalytic

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amplification of enzyme labels with additional electrochemical detections have been developed for meeting the demands of highly sensitive bioassays. Willner's group reported on the use of chronopotentiometry and Faradaic impedance spectroscopy for the detection of the biocatalytic precipitative accumulation of the insoluble reaction product on gold electrode surface (Katz et al., 2001; Alfonta et al., 2001). The application of carbon nanotubes (CNTs) as a dual amplification role both in the recognition process for loading numerous enzyme labels and in the transduction events for accumulating the product of enzymatic reaction was proposed to dramatically amplify enzyme-based bioaffinity electrochemical sensing of proteins and DNA (Wang et al., 2004). Hwang et al. (2005) developed a DNA sensor based on chemical accumulation of silver metal as an enzyme-catalyzed reduction product, a process termed as biometallizaion by the author, to achieve multiple amplifications and very low detection limits. Because the electro-active metallic product accumulates on the electrode during the enzymatic reaction, preventing the diffusing out of the enzyme-catalyzed product into the solution, the coupling of enzyme catalysis with metal deposition seems to be a promising strategy for highly sensitive bioaffinity assay.

Metal nanoparticles as a class of labels with many unique features such as optical, electronic, magnetic, and catalytic properties have been explored for potential applications in biomolecular detection (Katz et al., 2004). Based on these advantages, colloidal gold was first applied as TEM marker in 1971 (Faulk and Taylor, 1971), and was then introduced for SEM in 1975 (Horisberger et al., 1975). Recently, besides the application of metal nanoparticles in some analytical methods such as UV–vis (Schofield et al., 2006), Raman (Ni et al., 1999; Santos et al., 2004) and time-resolved fluorescence spectroscopy (Ipe and Thomas, 2004), SPR (He et al., 2000; Lyon et al., 1998) or QCM techniques (Zhou et al., 2000), a new electrochemical metalloimmunoassay by using colloidal gold as label was reported, which pushed the sensitivity of immunoassay to the picomolar domain (Dequaire et al., 2000). Further sensitivity enhancement can be obtained by the application of metal-enhanced gold nanoparticles, where functional gold nanoparticles act as catalysts to reduce gold or silver ions on themselves. Based on this principle, Mirkin's group has developed a scanometric DNA array (Taton et al., 2000), an electrical detection-based DNA array (Park et al., 2002) and Raman spectroscopic fingerprints for DNA and RNA detection (Cao et al., 2002). In these methods, the autocatalytic metal deposition procedure enlarges the size and darkens the color of nanoparticles, resulting in two to three orders of magnitude improvement in detection sensitivity of scanning electrochemical microscope (Wang et al., 2002), QCM (Su et al., 2001) or electrochemical stripping techniques (Wang et al., 2001; Cai et al., 2002; Liao and Huang, 2005; Chu et al., 2005). Anodic stripping voltammetry (ASV) has been proved to be a powerful approach for trace determination of metal ions (Jacobs, 1963; Dequaire et al., 2000). Its remarkable sensitivity is attributed to the preconcentration step during which the target metal is accumulated onto the surface of the working electrode through cathodical electro-deposition and the stripping step when the metal is stripped from the electrode by anodic oxidation. Such association of nanoparticle-promoted metal precipitation with the remarkable sensitivity of stripping metal analysis offers a dramatic enhancement of the signal response of immunoassay and DNA assays.

In this paper, a successively amplified electrochemical immunoassay is presented for the detection of human IgG based on coupling the biocatalytic deposition of silver nanoparticles with subsequent silver enhancement. As one of the most important enzymatic labels for ELISA due to its high turnover number and broad substrate specificity, alkaline phosphatase (ALP) was adopted to be the indicator for the analyte by using ascorbic acid 2-phosphate (AA-p) as substrate, which has been proved feasible for electrochemical detection (Kokado et al., 2000). After the sandwich type immunocomplex was formed, the ALP bound on the polystyrene microwells converted the AA-p into ascorbic acid. The latter, in turn, reduced the Ag(I) ions in the solution, leading to the deposition of a layer of yellow silver nanoparticles onto the surface of polystyrene microwells. These deposited silver nanoparticles subsequently catalyzed the spontaneous reaction of Ag(I) ions and hydroquinone in the silver-enhancer solution, resulting in the deposition of silver metal onto the particle surface and further enlargement of the size of nanoparticles, which are dissolved by HNO_3 and quantified by ASV. SEM was also utilized to characterize the silver nanoparticles formed. The successive amplification strategy provides a promising way for improving the sensitivity of electrochemical immunoassay as applied to biosensing.

2. Experimental

2.1. Materials and reagents

Goat anti-human IgG antibody, human IgG, and bovine serum albumin (BSA) were purchased from Beijing Dingguo Biotechnology Development Center (Beijing, China). Alkaline phosphatase (ALP) conjugated goat anti-human IgG antibody was provided by Beijing Zhongshan Biotechnology Reagents. Ascorbic acid 2- phosphate (AA-p) was obtained from Express Technology Co. Ltd. (Japan).

Buffers used in this study included 0.05 M $NaHCO_3$–Na_2CO_3 (pH 9.6) as coating medium and 0.05 M sodium phosphate-buffered saline (PBS, pH 7.4) as incubating and washing buffer. The enzyme reaction solution was a glycine–NaOH buffer containing 1.5 mM AA-p and 1 mM $AgNO_3$ (pH 9.0), while the silver-enhancer solution was a citrate buffer containing 2.0 mM $AgNO_3$ and 4 mg ml^{-1} hydroquinone (pH 3.5). Other reagents were of analytical purity, and ultra pure water of specific resistance 18 MΩ was used throughout the experiments.

2.2. Apparatus

Electrochemical measurements were performed with a three-electrode system comprising a carbon paste electrode (CPE) as working electrode, a saturated calomel electrode (SCE) as reference electrode, and a platinum wire as auxiliary electrode. The solid CPE was prepared by mixing the graphite (1.0 g) and melted paraffin (0.5 g) into a homogeneous paste and then