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Short communication

Electrochemical immunoassay for CD10 antigen using scanning electrochemical microscopy

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ABSTRACT

In the present study, a scanning electrochemical microscopic (SECM) method for imaging of antigen/antibody binding was proposed using CD10 antigen as the model. On the basis of anti-CD10 modified electrode, an electrochemical immunosensor for sensitive detection of CD10 antigen at low potential was developed by a multiple signal amplification strategy. Gold nanoparticles (AuNPs) served as carriers to load more secondary antibodies (Ab_2) and horseradish peroxidase (HRP). The tip ultramicroelectrode was used to monitor the reduction current, and the 3-D images were obtained simultaneously. Under optimized conditions, the approach provided a linear response range from 1.0×10^{-11} to 6.0×10^{-11} M with a detection limit of 4.38×10^{-12} M. SECM is a versatile system that can be used not only for quantitative current analysis but also for topographic imaging of binding reaction. In addition, specific binding of antigen–antibody could also be continuously and successfully monitored by SECM. This immunoassay provides a sensitive approach for detecting tumor marker, and has potential application in clinical diagnostics.

1. Introduction

The increasing demands of disease diagnostics and therapeutic analysis require the development of sensitive and accurate detection of disease-related proteins. Unfortunately, in many cases, it is still inconvenient to use conventional methods to detect some important protein biomarkers, due to their low abundance in bodyfluids or tissues. Hence, a large number of methods have been developed to detect the concentration of antigens, such as competitive exchange interactions of antibody–antigen (Monson et al., 2009), fluorescence detection method (Daxiang Cui et al., 2008), microfluidic fluorescence bead assay for accurately measuring antibody–antigen binding (Singhal et al., 2010), surface plasmon resonance (Chang et al., 2010), atomic force microscopy (Browning-Kelley et al., 1997), etc. Immunoassay based on the specific binding between antigen and antibody is one of the most important methods for the determination of proteins.

Employing specific binding of antigen–antibody, the electrochemical immunoassay has become a powerful analytical tool for highly sensitive and specific detection of clinical samples. Immunoanalytical methods using electrochemical detection has become increasingly popular due to their flexibility in terms of sensitivity and miniaturization of the fabricated sensors. The development of these methods is currently based on three main approaches. The first approach is that the electrochemical redox species are generated by a suitable enzyme conjugated to the antigen or the antibody (Bourdillon et al., 1999; Abad-Villar et al., 2000; Aguilar et al., 2002). The second approach makes use of the electroactive species directly attached to the antigen or antibody (Dequaire et al., 2000; Hayes et al., 1994; Wang et al., 1998). The third approach provides detection of binding between the antigen and antibody by electrochemical methods (Flores and Smyth, 1987; Maupas et al., 1997; Sÿnejdaŕkovaŕ et al., 2000).

SECM can be used either for electrochemically induced microfabrication or imaging the distribution of (electrochemical) reactivity on surfaces (Bard et al., 1994). In a feedback mode SECM experiment, the tip ultramicroelectrode is placed in a solution containing the oxidized (or reduced) form of a redox mediator. The reduction (or oxidation) of mediator species at the tip surface produces steady-state current. The image and topography are obtained simultaneously when the tip is positioned near immunosensor. Conventional electrochemical measurements based on the measure of substrate current may not uniquely isolate the contribution of the attached-to-substrate species. SECM is an interesting option because it allows a local characterization of substrate reactivity. These methods are being used increasingly for the study of different electrocatalytic processes such as oxygen reduction reaction (ORR), methanol interference during ORR, and oxygen evolution (Chen and Kucernak, 2004; Sanchez-Sanchez et al., 2008). Microspots of carcinoembryonic

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antigen (CEA) on glass substrates were characterized by (SECM) (Hitoshi et al., 1996), and the microscopic enzymatically active spots on an alkanethiolate-covered gold electrode were mapped using the imaging capabilities of SECM (Gunther and Wolfgang, 1997).

Recently, gold nanoparticles (AuNPs) have gained increasing attention in the clinical cancer screening and early diagnostic application. They have been used as excellent carriers for preparation of labels by loading numerous signal tags such as enzymes (Yu et al., 2006; Malhotra et al., 2010; Cui et al., 2008), quantum dots (Chen et al., 2009), oligonucleotides (Nam et al., 2003), and dyes (Cai et al., 2008). The prepared labels can amplify the transduction signal in bioassays.

In this work, a highly sensitive electrochemical immunosensor for detection of CD10 antigen at low potential was developed by using gold nanoparticles amplification signal strategy. The reduction current was monitored using the tip ultramicroelectrode, and the 3-D images were obtained simultaneously. SECM is used not only for quantitative current analysis but also for topographic imaging of binding reaction. As CD10 antigens are expressed on human Burkitt lymphoma cells, this immunoassay provides a sensitive approach for detecting tumor marker and has potential application for clinical diagnosis.

2. Experimental

2.1. Materials and reagents

CD10 antigen and anti-CD10 were purchased from Biosynthesis Biotechnology Co. (Beijing, China). The CD10 antigen and anti-CD10 were dissolved in pH 7.4 phosphate buffer solution (PBS) (0.01 M in phosphate, 0.1 M KCl) and stored at 4 °C prior to use. $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ were purchased from Tianjin Ruijinte Chemical Co., Ltd. (Tianjin, China). $HAuCl_4 \cdot 4H_2O$ was purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Other chemicals employed were all of analytical reagent grade and were used as received. Horseradish peroxidase (HRP) was supported by the Shanghai Generay Biotech Co., Ltd. (Shanghai China). L-Glutathione was purchased from Solarbio. S&T Co. (Beijing, China). H_2O_2 was supported by Tianjin Bodi Chemical Holding Co., Ltd. (Tianjin, China). CD10 antigen with different concentrations was prepared with PBS (pH 7.4). Double distilled water was used throughout the experiments.

2.2. Equipments and electrodes

The electrochemical cell was in a typical four-electrode configuration: the tip ultramicroelectrode (working electrode, WE), Pt wire counter electrode (CTR), Ag/AgCl, saturated KCl (RE), and substrate electrode (SE). The electrochemical impedance spectroscopy (EIS) was performed on the CHI 900C electrochemical working station (Shanghai CH Instrument, China). Transmission electron microscopy (TEM) images were taken with a JEOL JSM-1200EX instrument (Hitachi). Ultraviolet–visible (UV–vis) spectra were measured on a Cary 50 Ultraviolet spectrophotometer (Varian Pty Ltd., Australia).

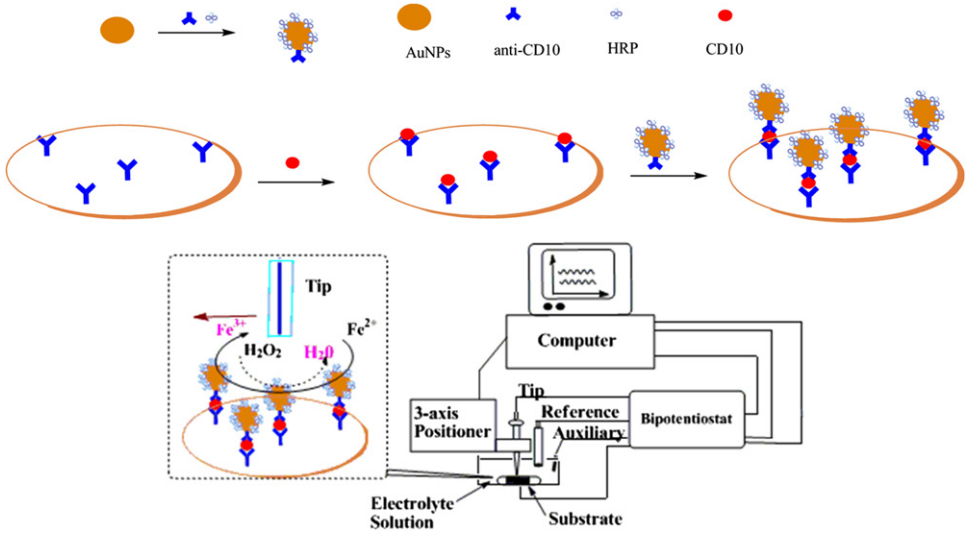
2.3. Fabrication of immunosensor

2.3.1. Preparation of the AuNPs

The AuNPs were prepared according to the method which has been reported previously (Shusheng et al., 2008). Firstly, $HAuCl_4$ (0.01%) and trisodium citrate (1.0%) solutions were filtered through a 0.22 μm microporous membrane filter accordingly, and then the $HAuCl_4$ solution was heated until boiling. With addition of 4.0 mL trisodium citrate and continuous stirring for another 10 min, the AuNPs were obtained and stored at 4 °C. The prepared AuNPs were characterized by TEM and UV–visible absorption spectrum.

2.3.2. Preparation of Ab_2/HRP/AuNPs

The prepared AuNPs were employed as the carrier for HRP and antibody, which could maintain the activity of proteins and amplify the signal of oxidation (Scheme 1). In order to link the HRP to the AuNPs, we used glutathione to activate the nanoparticles which forms an amide bond between the carboxylic group of capped AuNPs and amino groups present in the HRP. In this way, the enzyme is freely available in the solution while attached to the AuNPs with a linker.The AuNPs were activated with glutathione (Ahirwal and Mitra, 2009) for 10 min and excess glutathione was removed by centrifuging. Then, HRP (2 μL) and 110 μL of anti-CD10 (Ab_2) were added to AuNPs in PBS (pH 7.4) with 0.1 M KCl, which were mixed at 4 °C for 9 h. After centrifuging at 10,000 rpm for 30 min, the supernatant was discarded and the soft sediment was washed with PBS. After another centrifugation and discarding the supernatant, the resulting Ab_2/HRP/AuNPs were finally resuspended in PBS (pH 7.4) and stored at 4 °C.



Scheme 1. Schematic of SECM imaging for the binding reaction of CD10 and anti-CD10.