Electrochemistry Communications 13 (2011) 830–833

Contents lists available at ScienceDirect

Electrochemistry Communications

journal homepage: www.elsevier.com/locate/elecom

Electrochemical assay for saccharide–protein interactions using glycopolymer-modified gold nanoparticles

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ARTICLE INFO

Article history:
Received 1 March 2011
Received in revised form 11 May 2011
Accepted 12 May 2011
Available online 19 May 2011

Keywords:
Electrochemical assay
Mannose
Glycopolymer
Gold nanoparticles
Saccharide–protein interactions
Concanavalin A

ABSTRACT

A novel recognition element, made of electroactive glycopolymer-modified gold nanoparticles (GM-GNPs), was synthesized and used in an electrochemical assay of saccharide–protein interactions for the detection of concanavalin A (Con A). The electroactivity of the mannose-based GM-GNPs was investigated, and an electrochemical Con A assay system with a disposable screen printed carbon strips was proposed. A complex containing GM-GNPs was formed by a recognition reaction between the mannose-based glycopolymer and Con A. The complex was detected by the pre-oxidation of the GNPs and following reduction scans using differential pulse voltammetry (DPV). Under optimal conditions, a linear relationship between the DPV peak current intensity and Con A concentration was shown within an analytical range of 10–10,000 ng/mL. Our proposed new electric approach provides an interesting tool for the analysis of saccharide–protein interactions.

1. Introduction

Saccharides on cell surfaces play important roles in living systems and interact with proteins, cells, and viruses [1]. Therefore, saccharides and their derivatives are useful as recognition elements in biosensing [2]. In general, saccharide–protein interactions are weak but can be amplified by the multivalent effect of densely packed saccharides, the so-called glyco-cluster effect [3]. Thus, a densely packed saccharide structure in artificial glycopolymers can be used to increase the accuracy of detection of proteins. A saccharide assembly of a self-assembled monolayer (SAM) [4–6] and a Langmuir–Blodgett (LB) membrane [7] has been used as a biosensor. Recently, we synthesized many types of glycopolymers having a densely packed structure, such as a copolymer with a linear structure [8,9] and dendritic sugar [10,11], as new recognition elements for proteins.

The detection of saccharide–protein interactions using a quartz crystal microbalance (QCM) [5,6], surface plasmon resonance (SPR) [4,5,12], LSPR [13], evanescent-field fluorescence [14], impedance [5,15], and voltammetry has been reported by our group [16]. Recently, we successfully carried out a lateral flow assay on a nitrocellulose membrane, with results that were visible to the naked eye, using mannose-based glycopolymer-modified gold nanoparticles (GM-GNPs) [17]. This recognition element, which includes electroactive GNPs as shown in Fig. 1A, was used in combination with disposable sensor systems based on the three-electrode type of screen printed carbon strips (SPCS) (Fig. 1B) for the fabrication of a new sensing device. We have used the SPCS for the detection of enzyme activity [18], DNA [19], and proteins [20]. Electrochemical detection using SPCS seems to be a useful and low-cost tool for the analysis of saccharide–protein interactions.

In this communication, we propose a new approach to electrochemical assays using GM-GNPs for the quantitative detection of proteins. Fig. 1C shows a schematic illustration of this approach. A complex of mannose-based GM-GNPs and concanavalin A (Con A) was formed by saccharide–protein interactions, therefore the quantity of Con A was able to be measured as the quantity of the GM-GNPs. Then, the amount of GM-GNPs was detected by the pre-oxidation and following reduction scans by voltammetry on the disposable electrochemical device. The detection of saccharide protein interaction is important in terms of proteome analysis, and the protein–saccharide interaction has been reported to show specificity to the target protein [9], which is advantageous to the biosensor. In addition, the protein–saccharide interaction relates to the various serious pathogens. We propose the utilization of protein–saccharide interaction is a new potential methodology for biosensor of pathogen and proteome analysis.

2. Experimental

2.1. Materials and instruments

The following reagents were used as received: bovine serum albumin (BSA); Con A (Sigma-Aldrich Japan, Japan); rabbit anti-Con A

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doi:10.1016/j.elecom.2011.05.014

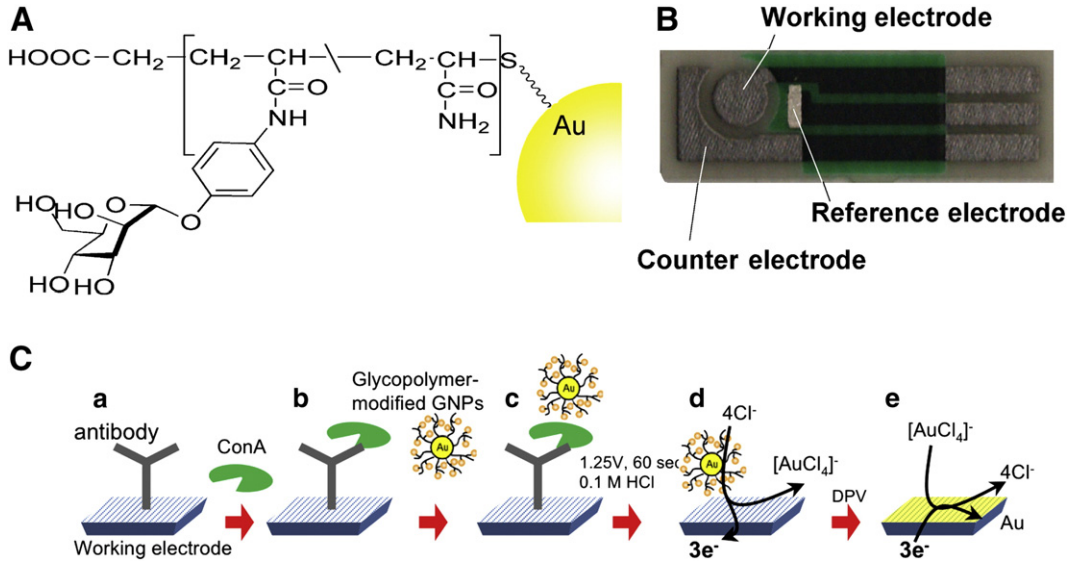

Fig. 1. (A) Structure of glycopolymer consisting of copolymer of *p*-acrylamidophenyl-α-D-mannoside (α-Man) and acrylamide. (B) Structure of SPCS. (C) Schematic illustration of GM-GNP-based electrochemical protein assay for detection of Con A. The Con A antibody was immobilized directly on the working electrode surface of the SPCS (a). A protein-containing sample was applied, and Con A was captured (b). GM-GNPs reacted with captured Con A and formed a protein–glycopolymer–modified GNP complex (c). A high potential of 1.25 V was applied to 0.1 M HCl for the oxidation of the GNPs (d). Then, the voltammetric measurement was performed (e).

antibodies (EY Laboratories, USA); wheat germ agglutinin (WGA) (J-oil Mills, Japan); and HCl, Na_2HPO_4, NaH_2PO_4, KCl, and NaCl (Wako Pure Chemical Industries Japan). A colloidal solution of gold nanoparticles having a diameter of 40 nm was purchased from Tanaka Kikinzoku (Japan). The other reagents were of analytical grade, and all the solutions were prepared and diluted using ultra-pure water (18.3 MΩ-cm) from a Millipore Milli-Q system.

Electrochemical measurements were performed using a potentiostat model 650A (Bio analytical Systems, USA). The planar SPCSs were purchased from BioDevice Technology (Japan) and consisted of a carbon working electrode with a geometric area of 2.64 mm^2, a carbon counter electrode, and an Ag/AgCl reference electrode.

2.2. Preparation of GM-GNPs and recognition device

The glycopolymer and the GM-GNPs were synthesized by our reported procedure [8]. A polyacrylamide derivative with a mannose was prepared via living radical polymerization using a reversible addition-fragmentation chain transfer reagent. A glycopolymer-to-mannose ratio of 6% was adopted in this assay because of its high affinity to Con A resulting from the lateral flow assay [17]. A GM-GNPs solution with an absorbance of $OD_{520} = 1.7$ was used as a stock solution and stored at 4 °C.

The assay device was fabricated by the previously reported procedure [20]. In brief, 2 μL of rabbit anti-Con A antibody solution at 100 μg/mL in 50 mM phosphate buffer saline (PBS, pH 7.4) was dropped onto the carbon electrode surface. After incubation at 4 °C for 12 h, the excess antibodies were rinsed with PBS. Following a blocking procedure using 1% BSA in PBS at 4 °C for 12 h and a rinsing process, the antibody-immobilized SPCS was stored as a Con A assay device at 4 °C until use.

2.3. Detection of saccharide–protein interactions

For the detection of saccharide–protein interactions, 2 μL of a sample solution containing Con A was applied to the working electrode of the sensor for 30 min at room temperature as the same manner of the previously reported [20]. After rinsing with PBS, 2 μL of the GM-GNPs solution was introduced onto the surface, incubated and rinsed using the procedure described above. A complex of Con A and GM-GNPs was formed on the electrode according to the amount of Con A.

The electrochemical detection of the GNPs was performed using 30 μL of 0.1 M HCl covering the entire three-electrode zone of the SPCS at room temperature. The GNPs was applied at a constant potential of 1.25 V for 60 s, called pre-oxidation, immediately followed by differential pulse voltammetry (DPV). The scanning potential ranged from 0.8 V to 0 V in steps of 4 mV. The pulse amplitude was 50 mV, and the pulse period was 0.2 s. The potentials were recorded against the reference electrode (Ag/AgCl) printed within the SPCS.

3. Results and discussion

3.1. Electrochemical characterization of GM-GNPs

The electrochemical property of the GM-GNPs was investigated. The electric behavior of 2 μL of the GM-GNPs solution in 30 μL of 0.1 M HCl was monitored using CV at 100 mV/s in a potential range of 0.0 to 1.0–1.5 V. The reduction signal for gold could be observed at a potential of 0.32 V when scanned up to 1.2–1.5 V. Fig. 2A shows two typical cyclic voltammograms at potential ranges of 0.0 to 1.0 V and 1.25 V. An arrow indicated the reduction signal of gold. As a result, it was found that the electric oxidation of GM-GNPs, required potential higher than 1.2 V under these conditions.

Next, the electrochemical sensing capability of the GM-GNPs was investigated using the pre-oxidation process and subsequent reduction scan using DPV. Fig. 2B shows the reduction peaks for 2 μL of GM-GNPs in 30 μL of 1 M HCl, measured by DPV with varying pre-oxidation times. The reduction peak at the potential was observed to be +0.46 V as a result of pre-oxidation, and the peak currents increased according to the pre-oxidation time. These observations suggest that the glycopolymer modification had no effect on the electrical oxidation and reduction of GNPs. We have already reported that the oxidative detection of gold nanoparticle-labelled antibodies with pre-oxidation is an effective sensitive detection of antigens using immunosensors [21].

On the other hand, the high affinity of the GM-GNPs to Con A has already been confirmed by our approach of lateral flow assay [17]. Therefore, the synthetic GM-GNPs can act as an electroactive