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Electrochemical determination of carbohydrate-binding proteins using carbohydrate-stabilized gold nanoparticles and silver enhancement

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ABSTRACT

A highly sensitive electrochemical lectin biosensor has been developed for the first time using carbohydrate-stabilized gold nanoparticles and silver-enhancement technique. A target lectin protein, Concanavalin A (Con A), was specifically bound to the self-assembled monolayer of thiolated mannose on a gold electrode. Mannose-stabilized gold nanoparticles were added to form a sandwich-type complex with the Con A and were followed by silver-enhancement process to coat the mannose-stabilized gold nanoparticles with silver metal. The coated metallic silver was dissolved in an acidic solution and the resulting silver ions were detected by anodic stripping voltammetry. The present lectin biosensor gave a linear response ($R^2 = 0.999$) for Con A concentration from 0.084 μg/mL to 50.0 μg/mL with a remarkable detection limit (S/N = 3) of 0.070 μg/mL, which is much lower compared to those obtained with the reported microgravimetric and colorimetric detection methods.

1. Introduction

Lectins are carbohydrate-binding proteins and are found in most biological organisms. It has been well known that lectin–carbohydrate interactions play key roles in a variety of important biological processes such as cell-surface recognition, cell–cell communications, cancer and host-pathogen infection (Dwek, 1996; Ritchie et al., 2002; Roth, 2002; Zachara and Hart, 2002). The detection of the carbohydrates and lectins in disease states and related lectin–carbohydrates interactions are thus of importance in the areas of glycomics and early diagnostics of cancer and other disease based on the determination of glycobiomarkers (Jelinek and Kolusheva, 2004).

Thus, the development of highly sensitive and simple detection methods for the carbohydrate–protein interactions is one of the most important analytical challenges. The carbohydrate–protein interactions have been probed by a variety of techniques, which include NMR, mass spectrometry, isothermal titration calorimetry and affinity chromatography (Nilsson, 2003). However, these methods are tedious, requiring complicated instrumentation and technical expertise. Therefore, there is a critical need for the development of simple glyco- and lectin biosensors that are rapid, sensitive, and cost-effective. To date, a number of researches have been directed towards the use of microgravimetric detection method based on quartz crystal microbalance (QCM) to study the lectin–carbohydrate interactions. For example, the interactions of specific carbohydrate and lectins have been studied using the thiol-tailored trisaccharide (Zhang et al., 2003) as well as azido sugars (Zhang et al., 2006) self-assembled monolayer (SAM) on Au QCM electrode. In addition, the interaction of carbohydrate and lectin Con A has been further applied to the selective detection of *E. coli* without any label since the microorganisms contain lectin-binding pockets at their surface (Shen et al., 2007). Our group also reported on a highly sensitive microgravimetric lectin biosensor to detect a target lectin Con A based on the use of mannose-stabilized gold nanoparticles (NPs) as a QCM signal amplifier (Lyu et al., 2008).

Optical transduction method was also employed in the lectin biosensor. Simple and rapid colorimetric detection of the lectin Con A using mannose-stabilized Au NPs was reported (Hone et al., 2003). The method is based on the measurement of a shift in the surface plasmon absorption band of the Con A-induced aggregation of the mannose-stabilized Au and silver NPs (Schofield et al., 2006). In order to enhance the sensitivity of the optical lectin biosensor, the lectin biosensor based on glycolipid modified gold nanoparticles immobilized on glass slide was also developed (Guo et al., 2007).

Electrochemical biosensors have been widely used for monitoring biomolecular interactions since it offers a certain advantages of cost-effectiveness and portability (possible point-of-care). Therefore, electrochemical biosensor could contribute enormously in the

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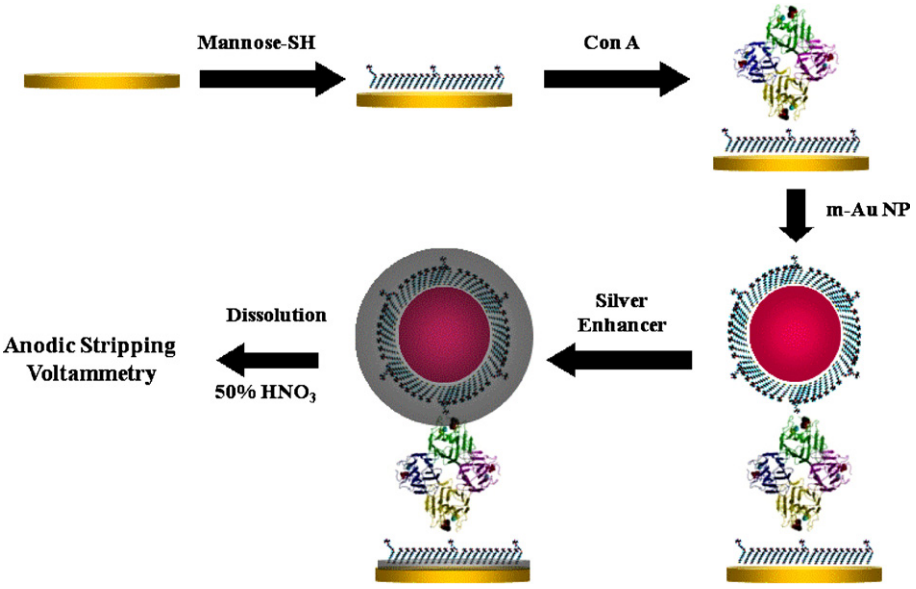


Scheme 1. Schematic illustration of the electrochemical lectin biosensor based on mannose-stabilized Au NPs and silver enhancement.

field of glycomics. However, the application of the electrochemical biosensor in the carbohydrate–lectin interaction is almost unexplored. Up to now, the detection of sugars was reported using lectin-immobilized electrode for the competitive binding of target sugar and sugar-binding CdS nanocrystal (Dai et al., 2006). The extent of the competition was monitored by the electrochemical detection method. Label free impedometric detection of glycan–lectin interactions was also reported (La Belle et al., 2007). In addition, lectin-based carbon nanotubes and Au NPs were used for highly sensitive electrochemical cytosensing (Cheng et al., 2008) and monitoring of dynamic carbohydrate expression on living cells (Ding et al., 2010).

Herein, we report for the first time on the electrochemical detection of lectin based on the carbohydrate-stabilized gold NPs and silver-enhancement reaction (Scheme 1). A target lectin protein Con A was specifically bound to the self-assembled monolayer (SAM) of thiolated mannose on a gold electrode. Con A from *Canavalia ensilformis* is well-known tetrameric protein with four carbohydrate binding sites, which specifically binds α-D-mannopyranosyl and α-D-glucopyranosyl groups (Dam and Brewer, 2002). The Con A is similar to a thick square (6.7 nm × 11.3 nm × 12.2 nm) and each binding site of Con A is located at each corner of a thick square (Bouckaert et al., 1996). Once the interaction between Con A and mannoside SAM on Au electrode is completed, the Au NPs stabilized with the mannoside are added to form the sandwich-type complex through the binding of the mannose-stabilized Au NPs to the remaining opposite binding sites of Con A. Silver-enhancement process was carried out to deposit the mannose-stabilized gold nanoparticles with silver metal, in which the Au NPs served as nucleation sites for silver deposition. Thus, the amount of deposited silver metal directly reflects the amount of the Con A binding to the mannose SAM-modified Au electrode. The deposited silver was dissolved in an acidic solution and the resulting silver ions were detected by anodic stripping voltammetry (ASV) on an external glassy carbon electrode, which resulted in the highly sensitive signal. Under optimal conditions, the present lectin biosensor results in the highly sensitive detection of lectin with a remarkable detection limit of 0.070 μg/mL Con A, which is much lower compared to those obtained with the reported microgravimetric and colorimetric detection methods.

2. Experimental

2.1. Reagents

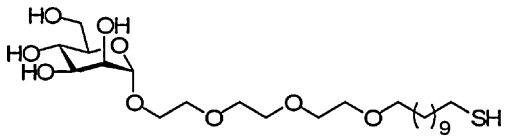
Concanavalin A was purchased from Vector Lab. (Cat. No. L-1000, USA). Au NPs (diameter: 10 nm) were obtained from British Biocell International Co (UK). Silver-enhancement solutions A and B, Human IgG and cholera toxin (from Vibrio choleraae Approx. 95%) were purchased from Sigma. $K_4Fe(CN)_6$, $K_3Fe(CN)_6$ and other reagents were purchased from Aldrich. Water for all solutions was purified using a Milli-Q water purification system (Millipore, USA).

2.2. Instrumentation

Cyclic voltammetry (CV), electrochemical impedance, and ASV experiments were performed with an EG&G 263A potentiostat and frequency response detector model 1025 (Oak Ridge, USA). A conventional three-electrode system was employed with a Pt wire as counter electrode, glassy carbon (GC, area: 0.196 cm^2) or gold electrode (area: 0.196 cm^2, Bioanalytical System, West Lafayette, USA) as a working electrode, and an Ag/AgCl (3 M NaCl) reference electrode. Quartz crystal microbalance (QCM) study was performed with an Au QCM electrode (Seiko EG&G, AT-cut, 9 MHz, area: 0.196 cm^2). The QCM electrode was mounted in a custom-made Kel-F flow cell. Centrifugation of mannose-stabilized Au NPs was carried out with HA-1000-3 centrifuge (Hanil Science Industrial, Seoul, Korea). UV/Vis spectra were obtained with UV-1650PC UV-Vis spectrophotometer (Shimadzu, Japan). Atomic force microscopy (AFM) images were obtained with Veeco diDimension™ 3100 atomic force microscope (Veeco Instruments Inc., USA) in tapping mode. The probe used in this study was made of silicon and the cantilever had a resonance frequency of 250 kHz.

2.3. Preparation of lectin biosensor

The mannoside containing a thiol functional group at the terminal position has been synthesized in a multistep sequence from a mannosyl trichoroacetimidate and an alcohol, and the prepared thiolated mannose was characterized by NMR and high-resolution mass spectrometer according to our previous report (Lyu et al., 2008).



Thiol-modified mannoside was self-assembled on a Au electrode with geometrical area of 0.196 cm^2 by placing the precleaned Au electrode in aqueous solution of thiolated mannose (20 μM, 1.0 mL) for 12 h in the room temperature. The formation of mannose SAM on Au electrode has been verified by CV and electrochemical impedance measurements. These experiments were carried out in 5.0 mM $K_4Fe(CN)_6/K_3Fe(CN)_6$ (1:1, v/v) solution in 0.1 M phosphate buffer at pH 7. The mannose SAM-modified Au electrode was used as the working electrode. A platinum wire and Ag/AgCl (3 M NaCl) were used as counter and reference electrodes. The impedance spectra were obtained in the frequency range from 100 mHz to 100 kHz at a bias potential of 0.0 V with alternating voltage of 5.0 mV. The impedance spectra were plotted in the form of impedance plane plot (Nyquist plots). Surface morphology of the bare Au electrode and the mannose SAM-modified Au electrode before and after the incubation in Con A solution was studied by AFM. The AFM images were obtained with a tapping mode in air. The scan rate was 0.5 Hz, and the resolution was 512 data points/line.

2.4. Preparation of mannose-stabilized Au NPs

The mannose-stabilized Au NPs were prepared by the displacement self-assembly process. Commercially available citrate-capped Au NPs (5.70×10^{12} particles/mL, 1.0 mL) were added in thiolated mannose aqueous solution (20 μM, 1.0 mL) for 12 h in the room temperature as descried earlier (Lyu et al., 2008). The excess thiol-modified mannoside was removed by centrifugation at 4000 rpm for 1 min and followed by successive centrifugation of the solution in 50 mM phosphate buffer (pH 7.0) with Centricon YM-100 (Millipore Co.) at 1000 rpm for 7 min. The final concentration of the Au NPs was determined to be 5.70×10^{12} particles per 1 mL by UV-Vis spectrophotometry.

2.5. Lectin biosensing experiment

The as-prepared mannoside SAM-modified Au electrode was placed in target lectin, Con A, solution (0.500 mL, prepared in 0.05 M phosphate buffer at pH 7.0) for 90 min. Once the interaction between Con A and mannoside SAM on Au electrode was completed, the mannose-stabilized Au NPs (0.50 mL) were added to form the sandwich-type complex through the binding of the mannose-stabilized Au NPs to the remaining opposite binding sites of Con A, which were then incubated for 90 min. Silver-enhancement process was carried out to coat the mannose-stabilized Au NPs with silver metal for 7 min, in which silver-enhancement reagent was prepared from 1:1 mixture of the silver staining solution A (silver salt) and solution B (hydroquinone initiator). After the silver-enhancement process, the biosensor was thoroughly washed with water to reduce the nonspecific silver staining background. The coated silver was dissolved in HNO_3 solution (50%, 50 μL) for 1 min and then KNO_3 solution (3.0 mL, 1.0 mM) was added. The resulting silver ions were electrochemically deposited onto the precleaned GC electrode at the applied potential of 0.0 V vs. Ag/AgCl (3 M NaCl) reference electrode for 10 min under the constant stirring at 1200 rpm. ASV on the silver-deposited GC electrode was performed by running potential scan from +0.20 V and +0.90 V at a scan rate of 100 mV/s. Selectivity test was performed with the 100 μg/mL of cholera toxin and human IgG solution in 50.0 mM phosphate buffer at pH 7.0 under the same condition and procedure with the Con A biosensing.

3. Results and discussion

3.1. Mannose-modified gold electrode for selective binding of lectin protein

The mannose-modified Au electrode was easily fabricated by self-assembly technique. Thiol-modified mannoside was self-assembled on a Au disk electrode by simply placing the precleaned Au electrode in 20 μM aqueous solution of thiolated mannose for 12 h. The formation of a compact mannose SAM on the Au electrode has been verified by CV and electrochemical impedance measurements. As shown in Fig. 1A, cyclic voltammogram on the bare Au electrode gave a reversible redox peaks of 5.0 mM $K_4Fe(CN)_6/K_3Fe(CN)_6$. The formation of the mannose SAM on Au electrode dramatically decreased the reduction peak from 3.40×10^{-5} A to 1.64×10^{-6} A. In addition, double layer charging capacitance was measured at the bare and the mannose SAM-modified Au electrode in 50 mM phosphate buffer at pH 7.0 without 5.0 mM $K_4Fe(CN)_6/K_3Fe(CN)_6$ solution (data not shown). The double layer charging capacitance was significantly decreased from 15 μF/cm^2 at the bare electrode to 2.0 μF/cm^2 at the mannose SAM-modified Au electrode. As shown in the Nyquist plots of Fig. 1B, the formation of a compact mannose SAM on the Au electrode drastically increased the electron transfer resistance (R_{et}) of

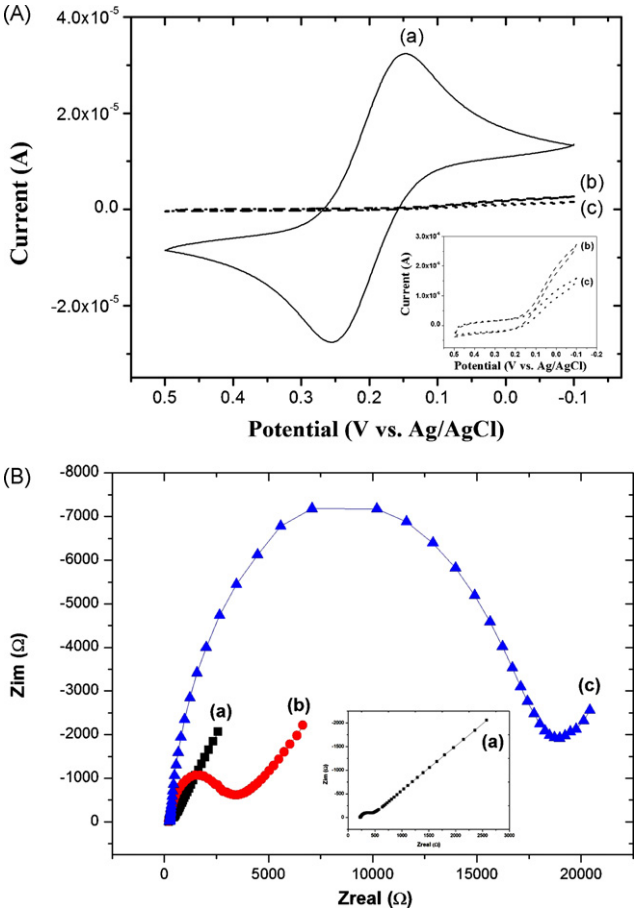


Fig. 1. The cyclic voltammograms (A) and Nyquist plots (B) of 5.0 mM $K_4Fe(CN)_6/K_3Fe(CN)_6$ solution in 50 mM phosphate buffer at a bare Au electrode (a) and at the mannose SAM-modified Au electrode before (b) and after the addition (c) of Con A (50 μg/mL).

the $K_4Fe(CN)_6/K_3Fe(CN)_6$ redox reactions from 0.28 kΩ to 3.28 kΩ. These results of CV and electrochemical impedance measurements indicate that a highly compact mannose SAM has been formed on the Au electrode surface and the resulting compact mannose SAM blocks the electrode surface, which impedes the electron transfer between the redox probes of $K_4Fe(CN)_6/K_3Fe(CN)_6$ and the electrode surface.

Since Con A is a tetrameric protein with four mannose binding sites and the association constant between the Con A and the mannose SAM in the present system is quite large as studied in our previous work (*ca.* $2.24 \times 10^7\ M^{-1}$, Lyu et al., 2008), a target lectin protein Con A is specifically bound to the mannose SAM on a Au electrode. Cyclic voltammograms and Nyquist plots were obtained at the mannose SAM-modified electrode in 5.0 mM $K_4Fe(CN)_6/K_3Fe(CN)_6$ solution after its exposure to 50 μg/mL of Con A. As shown in Fig. 1A, the binding of the Con A to the mannose SAM-modified Au electrode dramatically decreased the reversible redox peak from 1.64×10^{-6} A to 8.71×10^{-7} A at the potential of 0.0 V. In addition, the electron transfer resistance of the $K_4Fe(CN)_6/K_3Fe(CN)_6$ redox reactions was attenuated from 3.28 kΩ to 18.73 kΩ as shown in Fig. 1B. These results indicate that the binding of the thick tetrameric Con A protein to the mannose SAM-modified Au electrode further blocks the electrode surface for the redox reactions and increases the electron transfer barrier, thus leading to both the decreased redox current in CV and the increased electron transfer resistance in electrochemical impedance measurements.

Surface morphology was studied with tapping mode AFM. Fig. 2 shows the AFM images of the bare Au electrode (A) and the mannose SAM-modified Au electrode before (B) and after the binding with Con A (C). Prior to the AFM measurement, the Con A-bound mannose SAM electrode was thoroughly rinsed with deionized water and PBS buffer. There was little change after the formation of mannose SAM on Au electrode in comparison to the bare Au electrode. Since the Au QCM electrode used in the AFM study is not atomically flat, it was difficult to resolve individual thiolated mannose molecules in our experimental conditions. However, significant visible change was observed after the incubation of the mannose SAM-modified electrode in the Con A solution (50 μg/mL) as shown in Fig. 2C. The maximum peak to valley distance was ~7 nm, which was similar to the size of Con A obtained with X-ray crystallography (Bouckaert et al., 1996) and tapping mode AFM (Yonzon et al., 2004). In addition, the roughness of Con A-bound mannose SAM-modified electrode was consistently different from that of mannose SAM electrode in the full range of the AFM image, which indicates an even distribution of the Con A molecules on the mannose SAM on Au electrode. This result was similar to the contact mode AFM observation with the Con A bound-azido mannose SAM-modified electrode (Zhang et al., 2006). The QCM study was performed in order to find out the surface coverage of the Con A bound onto the mannose SAM-modified electrode. The addition of Con A at a concentration of 50 μg/mL generated a frequency change of ~110 ± 5 Hz (data not shown), which corresponds to approximately $(5.6 \pm 0.6) \times 10^{-12}$ mol/cm^2 of Con A coverage on the mannose SAM-modified electrode. This coverage is good enough to evenly cover the mannose SAM-modified electrode with Con A.

In order to confirm that the binding of the lectin Con A to the mannose SAM is specific rather than simple nonspecific adsorption, the control experiments were carried out with cholera toxin (specifically binding to galactose) and human IgG as model proteins. When cholera toxin and the human IgG at 100 μg/mL concentration were added to the mannose-modified Au electrode, there were negligible redox current change. The results indicate that the target lectin protein Con A was specifically bound to the self-assembled monolayer of mannose on a gold electrode.

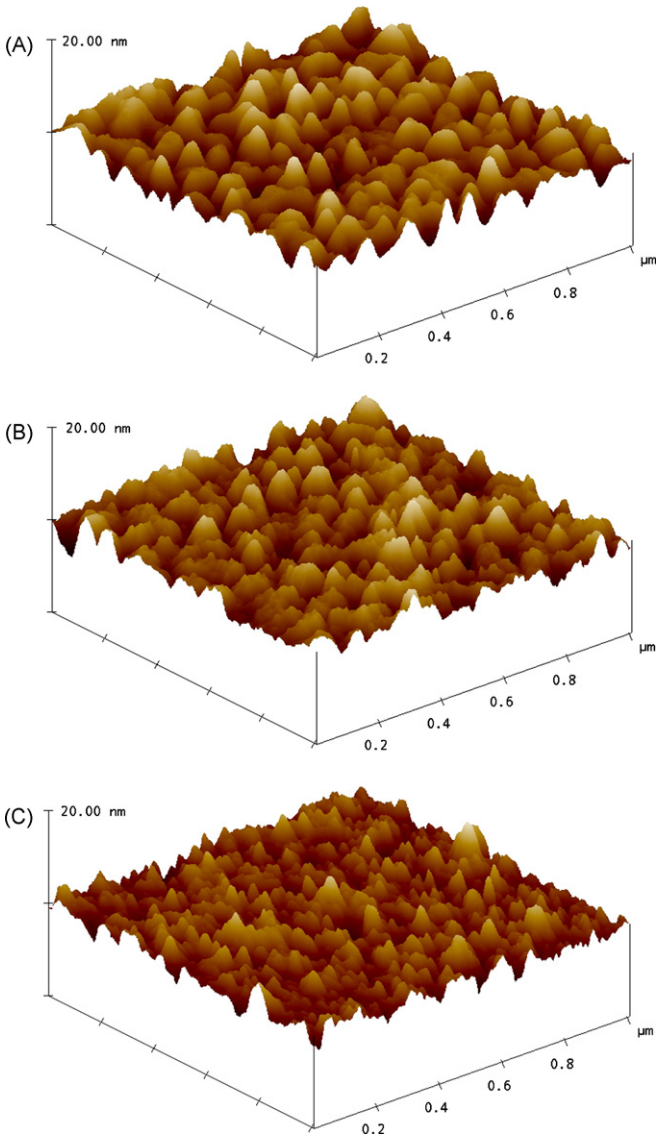


Fig. 2. AFM 3D topography images (1 μm × 1 μm × 20 nm) of the bare Au electrode (A) and the mannose SAM-modified Au electrode before (B) and after (C) the binding of Con A (50 μg/mL).

3.2. Mannose-stabilized Au NPs for the formation of sandwich-type complex

The mannose-stabilized Au NPs were prepared by the displacement self-assembly of commercially available citrate-capped Au NPs (*ca.* 10 nm diameter) with thiol-modified mannoside according to our previous work (Lyu et al., 2008). The surface coverage of the Au NPs with mannose was 196 pmol/cm^2, which was calculated according to the literature (Leff et al., 1995). The mannose-stabilized Au NPs were stable in the phosphate buffer solution. Since the Con A is tetramer with four binding sites, the addition of Con A into the mannose-stabilized Au NPs solution induces the aggregation of the mannose-stabilized Au NPs (Hone et al., 2003). The changes in UV/Vis spectra of the mannose-stabilized Au NPs before and after the addition of Con A in the phosphate buffer at pH 7.0 were monitored in order to confirm the formation of the mannose-stabilized Au NPs. It is well known that the aggregation of Au NPs leads to the change in the surface plasmon absorption band to longer wavelengths (Hone et al., 2003). Fig. 3 shows the UV/Vis spectra of the mannose-stabilized Au NPs before (- - -) and after the

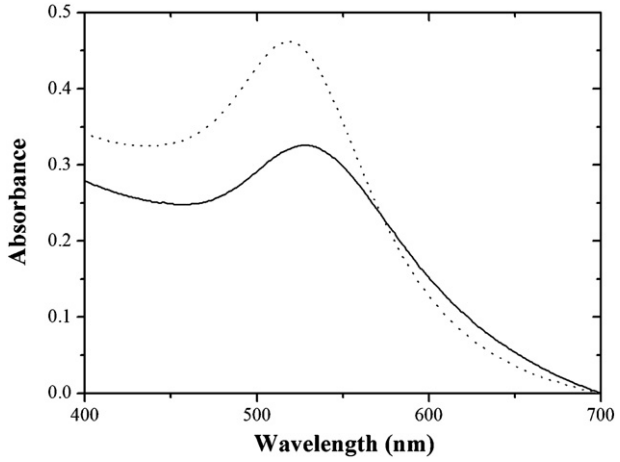


Fig. 3. Changes in the UV/Vis spectra of mannose-stabilized Au NPs before (- - -) and after the addition of 50 μg/mL of Con A (–) after the period of 30 min.

addition of 50 μg/mL Con A (–) in the phosphate buffer at pH 7.0 after the period of 30 min. The addition of Con A into the mannose-stabilized Au NPs certainly shifted the surface plasmon absorption band to longer wavelengths due to the Con A-induced aggregation of the mannose-stabilized Au NPs. In order to confirm that the binding of the mannose-stabilized Au NPs to the lectin Con A is specific rather than simple electrostatic interactions, the control experiments were carried out with cholera toxin and human IgG. When the identical concentrations of cholera toxin and human IgG were added to the mannose-modified Au electrode, there were negligible change in the UV/Vis spectra between 500 nm and 700 nm. The results indicate that the target lectin protein Con A was specifically bound to the self-assembled monolayer of mannose on Au NPs.

3.3. Electrochemical detection of lectin

Upon binding of the target Con A on the mannose SAM-modified Au electrode, the sensor was further treated with the mannose-stabilized Au NPs to form a sandwich-type complex. Then, silver-enhancement process was carried out to coat the mannose-stabilized Au NPs, which served as nucleation sites for silver deposition. Thus, the amount of deposited metallic silver directly reflects the amount of the Con A binding to the mannose SAM-modified Au electrode. The silver staining was well known and widely used in the bioassay (Wang et al., 1998; Taton et al., 2000; Su et al., 2001). In the present study, silver enhancement was carried out by the silver-enhancement reagent prepared from 1:1 mixture of the silver staining solution A (silver salt) and solution B (hydroquinone initiator). To reduce the nonspecific silver staining background, the silver staining process was carried out for 7 min.

The deposited metallic silver was dissolved in HNO_3 solution (50%, 50 μL) for 1 min and then KNO_3 solution (3.0 mL, 1.0 mM) was added. The resulting silver ions were electrochemically deposited onto the precleaned GC electrode at the applied potential of 0.0 V vs. Ag/AgCl (3 M NaCl) reference electrode for 10 min under the constant stirring at 1200 rpm. ASV on the silver-deposited GC electrode was performed by running potential scan from +0.20 V and +0.90 V at a scan rate of 100 mV/s. Fig. 4 shows anodic stripping voltammograms in the presence of control solution (no target Con A) and 0.5 μg/mL Con A. The addition of Con A distinctly results in larger stripping peak. Under the optimal conditions, calibration curve for lectin Con A has been constructed. As shown in Fig. 5, calibration curves are plotted on logarithmic axes to show wide dynamic ranges. Each point is a mean of four or more ASV signals obtained with each separate experiment at a given concentration.

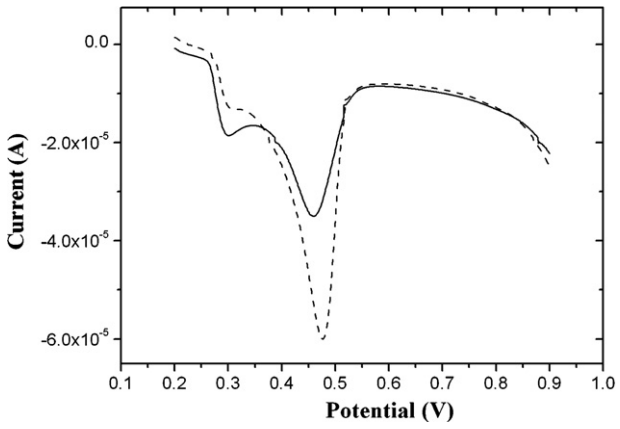


Fig. 4. Anodic stripping voltammograms in the presence of control solution (no target Con A, –) and 0.5 μg/mL Con A (- - -).

The present lectin biosensor gave a linear response ($R^2 = 0.999$) for Con A concentration from 0.084 μg/mL to 50.0 μg/mL with a remarkable detection limit (S/N = 3) of 0.070 μg/mL, which is much lower compared to those obtained with the reported microgravimetric and colorimetric detection methods as summarized in Table 1. The QCM analysis of Con A based on the azido mannose SAM-modified electrode reported the detection limit of 9.2 μg/mL (Zhang et al., 2006). The colorimetric detection of the lectin Con A using mannose-stabilized Au and Ag NPs resulted in the detection limit of 10.3 μg/mL (Hone et al., 2003) and 4.1 μg/mL, respectively (Schofield et al., 2006). The lectin biosensor based on glycolipid modified Au NPs immobilized on glass slide exhibited slightly better detection limit of 0.010 μg/mL (Guo et al., 2007) than the present result. However, the reported optical lectin biosensing strategy suffers from short dynamic range (less than 2 orders of magnitude). Although the sensitivity with the present biosensing system is impressive, sensitivity and detection limit can be further enhanced by increasing the silver deposition time more than 7 min in order to increase the anodic stripping peak.

It is of interest to find out if the present lectin Con A biosensor has a selectivity against other proteins. Therefore, the identical biosensing procedures were carried out for 100 μg/mL of cholera toxin and human IgG as model proteins. As shown in Fig. 6, the background corrected ASV signals of cholera toxin and human IgG were 6.67 ± 0.33% ($n = 5$) and 3.67 ± 0.17% ($n = 5$), respectively, compared to that measured from 100 μg/mL Con A. The change is almost negligible in consideration of such high concentration of cholera toxin and human IgG. The result indicates that the present lectin biosen-

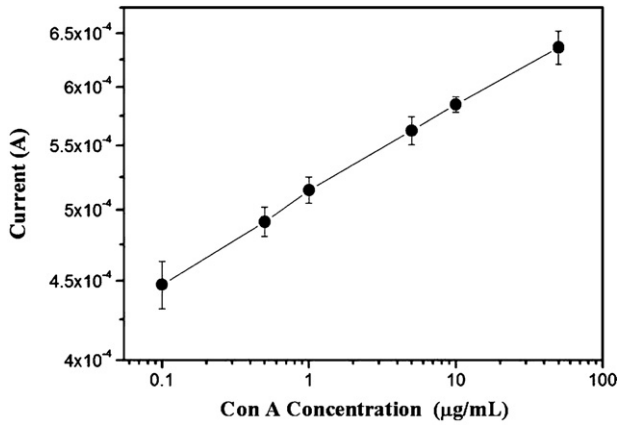


Fig. 5. Calibration curve for Con A obtained with the present biosensor. Error bars represent SD, $n = 4$.

Table 1
Comparison of the figure of merits for Con A determination with various lectin biosensing methods.

Lectin biosensing method	Linear dynamic range (μg/mL)	Detection limit (μg/mL)	References
Colorimetric/Con A-induced mannose-Au NPs aggregation	19.7–39.5	10.3	Hone et al. (2003)
Colorimetric/Con A-induced mannose-Ag NPs aggregation	8.2–26.7	4.1	Schofield et al. (2006)
Colorimetric/mannose bilayers on nano-Au glass slide	0.010–0.48	0.010	Guo et al. (2007)
Microgravimetric/no signal enhancement	ND	9.2	Zhang et al. (2006)
Microgravimetric/mannose-Au NPs induced signal enhancement	0.020–6.2	0.013	Lyu et al. (2008)
Electrochemical/mannose-Au NPs and silver enhancement	0.084–50.0	0.070	Present study

ND: not determined.

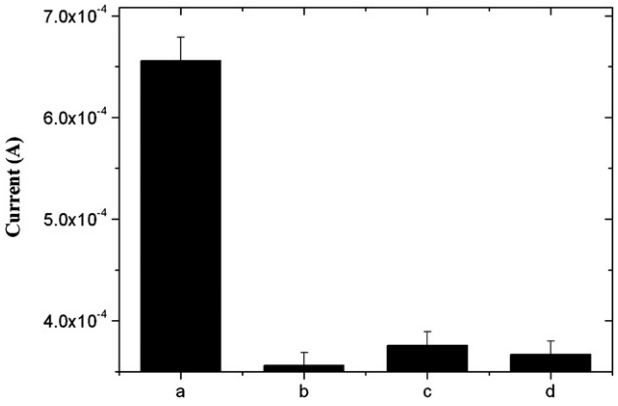


Fig. 6. Selectivity study showing ASV signals of the present lectin biosensor for 100 μg/mL Con A (a), blank solution (b), 100 μg/mL cholera toxin solution (c) and 100 μg/mL human IgG solution (d). Error bars represent SD, $n = 4$.

sor has good selectivity towards Con A as already proven by UV-Vis spectrophotometric studies on aggregation experiments.

The sensor-to-sensor reproducibility has been examined by measuring the ECL responses of 10 replicate lectin biosensors to Con A concentrations from 84.4 ng/mL to 50.0 μg/mL. The relative standard deviation was less than 3.5%, indicating the good reproducibility of the present biosensing system.

4. Conclusions

We have developed for the first time a highly sensitive electrochemical lectin biosensor, which relies on the use of highly selective carbohydrate-modified electrode, carbohydrate-stabilized Au NPs and silver-enhancement technique. Mannose-stabilized Au NPs on the sandwich-type complex of the Con A serves as the nucleation sites for silver-enhancement process and thus greatly amplified the electrochemical ASV signals, which are dependent upon Con A concentration. Based on this detection method, the present lectin biosensor exhibited a wide linear dynamic range of almost three orders of magnitude for Con A concentration along with a remarkable detection limit (S/N = 3) of 0.070 μg/mL. The present biosensing method is simple, rapid, sensitive and inexpensive. Thus it should complement other methods such as ELISA in a favorable manner and provide a versatile tool for the analysis of clinically important lectin proteins containing several binding sites. In addition, the present method can be extended to the monitoring of glycan–lectin interactions and to bioassays of disease or cancer-related glycans based on competitive binding. The relevant clinical application is under investigation.

Acknowledgements

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