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FULL PAPER

Electrochemical detection of glycan and protein epitopes of glycoprotein in serum

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ABSTRACT

Aberrant protein glycosylation is associated with a range of pathological conditions including cancer and possess diagnostic importance. Translation of glycoprotein biomarkers will be facilitated by development of a rapid and sensitive analytical platform that simultaneously interrogates both the glycan and protein epitopes of glycoproteins in body fluids such as serum or saliva. To this end, we developed an electrochemical biosensor based on the immobilization of a lectin on the gold electrode surface to recognize/capture target glycan epitope conjugated to glycoproteins, following with detection of the protein epitope using target protein-specific antibody. Electrochemical signals are generated by label-free voltammetric or impedimetric interrogation of ferro/ferricyanide redox couple (e.g. $[Fe(CN)_6]^{3-/4-}$) on the sensing surface, where the change in voltammetric current or interfacial electron transfer resistance were measured. The detection system was demonstrated using the model glycoprotein chicken ovalbumin with *Sambucus nigra* agglutinin type I (SNA lectin), and exhibits femtomolar sensitivity in the background of diluted human serum. The results obtained in this proof-of-concept study demonstrate the possibility of using electrochemical detection for developing cheap point-of-care diagnostics with high specificity and sensitivity for blood glycoprotein biomarkers.

KEYWORDS

Electrochemical biosensor, label-free detection, Lectin, Glycoprotein biomarker, Serum, Ovalbumin

INTRODUCTION

Differential protein glycosylation is a feature of diverse pathological conditions such as atherosclerosis, ulcerative colitis, rheumatoid arthritis, microbial infection, Alzheimer's disease and cancer.[1] Monitoring specific glycosylation changes for glycoprotein biomarker candidates such as fucosylated haptoglobin (pancreatic cancer) and sialylated prostate-specific antigen (prostate cancer) provides higher diagnostic power compared to changes in total glycoprotein levels.[2] In fact, measurement of fucosylated α-fetoprotein in blood (AFP-L3 test) has been approved by the FDA for early detection of hepatocellular carcinoma.[3, 4] Over the past few years, various glycoprotein enrichment platforms have been coupled with mass spectrometric and nuclear magnetic resonance spectroscopic techniques to uncover disease specific glycosylation changes for candidate glycoprotein biomarkers. These include single, serial and multi-lectin affinity chromatography,[5, 6] lectin magnetic bead array (LeMBA),[7, 8] boronic acid[9] and hydrazide chemistry[10] based extraction methods. While all these methods have excellent analytical performance in detecting candidate cancer glyco-biomarkers, they are poorly suited for routine clinical use due to high maintenance/running cost, requirement of technical expertise, long assay time and complicated data analysis procedure. The development of detection methods that are user-friendly, robust, sensitive, quick and cheaper than currently available methods is warranted to fulfill important needs for developing future diagnostics.

Electrochemical (EC) detection methods offer elegant ways for interfacing bio-recognition and transduction events and represent a substantial driver to achieve rapid, cost-effective, sensitive, selective and accurate quantification of biomolecules.[11-15] Among many EC methods, Faradaic electrochemical impedance spectroscopy (F-EIS) is one of the most effective methods for the label-free detection of biomolecules and for probing the build-up of the biomaterials sensing film

on the electrodes.[16-18] In F-EIS, the binding of a target protein with its ligand (i.e., specific antibody) on electrode surface can be detected, where the change in impedance of the electrode surface and its interface to the electrolyte solution containing a redox probe (e.g. $[Fe(CN)_6]^{3-/4-}$) is measured in the form of its electron transfer resistance (R_{ct}).[19-21] This interfacial electron transfer reaction of the redox process can also be measured via voltammetric technique, where the presence or absence of the target proteins will alter the voltammetric current of the redox process at the sensing surface. More recently, the differential pulse voltammetric (DPV) interrogation of interfacial electron transfer reaction of the $[Fe(CN)_6]^{3-/4-}$ process generated upon protein binding has been used as an effective label-free tool for protein detection.[22, 23]

In past few years, much attention has been focused on development of label-free electrochemical biosensors to monitor glycosylation changes (e.g. mannosylation, galactosylation, sialylation and fucosylation) in complex biological fluids[24-27] using naturally occurring lectin as glycan recognition element. These biosensors detect overall changes in glycan profile without monitoring specific glycoprotein to which glycan is attached. To increase specificity and diagnostic value, analytical methods are required to detect not only overall status of glycosylation in the sample but also aberrant glycosylation for a specific glycoprotein. To overcome this limitation, we investigated label-free electrochemical detection methods for the simultaneous interrogation of specific glycan on target glycoprotein.

Here we describe the development of a simple method that can efficiently and reproducibly detect both glycan and protein epitopes of a glycoprotein in background of diluted serum sample at femtomolar concentration. The method consists of a gold macrodisk electrode that is biochemically functionalized with *Sambucus nigra* agglutinin type I lectin (SNA lectin) [specific to recognize terminal sialic acid attached to galactose through α-(2-6) linkage].[28] The attachment

of the target glycoprotein chicken egg albumin (ovalbumin) on SNA lectin functionalized electrode and complexation with polyclonal anti-ovalbumin antibody (Figure 1 scheme) was followed by the F-EIS and DPV measurements. Each bimolecular layer on the sensor surface acts as a barrier for the interfacial electron transfer reaction of the $[Fe(CN)_6]^{3-/4-}$ process, resulting in an increase in R_{ct} or decrease in DPV current response. The presence of target glycoprotein ovalbumin and subsequent complexation with polyclonal anti-ovalbumin antibody appear to further block the $[Fe(CN)_6]^{3-/4-}$ process from accessing the electrode surface effectively. When we monitored R_{ct} or DPV current responses generated from the $Fe[(CN)_6]^{3-/4-}$ process before and after ovalbumin capture, there was a clear correlation between the presence of the target ovalbumin and changes in R_{ct} or DPV current response. To mimic clinical scenario, we spiked ovalbumin into serum and showed linear changes in current readout with concentration range from 10 pg mL^{-1} to 500 pg mL^{-1}. To our knowledge, this is the first electrochemical method that simultaneously interrogates specific glycans and the target protein on which the glycan is attached. Moreover, we believe the simplicity of this technology could facilitate translation of current glycoprotein biomarker research.

MATERIALS AND METHODS

Materials. Biotin labeled *Sambucus nigra* agglutinin type I **(**SNA-I) lectin was purchased from Vector Laboratories (USA). Lyophilized chicken egg albumin (ovalbumin) (#A5503), polyclonal anti-chicken egg albumin antibody produced in rabbit (#C6534), potassium ferrocyanide, potassium ferricyanide, and KCl were purchased from Sigma (Australia). Biotinylated BSA was purchased from Thermo Scientific (USA) while multivalent streptavidin was purchased from Invitrogen (USA). Human blood sample was collected from a healthy

volunteer with consent and ethics approved by The University of Queensland Human Ethics Committee.

Cleaning gold disk electrodes. Gold disk working electrodes (diameter = 3 mm) were purchased from CH Instruments (Austin, USA). The electrodes were reused after cleaning and regeneration. The electrodes were cleaned using piranha (H_2SO_4:H_2O_2 = 3:1) on a sonicator water-bath for 30 sec - 1 min (Note: piranha is a highly toxic and hazardous chemical. It is to be handled in fume-hood with adequate personal protective equipments. University safety guidelines were followed for its disposal). The electrodes were then physically polished with 1 micron alumina and subsequently with 0.05 micron alumina slurry. Prior to electrochemical cleaning, electrodes were sonicated in acetone for 20 min. Electrochemical cleaning was performed in 0.5 M H_2SO_4 until reproducible characteristic gold electrode profiles were achieved.

Construction of biosensor. Immediately before functionalization, thoroughly cleaned gold disk electrodes were dried under a flow of nitrogen gas. The electrodes were incubated with 500 μg mL^{-1} biotin-BSA in 1× PBS (137 mM NaCl, 2 mM KCl, 10 mM phosphate buffer, pH 7.4) on thermo shaker set at 25 C, 300 rpm for 45 min. The electrodes were washed with 1× PBS after each incubation step for 3 times. The electrodes were then incubated with multivalent streptavidin (500 μg mL^{-1} in 1× PBS, at 25 C, 300 rpm for 45 min), followed by biotinylated SNA lectin (500 μg mL^{-1} in 150 mM NaCl containing 0.1 mM Ca^{2+}, at 25 C, 300 rpm for 45 min) to form the bio-recognition layer of lectin on electrode surface. Ovalbumin stock solution was made at concentration of 2 mg mL^{-1} and stored at -30 C in aliquots. Designated concentration of ovalbumin was freshly made (in 150 mM NaCl solution containing 0.1 mM Ca^{2+}) prior to use and protein capture was performed for 45 min with gentle agitation using

intelli-mixer (PCOD Scientific). 1 in 1000 dilution of polyclonal anti-ovalbumin antibody in 1× PBS for 15 min was used for detection of captured chicken egg albumin. Designated concentrations of ovalbumin were spiked in diluted serum (1 in 1000 dilution of serum in 150 mM NaCl solution containing 0.1 mM Ca^{2+}).

After modification of the sensor surfaces (up to SNA lectin attachment), the actual detection steps include capture of the ovalbumin antigen followed by the anti-ovalbumin detection. Both of these steps are directly followed by the label-free impedance (or DPV) technique. Thus once electrode is modified with SNA lectin, overall detection process takes approximately 2 hr.

Electrochemical measurement. All electrochemical experiments were conducted at room temperature (25 ± 1°C) in a standard three-electrode electrochemical cell arrangement using an electrochemical workstation CHI 650D, CH Instruments (Austin, USA). The electrochemical cell consisted of gold disk electrode sensor as a working electrode, platinum wire counter electrode and Ag/AgCl (3M KCl) reference electrode. Electrochemical signals were measured in 1× PBS buffer (pH 7.4) containing 2.5 mM $[Fe(CN)_6]^{3-}$/ $[Fe(CN)_6]^{4-}$ (1:1) and 0.1 M KCl. DPV signals were obtained with a potential step of 4 mV, pulse amplitude of 50 mV, pulse width of 200 ms, sampling width of 16.7 ms and pulse period of 500 ms. The F-EIS spectra were recorded in 1× PBS buffer (pH 7.4) containing 2.5 mM $[Fe(CN)_6]^{3-}$/ $[Fe(CN)_6]^{4-}$ (1:1) and 0.1 M KCl using an alternating current voltage of 5 mV, with the frequency range of 0.1 (or 1) Hz – 100 kHz. During run, bias DC current was applied below frequency of 100 Hz. The faradic current generated by the $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ probe accounts for the presence of a biomolecule. The current changes corresponding to ovalbumin detection were calculated as follows:

$$\% \text{ Decrease of DPV peak current} = (I_{before} - I_{after}) \times 100 / I_{before} \quad \ldots\ldots\ldots \quad (1)$$

where I_{before} = peak current at ovalbumin capture step [*e.g.*, current recorded at biotin-BSA/multivalent streptavidin/biotinylated SNA lectin/designated concentration of ovalbumin (either in buffer or spiked into 1 in 1000 serum)], I_{after} = peak current at detection step [*e.g.*, current recorded at biotin-BSA/multivalent streptavidin/biotinylated SNA lectin/designated concentration of ovalbumin (either in buffer or spiked into 1 in 1000 serum)/1 in 1000 anti-ovalbumin polyclonal antibody].

For each electrode, peak current was normalized with DPV response obtained at initial step when electrodes were clean.

RESULTS & DISCUSSION

Construction of biosensor. Figure 1 describes assembly scheme of the biosensor. As reported previously,[18] biotin labeled BSA is used for coating gold electrode surface. Subsequently, multivalent streptavidin was used as a linker to immobilize biotin labeled SNA lectin to form bio-recognition layer on the electrode surface. The formation of bio-recognition layer on electrode surface affects analytical performance of the biosensor and in our experiments this formation is controlled by attachment of biotin-BSA, streptavidin and biotinylated SNA lectin. To achieve maximal analytical performance, we optimized binding conditions for all three biomolecules one after another by incubating electrodes with three different concentrations of biotin-BSA, multivalent streptavidin and biotinylated SNA lectin.

Firstly to determine optimal biotin-BSA concentration, we incubated thoroughly cleaned gold electrodes with 100 μg mL^{-1}, 500 μg mL^{-1} and 1000 μg mL^{-1} solution of biotin-BSA for 45 min. The electrodes were washed three times with 1× PBS, followed by F-EIS measurements to

determine optimal biotin-BSA concentration. As compared to 100 μg mL^{-1}, incubation of electrodes with 500 μg mL^{-1} concentration of biotin-BSA showed increase in size of the semicircle on the Nyquist plot (Figure 2A, i vs. ii) suggesting increased electron transfer resistance (R_{ct}). There was no further increase in R_{ct} at 1000 μg mL^{-1} biotin-BSA concentration as compared to 500 μg mL^{-1} (Figure 2A, ii vs. iii). As 500 μg mL^{-1} biotin-BSA showed best F-EIS i.e. maximum R_{ct} response, the same binding condition was used for subsequent experiments. Next, F-EIS for three different concentrations 100 μg mL^{-1}, 500 μg mL^{-1} and 1000 μg mL^{-1} of multivalent streptavidin under optimal biotin-BSA binding conditions was obtained. As shown in Figure 2C, incubation of electrode with 500 μg mL^{-1} concentration of multivalent streptavidin showed maximum R_{ct} under optimal biotin-BSA binding condition. Similarly, as shown in Figure 2E, 500 μg mL^{-1} of biotinylated SNA lectin showed best F-EIS response under optimal biotin-BSA and multivalent streptavidin binding conditions. To confirm F-EIS results we conducted parallel DPV measurements and as shown in Figure 2B, 2D and 2F, there was decrease in peak DPV current corresponding with increase in R_{ct} suggesting reduction in actual electron transfer between electrode/redox electrolyte double layer with increased resistance. For all three biomolecules, maximum R_{ct} and minimal DPV peak current was observed at 500 μg mL^{-1} concentration. Moreover, there was no further increase in R_{ct} at higher concentration i.e. 1000 μg mL^{-1}. This suggests that electrode surface is getting saturated when incubated with 500 μg mL^{-1} concentration of biomolecules and there is no non-specific binding of the protein directly on the electrode surface at higher concentration. As the protein binding to electrode surface is showing saturation without any non-specific binding, it is anticipated that there will be minimal or no non-specific binding of biomolecule of interest directly to the electrode surface during further stages of experiment.

Glycoprotein capture and detection. After optimizing conditions for SNA lectin immobilization on electrode surface, next we captured glycoprotein ovalbumin in 150 mM NaCl solution containing 0.1 mM Ca^{2+}. The formation of bio-recognition layers and capture of ovalbumin was followed by electrochemical detection. As shown in Figure 3A, biotin-BSA coated electrode gave rise to smallest semicircle (Figure 3Ai), followed by streptavidin (Figure 3Aii), SNA lectin (Figure 3Aiii) and ovalbumin (Figure 3Aiv) showed gradual increase in semicircle indicating increase in electron transfer resistance. In line with changes observed in impedance, we observed corresponding decrease in peak DPV current (Figure 3B). These results firstly confirm results shown in previous section for successful formation of bio-recognition layers on electrode surface. Next, it demonstrates successful capture of target glycoprotein ovalbumin on SNA biosensor with electrochemical detection.

Lectins generally recognize glycan structures with low affinity but with high avidity mainly through hydrogen bonding, hydrophobic interactions and van der Waals forces.[29] In fact, glycan structure specific interactions of a glycoprotein with lectins are very well demonstrated in the recent past mainly using sialic acid binding lectin biosensors.[25-27, 30] Incubation of SNA lectin biosensor with asialofetuin, a non-sialic acid expressing glycoform variant of glycoprotein fetuin, showed very minimal change in the baseline impedance.[25-27, 30] On contrary, a linear increase from baseline impedance was observed when SNA lectin biosensor was incubated with increasing concentration of fetuin expressing correct glycan epitope.[25-27, 30] Apart from showing very high binding specificity towards glycan epitope, these SNA lectin biosensors have demonstrated up to attomolar sensitivity for the glycoprotein detection (e.g. fetuin).[25-27, 30] SNA along with other electrochemical lectin biosensor developed so far have been applied to glycan profiling of healthy and rheumatoid arthritis human serum samples,[27] monitor glycosylation

changes between normal and cancerous pancreatic cell extracts,[25] dengue diagnosis[31] and microorganism recognition,[32] without detecting specific glycoproteins. Detection of both glycan and protein epitopes of a glycoprotein is required to provide the specificity to measure glycosylation-specific biomarker, for example fucosylated alpha-fetoprotein. Therefore, following capture of ovalbumin glycoprotein using SNA lectin biosensor, we interrogated ovalbumin protein backbone using polyclonal anti-ovalbumin antibody. Upon capturing ovalbumin on SNA biosensor, electrodes were incubated with 1 in 1000 dilution of anti-ovalbumin antibody, followed by F-EIS and DPV measurements. As compared to ovalbumin capture step (Figure 3Aiv) incubation of electrode with anti-ovalbumin antibody (Figure 3Av) showed increase in impedance, with corresponding decrease in DPV peak current (Figure 3B). These results indicate stepwise construction of bio-recognition layer, capture of ovalbumin on SNA biosensor and detection of ovalbumin using polyclonal anti-ovalbumin antibody. As F-EIS and DPV techniques showed an excellent agreement between the results obtained for stepwise binding of biomolecules on the sensor surface, we presented only DPV responses for remaining all experiments.

As antibodies are glycoprotein themselves, they may directly interact with lectin which may lead to false positive results. Therefore, contribution of false positive response due to direct interaction between SNA lectin and polyclonal anti-ovalbumin antibody was tested. The gold electrode modified with biotin-BSA/streptavidin/SNA lectin was directly incubated with anti-ovalbumin antibody to allow any possible direct interaction between them. As shown in Figure 3C we did not observe any change in DPV response between biotin-BSA/streptavidin/SNA lectin and biotin-BSA/streptavidin/SNA lectin incubated with anti-ovalbumin antibody. This finding indicates that there was no direct interaction between ovalbumin antibody with SNA lectin.

Thus, we demonstrate stepwise formation of bio-recognition layer, capture of glycoprotein ovalbumin on SNA biosensor surface followed by detection of ovalbumin using anti-ovalbumin antibody and label-free electrochemical detection using F-EIS and DPV measurements. Moreover, we did not observe any direct interaction between lectin and antibody which excludes possibility for false positive results. To the best of our knowledge, this is the first electrochemical biosensor which interrogates both glycan epitope of a glycoprotein using lectin and protein epitope using anti-protein antibody with label-free electrochemical monitoring.

Detection of ovalbumin in serum. To evaluate the biosensor performance under more physiological conditions, we conducted a series of experiments in which ovalbumin was spiked into diluted human serum collected from healthy donor. To mimic biomarker detection condition, ovalbumin at final concentration of 1 ng mL^{-1} was spiked into 1:1000 serum sample. Similar dilution of serum sample has been recommended to study overall changes in glycan profiles using electrochemical detection.[27] Blood is very heterogeneous in terms of its protein composition, consisting of proteins with abundance varying several orders of magnitude. Top six abundant plasma proteins account for 85% of the total plasma proteome.[33] Out of them, five are found to be glycosylated (transferrin, haptoglobin, α1-antitrypsin, IgG, and IgA) and show varying degree of binding to SNA lectin. This means that one would expect significant amount of glycoprotein capture when electrode immobilized with SNA lectin is incubated with serum/plasma sample. As can be seen in Figure 4A, DPV peak current was significantly reduced when SNA lectin modified electrode was incubated with serum sample (Figure 4A, i vs. ii). However, there was no further decrease in DPV response with anti-ovalbumin antibody incubation suggesting the antibody did not bind non-specifically with any SNA lectin-bound human serum proteins (Figure 4A, ii vs. iii).

When ovalbumin was spiked into serum, incubation of electrode with polyclonal anti-ovalbumin antibody (Figure 4Bii) showed significant reduction in peak DPV response as compared to DPV peak current resulted at the ovalbumin capture step in serum (Figure 4Ai). This data clearly demonstrates that our method is highly effective for specific detection of ovalbumin glycoprotein in the presence of large excess of several other SNA lectin binding proteins in serum.

To assess the dynamic range and lower limit of detection of our method, a dilution series of ovalbumin protein (1 pg mL^{-1} to 10 ng mL^{-1}) was spiked in diluted serum. For each ovalbumin concentration, electrochemical response was measured at the step of glycoprotein capture and detection step using anti-ovalbumin antibody. The percentage change in peak current between these two measurements is attributed to the amount of ovalbumin present in the sample. As shown in Figure 4C, there was a gradual increase in percentage change in DPV peak current with increasing concentration of ovalbumin spiked in serum ranging from 10 pg mL^{-1} to 500 pg mL^{-1}. Beyond 500 pg mL^{-1}, there was no further increase in DPV response suggesting saturation of the glycoprotein capture on electrode surface. The lower limit of detection of 10 pg mL^{-1} described here is comparable with previous electrochemical glycan profiling studies.[25, 27] This 10 pg mL^{-1} lower limit of detection in 1000 fold diluted serum sample will actually translate into 10 ng mL^{-1} in the actual undiluted sample. Previously published lectin electrochemical biosensors showed femtomolar sensitivity and outperformed conventional lectin-ELISA and lectin-microarray by several fold in terms of lower limit of detection.[25, 27] Unlike previous studies which only interrogated glycan, here we interrogated both glycan as well as protein epitope of a glycoprotein in background of diluted serum with detection limit of 10 pg mL^{-1}. Detection limit of our assay could be further improved via incorporating and optimizing the device geometry and

experimental parameters.[34] Moreover, designing multiplexed electrochemical biosensor[25, 34] would help to monitor panel of clinically relevant glycoprotein biomarkers in parallel.

CONCLUSION

In this proof-of-concept study we demonstrated interrogation of glycan along with protein epitope of a glycoprotein ovalbumin using SNA lectin and anti-ovalbumin antibody respectively followed by label-free electrochemical detection in background of diluted serum. Our lectin-antibody biosensor detects specific glycan and the glycoprotein to which the glycan is attached, hence providing increased specificity over existing lectin biosensors. Furthermore, with detection limit of 10 pg mL^{-1}, lectin-antibody electrochemical biosensor described here could be developed further to achieve point-of-care diagnosis for clinically relevant glycoprotein biomarkers.

Notes

The authors decleare no competing financial interest.

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FIGURE CAPTIONS

Figure 1. (A) Schematic illustration of the preparation of an SNA lectin immunosensing layer. **(B)** Schematic view of the glycoprotein ovalbumin capture and label-free detection using anti-ovalbumin antibody which results in increased charge transfer resistance (R_{ct}) and corresponding decrease in DPV peak current.

Figure 2. Optimization of biosensor construction. Nyquist plots of a gold electrode modified with (A) biotin-BSA, (C) biotin-BSA/streptavidin, and (E) biotin-BSA/streptavidin/SNA lectin in 1× PBS buffer containing 2.5 mM $K_3[Fe(CN)_6]$, 2.5 mM $K_4[Fe(CN)_6]$. $3H_2O$ and 0.1 M KCl. (B, D, F) Corresponding DPV responses obtained at a gold electrode modified with (B) biotin-BSA, (D) biotin-BSA/streptavidin, and (F) biotin-BSA/streptavidin/SNA lectin, respectively. The concentration of biotin-BSA in Figures A & B, multivalent streptavidin in Figure C & D (following incubation with 500 μg mL^{-1} of biotin-BSA), and SNA lectin in Figure E & F (following incubation with 500 μg mL^{-1} biotin-BSA and 500 μg mL^{-1} streptavidin each) were (i) 100 μg mL^{-1}, (ii) 500 μg mL^{-1}, and (iii) 1000 μg mL^{-1}.

Figure 3. (A) The Nyquist plots and **(B)** Differential pulse voltammetric response for monitoring (i) Biotin-BSA (ii) Biotin-BSA/Multivalent Streptavidin (iii) Biotin-BSA/Multivalent Streptavidin/SNA lectin (iv) Biotin-BSA/Multivalent Streptavidin/SNA lectin/1 ng mL^{-1} ovalbumin and (v) Biotin-BSA/Multivalent Streptavidin/SNA lectin/1 ng mL^{-1} ovalbumin/anti-ovalbumin antibody in 1× PBS buffer containing 2.5 mM $K_3[Fe(CN)_6]$, 2.5 mM $K_4[Fe(CN)_6]$.3H2O and 0.1 M KCl. **(C)** Differential pulse voltammetric response to detect any non-specific binding of anti-ovalbumin antibody with SNA lectin (i) Biotin-BSA/Multivalent

Strepatavidin/SNA lectin and (ii) Biotin-BSA/Multivalent Streptavidin/SNA lectin/anti-ovalbumin antibody.

Figure 4. Differential pulse voltammetric response for **(A)** Detection of SNA binding proteins present in human serum (i) Biotin-BSA/Multivalent Streptavidin/SNA lectin (ii) capturing SNA lectin binding glycoproteins present in human serum (1 in 1000 dilution) and (iii) detection of any non-specific recognition of SNA bound serum glycoprotein using anti-ovalbumin antibody. **(B)** Detection of ovalbumin spiked in human serum (i) capturing 1 ng mL^{-1} ovalbumin in human serum (1 in 1000 dilution) using SNA lectin and (ii) its detection using anti-ovalbumin antibody. **(C)** Calibration plot for % change in differential pulse voltammetric response for detection of designated concentration of ovalbumin spiked in human serum (1 in 1000 dilution) using anti-ovalbumin antibody. Each data point represents average ± SD (standard deviation) of 3 independent experiments.

FIGURES

Figure 1

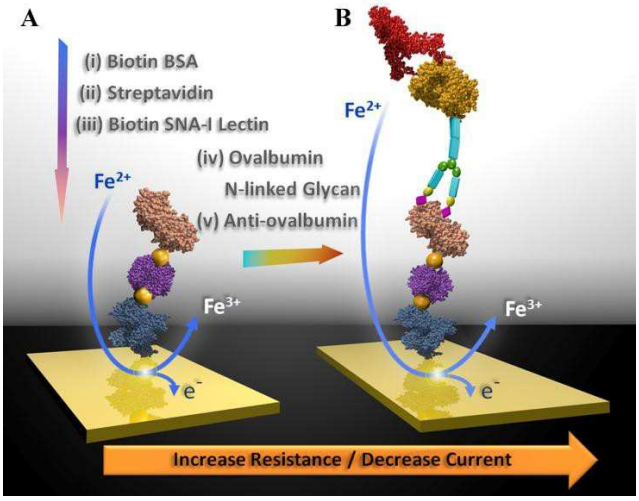


Figure 2

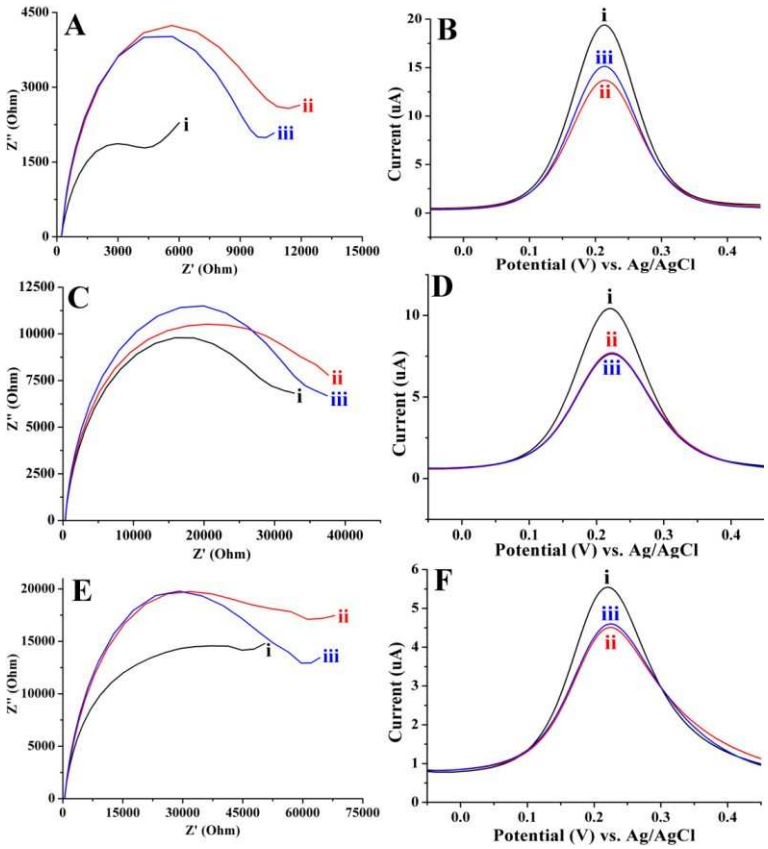


Figure 3

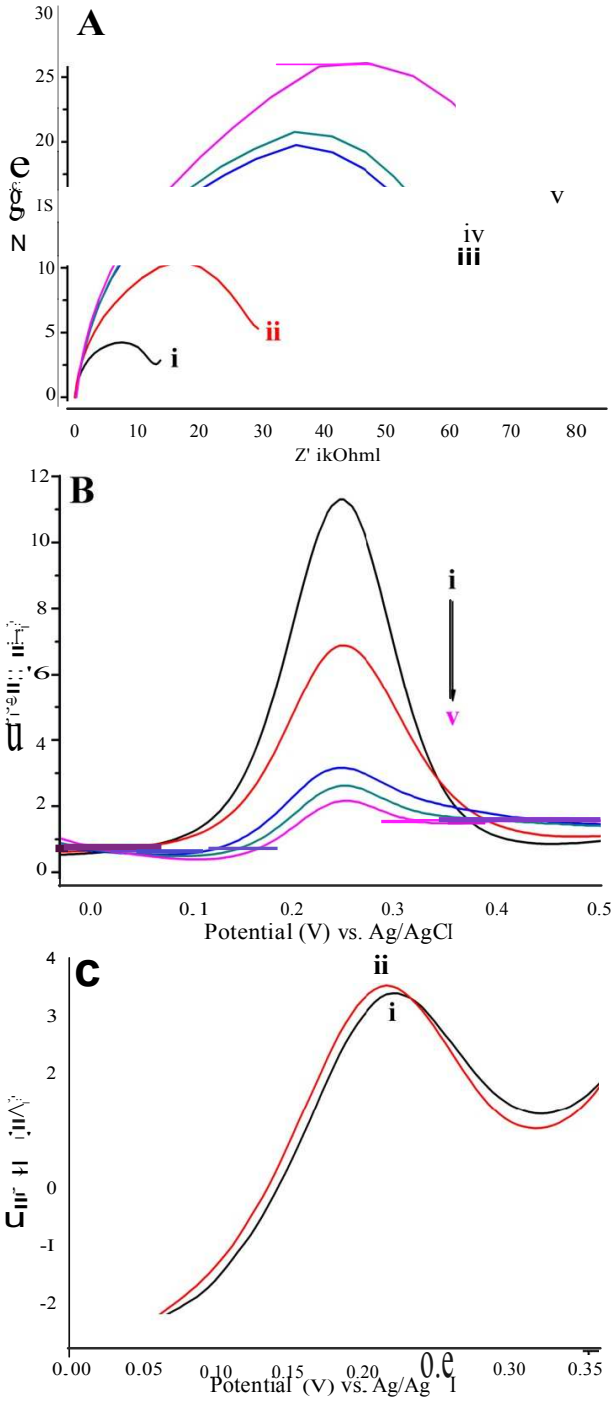


Figure 4

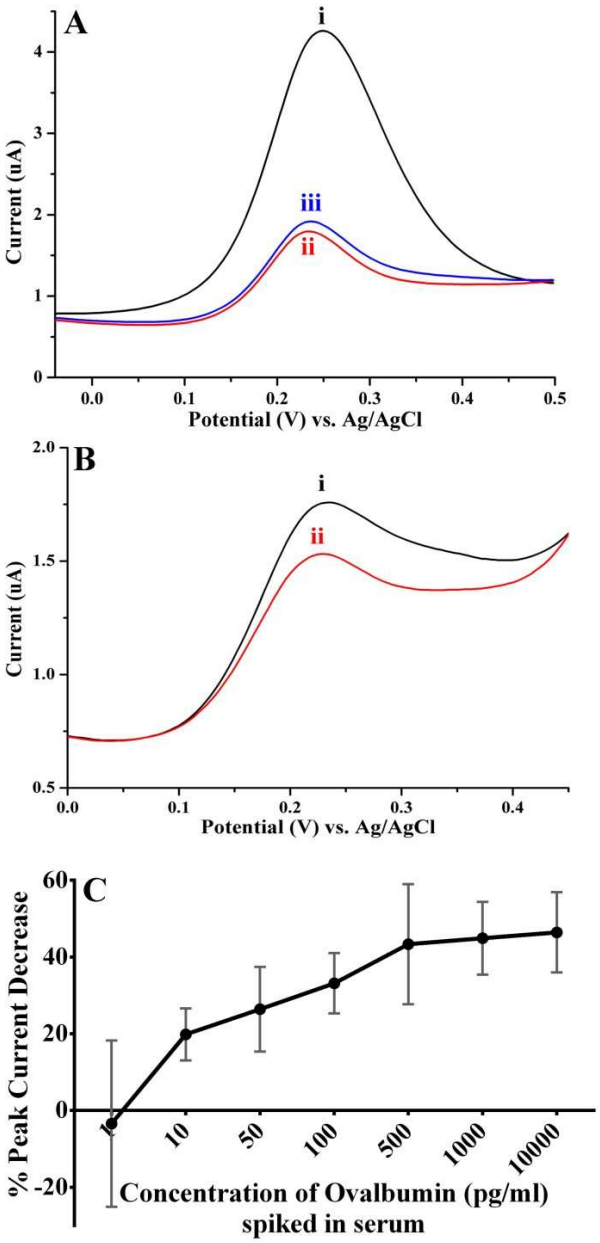
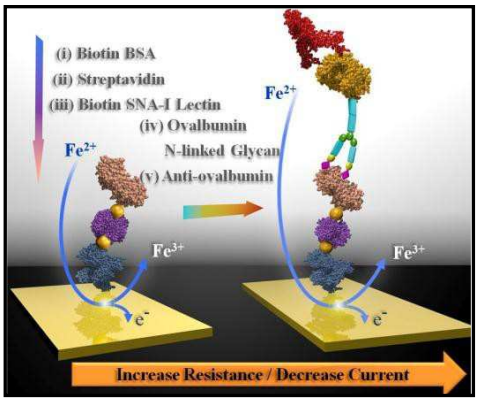


Table of contents entry



Description: Electrochemical interrogation of glycan and protein epitopes of glycoprotein chicken ovalbumin using SNA lectin and anti-ovalbumin antibody in serum