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

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## **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.

*Key words:* Electrochemical assay, mannose, glycopolymer, gold nanoparticles, saccharide-protein interactions, concanavalin A

## 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 Figure 1A, was used in combination with disposable sensor systems based on the three-electrode type of screen printed carbon strips (SPCS) (Figure 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. Figure 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 antibodies (EY Laboratories, USA); wheat germ agglutinin (WGA) (J-oil Mills, Japan); and HCl, Na<sub>2</sub>HPO<sub>4</sub>, NaH<sub>2</sub>PO<sub>4</sub>, 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 M $\Omega$ -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<sup>2</sup>, 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<sub>520</sub> = 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  $\mu$ L of rabbit anti-Con A antibody solution at 100  $\mu$ g/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  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  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 was 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 behaviour of 2  $\mu\text{L}$  of the GM-GNPs solution in 30  $\mu\text{L}$  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. Figure

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. Figure 2B shows the reduction peaks for 2  $\mu\text{L}$  of GM-GNPs in 30  $\mu\text{L}$  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 recognition element for Con A when applied to an electrochemical Con A sensor using disposable SPCS.

### *3.2 Electrochemical Con A detection*

The interaction between GM-GNPs and ConA has been monitored by measuring the relationship between the electrochemical peak current and incubation time of GM-GNPs solution on the Con A modified electrode. Peak current was increased time dependently and reached maximum at 10 minutes as shown in Figure 3A, and 20 minutes incubation



was adopted in the following experiments.

As the optimal conditions for electrochemical assay, the pre-oxidation potential and time were investigated. Figure 3B shows the reduction peak currents on the applied potentials of 1.25 V and 1.3 V during 0–80 s for the pre-oxidation in the presence of 10  $\mu\text{g/mL}$  Con A. At a potential of 1.25 V, the reduction peak currents increased gradually, reaching a maximum at 60 s. In contrast, the reduction peak currents reached a maximum at 20 s under the potential of 1.30 V and then decreased with an increase in the pre-oxidation time because of the diffusion process of the oxidized Au ions. From these observations, 1.25 V and 60 s were adopted as the optimal pre-oxidation potential and time, respectively.

The sensor calibration curve for Con A is shown in Figure 4C. The reduction peak currents depended linearly on the concentration of Con A from 10 ng/mL to 10  $\mu\text{g/mL}$ , and the correlation coefficient was 0.99. The analytical range and sensitivity were estimated as 10 ng/mL (96 pM) to 10  $\mu\text{g/mL}$  (96 nM) and 10 ng/mL (96 pM), respectively. At the same time, other proteins were measured to investigate the detection specificity. When 1% WGA and/or BSA in PBS (pH 7.4) was applied, no GNP reduction peak was observed (data not shown).

Generally, saccharide-protein interaction has been investigated using measurements based on a QCM, SPR or impedance in an electric method. However, these sensing devices are very expensive, giving electrochemical detection an advantage in the development of low cost devices. Using the DPV technique, amyloid beta peptide interaction and sialic acid interaction could be detected by the oxidation signal of tyrosine in our laboratory [16]. However, the sensitivity of the assay was the 1  $\mu\text{M}$  order.

With this Con A assay, a detection limit of 96 pM was estimated. This value is ten thousands times higher than the DPV detection of the amyloid beta protein [16] and nearly equal to a lateral flow assay using the same structure of GM-GNPs [17]. In addition, a series of procedures could be performed on the surface of a disposable electrode, and the required sample volume was only 2  $\mu$ L. Our proposed process offers the potential for further applications by changing the synthetic glycopolymer, which would allow, not only a basic analysis of saccharide-protein interaction, but also the detection of various pathogens having high affinities to saccharides such as Shiga toxins and influenza viruses.

#### **4. Conclusion**

Mannose-based GM-GNPs were synthesized for the electroactive recognition of Con A, and an electrochemical assay system for Con A was fabricated using disposable SPCS. This is the first report of electrochemical detection of saccharide-protein interactions using a GNP-labelled glycopolymer. We expect that this study will lead to additional exciting investigations on a wide variety of glycobiological events.

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## Figure captions

**Figure 1.** (A) Structure of glycopolymer consisting of copolymer of *p*-acrylamidophenyl- $\alpha$ -D-mannoside ( $\alpha$ -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).

**Figure 2.** A) Cyclic voltammograms of GM-GNPs in 0.1 M HCl at 100 mV/s. The potential was ranged from 0.0 to 1.0 V (a) and 1.25 V (b). B) Differential pulse voltammograms of GM-GNPs in 0.1 M HCl at 20 mV/s for various pre-oxidation times. Pre-oxidation was performed at 1.25 V for 0, 20, 40, 70, and 100 s.

**Figure 3.** A) Relationship between the electrochemical peak current and incubation time of GM-GNPs solution on the Con A modified electrode (10  $\mu$ g/mL Con A). Potential of 1.25 V during 60 s was applied for the pre-oxidation. (B) Peak current of Con A sensor (10  $\mu$ g/mL Con A) under different pre-oxidation conditions. The plots show the dependence of the peak current on the applied potentials of 1.25 V ( $\bullet$ ) and 1.3 V ( $\circ$ ) during the pre-oxidation time. (C) Calibration curves for Con A measured for peak current intensity of Con A sensor. Error bars indicate the relative standard

deviation of the three measurements ( $n = 3$ ) performed with three different samples.







