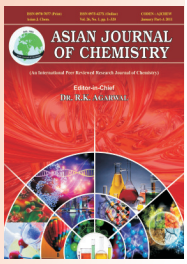
Asian Journal of Chemistry; Vol. 26, No. 19 (2014), 6357-6363

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16372



Ultrasensitive Impedimetric Lectin Biosensor with Efficient Antifouling Properties Applied in Determination of α-Fetoprotein on Mixed Self-Assembled Monolayer on Gold

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Received: 14 September 2013; *Accepted*: 8 January 2014; *Published online*: 16 September 2014; AJC-15917

A sensitive and attractive antifouling impedimetric lectin biosensor for the determination of α-fetoprotein in human serum samples was developed. The biosensor was fabricated by co-assembling 11-mercapto-undecanoic acid for covalent immobilization of *wheat-germ agglutinin* and dithiothreitol to resist nonspecific interactions on the surface of gold electrode. In the measurement of α-fetoprotein, the change in interfacial electron transfer resistance of biosensor was monitored using a ferri/ferrocyanide redox couple. Owing to the specific recognition of *wheat-germ agglutinin* with N-glycan on α-fetoprotein, the increase in the electron transfer resistance was linearly proportional to the logarithm of the concentration of α-fetoprotein in the range from 1.0×10^{-11} to 8×10^{-10} g mL^{-1} and a detection limit of 9×10^{-12} g mL^{-1} α-fetoprotein was achieved. The fabricated biosensor allowed direct quantification of extrinsic α-fetoprotein in serum samples. This work provides a promising strategy for clinical application with impressive sensitivity and antifouling characteristics.

Keywords: Impedimetric, Lectin biosensor, Mixed self-assembled monolayer, α-Fetoprotein.

INTRODUCTION

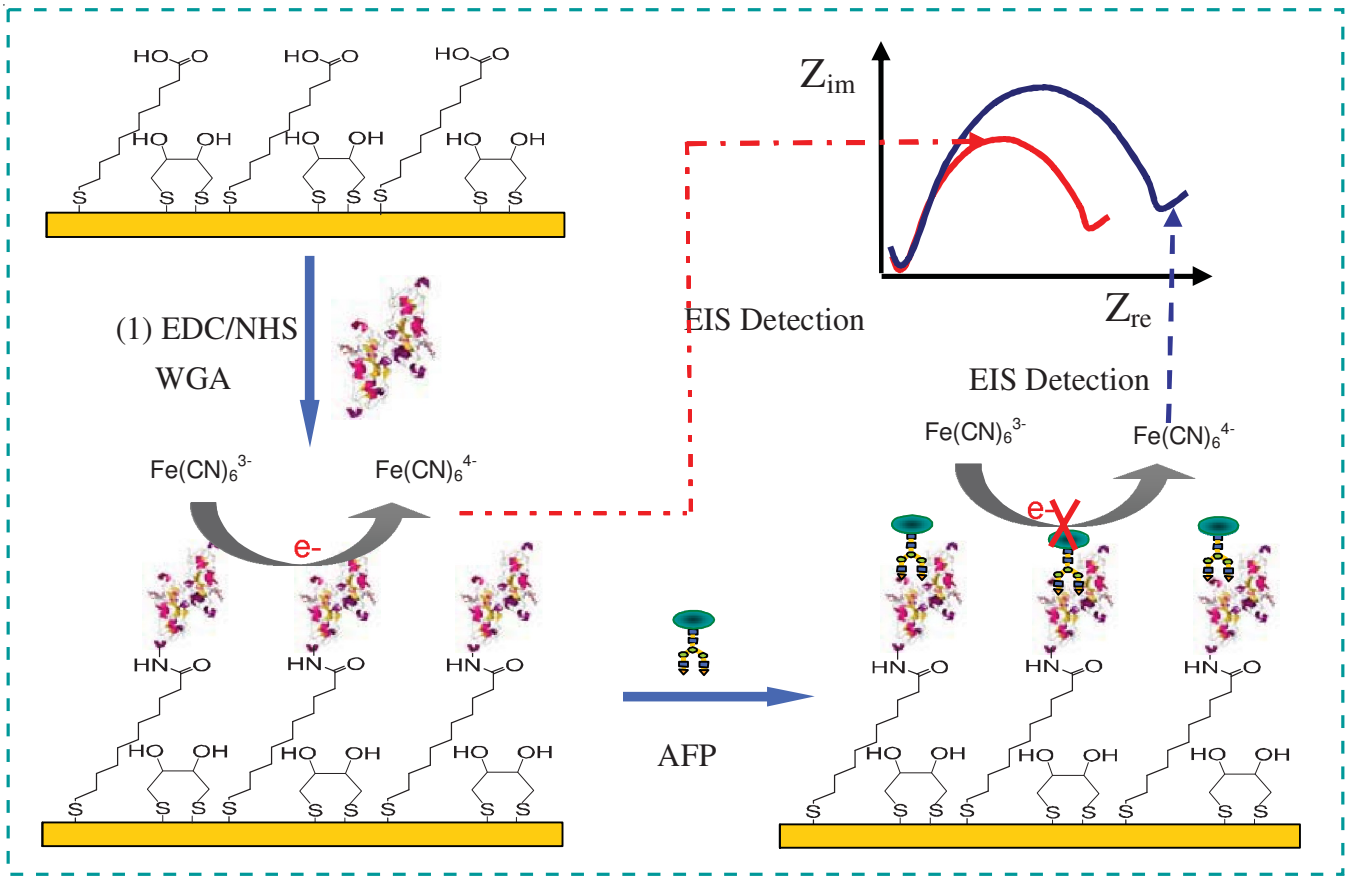
Development of simple, sensitive and selective method for the determination of specific proteins in complex biological matrices such as blood plasma or serum has received more and more attention in clinical and biological fields[1]. Glycosylation is one of the most common modifications to proteins and its presence affects protein-protein interactions, cell-cell recognition, adhesion and motility[2]. Mounting evidence suggests that glycosylation is altered in disease states such as cancer and associated with disease development[3]. Most clinical biomarkers are glycoproteins. Therefore, analysis of glycobiomarkers is expected to improve the specificity of disease diagnosis. Biosensors are simple, inexpensive analytical devices and provide escalating quantities of proteins information[4]. Extensive efforts have been devoted to the design of novel biosensors for glycobiomarkers detection, including the employment of novel biological recognition molecules as well as highly sensitive detection techniques, such as electrogenerated chemiluminescence immunoassay[5,6] and electrochemical immunoassay[7-13] and fluorescence immunoassay[14]. Despite the extensive development of these methods, the antibodies that are widely used have some limitations such as the challenging production of antibodies *in vivo*[15]. Approaches based on lectin open an avenue for analysis of glycans on biomarkers. Taken as molecular recognition elements, lectins can strongly bind to specific carbohydrate moieties on the surface of glycoproteins, have received considerable interest due to the advantages, such as easy production and intrinsic stability compared with antibodies[16]. Compared with antibody-based immunoassay, the lectin-based bioassay could detect the protein content and discriminate the aberrant glycosylation of the tumour biomarkers, which could increase the specificity for cancer diagnosis since the aberrant glycosylation is a fundamental characteristic of progression of cancer[17,18].

As a result, a lot of lectin-based assays for the detection and discrimination the glycan expression of glycoproteins have been proposed in the past few years, such as mass spectrometry[19], fluorescence[20], electrochemistry[21,22] and electrochemiluminescent[23], *etc*. Most reported methods are based on a labeling (analytes or recognition elements) strategy, which not only requires a complicated labeling procedure but also reduces the bioaffinity of the recognition elements. Electrochemical technique, especially electrochemical impedance spectroscopy (EIS) technique, becomes increasingly popular in a variety of protein biosensing because it offers several advantages such as simplicity, high sensitivity and serving as an elegant way to interface biorecognition events and signal

transduction[24]. Various EIS lectin-based sensors have been designed for determination of biomarkers or cancer cells. Oliverira *et al.*[25] reported a biosystem to analyze the interactions between *CramoLL* lectin and fetuin for the detection of glycoprotein in the serum of patients contaminated with dengue serotypes 1, 2 and 3. Belle *et al.*[22] developed a label-free EIS method for the detection of glycan-lectin interactions by immobilizing lectins of *Sambucus nigra agglutinin and peanut agglutinin* on layered Cu/Ni/Au printed circuit board electrodes. The effort is mainly focused on the improvement of sensitivity by employing various amplification strategies and reducing background strategies[26]. However, these strategies are largely limited due to the challenge of the interference of nonspecific binding and a high background[27]. Therefore, how to specifically and sensitively recognize target protein in the presence of non-target proteins is a key problem in the clinical bioassay[28].

To tackle the specificity issues, several approaches have been developed and reported. Surface modifications with an antifouling mixed self-assembled monolayer and polymer brushes were employed to reduce or suppress the non-specific adsorption. For example, mixed self-assembled monolayer of alkane thiolates on gold to reduce the nonspecific adsorption of undesired biological entities[29,30]. The incorporation of both thiols in a mixed self-assembled monolayer enables the attachment of receptor molecules and induces specific interaction of the immobilized molecules instead of a nonspecific adsorption of undesired biospecies on the surface[31]. Qi *et al.*[29] reported an EIS apta sensor by co-assembling anti-thrombin binding aptamer and dithiothreitol, followed by assembling of 6-mercapto-1-hexanol on the surface of gold electrode to form a ternary monolayer to determinate of thrombin and the mixed self-assembled monolayer offered high sensitivity and direct measurement thrombin in undiluted serum with good anti-fouling characteristics. Lieven *et al.*[32] reported an immunoassay for prostate-specific antigen based on a mixed self-assembled monolayer to minimize non-specific adsorption of analyte.

With the foregoing analysis in mind, in this paper, we describe a simple, selective and sensitive impedimetric lectin biosensor for the determination of protein in diluted serum sample using the lectin *wheat-germ agglutinin* as the recognition element. α-Fetoprotein, a tumor marker for hepatocellular carcinoma which contains one N-glycosylation site at asparagine 232, was chosen as model protein in this work[17]. Schematic representation of biosensor with fabrication steps and performance is showed in **Scheme-I**. The biosensor was designed by co-assembling 11-mercapto-undecanoic acid available for covalent immobilization of lectin and dithiothreitol forming an interfacial layer blocking nonspecific interactions on the surface of gold electrode to form a binary monolayer. The introduction of target α-fetoprotein forming a *wheat-germ agglutinin*-carbohydrate conjugate increased electron transfer resistance of biosensor. The characteristics and analytical performance of the *wheat-germ agglutinin* biosensor for the detection of α-fetoprotein are reported. The fabricated biosensor was also applied to detect α-fetoprotein in both buffer solution and diluted serum sample.



Scheme-I: Schematic representation of the fabrication of *wheat-germ agglutinin* biosensor and detection of α-fetoprotein

EXPERIMENTAL

Wheat-germ agglutinin (*wheat-germ agglutinin*, from Triticum vulgarist), mannose, galactose, N-acetylglucosamine (GlcNAc), N-acetylgalctosamine (GalNAc), 11-mercapto-undecanoic acid, 6-mercapto-1-hexanol, dithiothreitol, N-(3-dimethylamino-propy)-*N'*-ethylcarbodimide hydrochloride (EDC), N-hydroxy-succinimide (NHS) were obtained from Sigma-Aldrich (St. Louis, USA). Human α-fetoprotein (AFP, M803209) was obtained from Fitzgerald Industries International, Inc. (USA). Bovine serum albumin (BSA) was obtained from Sinopharm Chemical Reagent Co., Ltd. (China). All reagents were of analytical grade and used as received. Millipore Milli-Q water (18.2 MΩ cm) was used to prepare all the solutions.

10 mM phosphate buffer saline (PBS, pH 7.4, 10 mM Na_2HPO_4/NaH_2PO_4 and 100 mM NaCl) was used as lcetin immobilization buffer. 10 mM PBS (pH 7.4) was used as washing buffer. 10 mM PBS containing 1 mM $CaCl_2$ and 1 mM $MnCl_2$ was used as binding buffer. α-Fetoprotein solutions and all carbohydrate solutions (mannose, galactose, GlcNAc and GalNAc) were prepared by binding buffer.

A CHI 660 electrochemical workstation (Shanghai Chenhua Instrument Co. Ltd., Shanghai, China) was used for all electrochemical measurements. All experiments were carried out using a conventional three-electrode system with a fabricated lectin biosensor or a gold electrode as working electrode, a platinum wire as counter electrode and an Ag/AgCl (sat. KCl) as reference electrode. The gold electrode (2 mm diameter) was polished to a mirror finish using 0.3 and 0.05 μm alumina slurry (Beuhler) followed by thorough rinsing with water and drying under flowing nitrogen gas. All potentials were referred to this reference electrode.

Fabrication of biosensor: A cleaned gold electrode was immersed into 100 μL solution of 1 μM 11-mercapto-undecanoic acid and 10 μM dithiothreitol, then incubated 14 h at 4 °C in a humidified chamber to obtain the DTT/MUA/Au electrode. The substrates were thoroughly rinsed with washing buffer and dried under a stream of nitrogen. After washing the DTT/MUA/Au was activated in 100 μL of freshly prepared solution containing 2 g L^{-1} EDC and 5 g L^{-1} NHS for 0.5 h and rinsed with washing buffer. The activated electrode was then immersed into 100 μL of 1 g L^{-1} *wheat-germ agglutinin* for 1 h at -25 °C. Finally, the *wheat-germ agglutinin* biosensor (WGA/ DTT/MUA/Au) was obtained. The EIS biosensor was rinsed with washing buffer and stored at 4 °C in the dark.

Serum samples preparation: α-Fetoprotein serum samples were prepared by adding small volume of extrinsic α-fetoprotein solution in excess volume of diluted serum samples. The healthy human serum samples were from local hospital. Blood samples serum were transferred into separate tubes and stored at -20 °C. Before analyses, the human serum samples were diluted by biding buffer. In the case of the serum samples analysis, the blank assays corresponded to the serum sample without added extrinsic α-fetoprotein and the recovery experiments prepared by adding small volume of extrinsic α-fetoprotein solution in excess volume of diluted serum samples.

Electrochemical measurement: The fabricated biosensor was immersed in 100 μL of 10 mM PBS containing different concentrations of α-fetoprotein or serum samples for 60 min and then rinsed with washing buffer. Electrochemical measurements were carried out in 3 mL of 10 mM PBS containing 5 mM $K_4Fe(CN)_6$ and 5 mM $K_3Fe(CN)_6$ at the equilibrium potential of $[Fe(CN)6]^{3-/4-}$ (0.24 V *vs*. Ag/AgCl) with a 5 mV sinusoidal excitation amplitude. The impedance spectra were recorded within a frequency range from 100 KHz to 1 Hz with a sampling rate of 12 points per decade. The concentration of α-fetoprotein was quantified by an increase in the electron transfer resistance ΔR_{et} ($\Delta R_{et} = R_{et.i} - R_{et.0}$), where $R_{et.0}$ and $R_{et.i}$ are the electron transfer resistance values before and after incubation with α-fetoprotein, respectively. All electrochemical experiments were carried out at room temperature (25 ± 1 °C).

RESULTS AND DISCUSSION

The different electrodes in the fabrication of the *wheat-germ agglutinin* biosensor were characterized by electrochemical impedance spectrometry and cyclic voltammetry in the presence of the ferri/ferrocyanide redox couple as redox probe. As shown in Fig. 1a, the R_{et} measured was 300 Ω for Au electrode and 4957 Ω for the DTT/MUA/Au (curve a and curve b), 9304 Ω for the WGA/DTT/MUA/Au (curve c). The R_{et} continued to increase to17050 Ω (curve d) after 5×10^{-11} g mL^{-1} α-fetoprotein incubating with *wheat-germ agglutinin* immobilized on the surface of gold electrode. It can be also seen from Fig. 1b that when the *wheat-germ agglutinin* is immobi lized and α-fetoprotein is captured by the biosensor, the current decreased. This is mainly attributed to the fact that the big protein immobilized on the surface of the electrode prohibits the mass transfer of $[Fe(CN)_6]^{3-/4-}$ from the solution to the surface of electrode. The results indicate that *wheat-germ agglutinin* is modified onto a DTT/MUA platform by the carboxylic groups of 11-mercapto-undecanoic acid which are activated for *wheat-germ agglutinin* coupling with EDC and NHS and *wheat-germ agglutinin* can specifically recognize the GlcNAc fragment of N-glycan on α-fetoprotein[33,34].

In the fabrication of biosensor, the interfacial properties of the mixed monolayer play an important role in the analytical performance of biosensor[32]. It is reported that the mixed self-assembled monolayer with antifouling characters were employed to reduce or suppress the non-specific adsorption. As to estimate the degree of non-specific binding of the fabricated biosensor, non-carbohydrate protein BSA was chosen as non-specific protein, which would not be recognized by *wheat-germ agglutinin*. After incubated with 0.1 % BSA, the R_{et} value increases only 5.2 % for the mixed binary layer indicted that the inclusion of dithiothreitol during *wheat-germ agglutinin* immobilization can highly effectively prevent nonspecific adsorption of BSA. Dithiothreitol is a four carbon α-ω dithiol with hydroxyl groups on the second and third carbons. The two hydroxyl groups on dithiothreitol exposed at molecule outer surface provide a hydrophilic microenvironment which is favourable for binding reaction and which also enhances the antifouling properties of the monolayer[29,35].

Optimization of ratio of mixed self-assembled monolayer and *wheat-germ agglutinin* concentration: Experimental parameters including the ratio of mixed self-assembled monolayer during fabrication and the incubation time were optimized. As expected, at a fixed immobilization time, the ratio

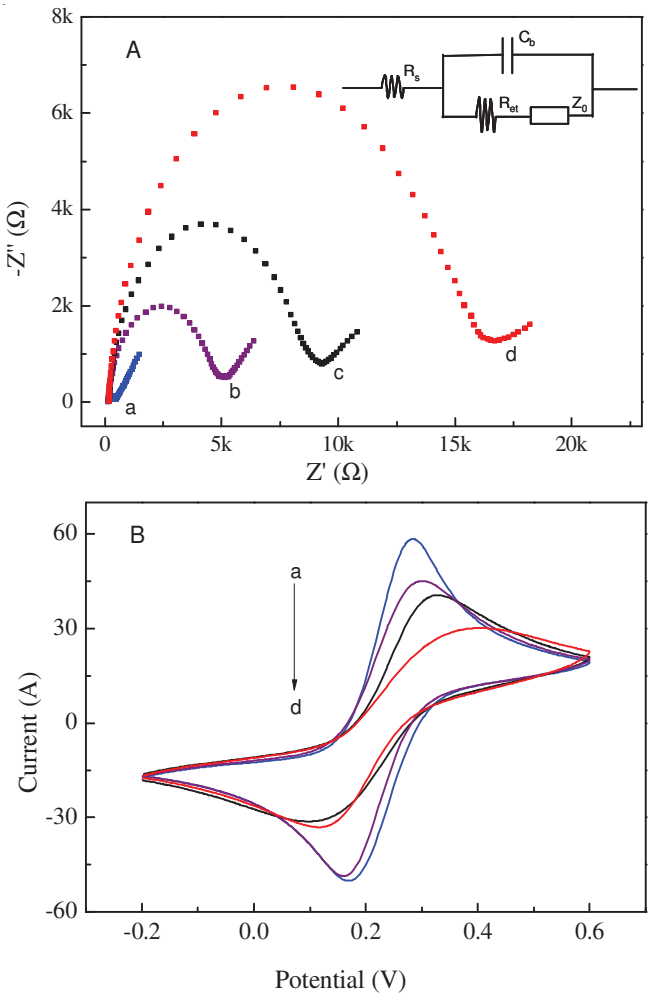


Fig. 1. (A) Nyquist plots and (B) cyclic voltammetry obtained in 10 mM PBS (pH 7.4) containing 5 mM $K_3[Fe(CN)_6]$/5 mM $K_4[Fe(CN)_6]$-0.1 M KCl at (a) a bare gold electrode, (b) DTT/MUA/Au, (c) WGA/DTT/MUA/Au, (d) WGA/DTT/MUA/Au after incubation with 5×10^{-11} g mL^{-1} α-fetoprotein for 60 min. The biased potential was 0.24 V *vs.* Ag/AgCl. The frequency was from 1 Hz to 100 kHz and the amplitude was 5 mV for EIS. The insert in (a) was the equivalent circuit. Scan rate in cyclic voltammetry was 100 mv s^{-1}

of mixed alkane thiolates directly influences the surface coverage of the recognition element on the DTT/MUA/Au and further affects the sensitivity of the biosensor. The performance of the biosensor was tested on five different surfaces prepared from a mixture of MUA/DTT having different molar rations in a liquid phase (1:0, 1:0.5, 1:1, 1:10, 1:30, 1:50). 11-mercapto-undecanoic acid carries a functional group to covalently immobilize the recognition molecule, whereas dithiothreitol minimizes non-specific adsorption. Different levels of *wheat-germ agglutinin* immobilization could be achieved by varying the amounts of 11-mercapto-undecanoic acid and dithiothreitol used to prepare the mixed self-assembled monolayer solution (expressed in n/n ratios). Fig. 2b shows the influence of different ration of MUA/DTT on the normalized EIS values (S/N = $\Delta R_{et}/R_{et,0}$) with 1 g L^{-1} *wheat-germ agglutinin* immobilization for the biosensor fabrication. After the recognition molecular immobilization, the biosensor incubated with 5×10^{-11} g mL^{-1} and 5×10^{-10} g mL^{-1} α-fetoprotein, respectively. Apparently, incubation with α-fetoprotein, the normalized EIS values increase as the ratio increases from 1:0 to 1:10 and then decreased with ratios from 1:10 to 1:50. The highest signal was achieved on the 1:10 MUA/DTT mixed self-assembled monolayer. Since the carboxylic groups on the 1:10 11-mercapto-undecanoic acid mixed self-assembled monolayer are less imbedded in its two-dimensional structures compared to a 1:0 MUA/DTT monolayer, they are believed to be more accessible for immobilization[32]. This could explain the high immobilization degree observed on the 1:10 MUA/DTT mixed self-assembled monolayer. The results show clearly that the steric crowding of recognition molecular immobilization at the electrode surface inhibits the interaction between lectin and carbohydrate[36]. Therefore, a 1:10 ratio of the MUA/DTT was chosen in the following experiments.

Fig. 2b shows the influence of incubation time on the R_{et} values when the biosensors are incubated with 5×10^{-11} g mL^{-1} α-fetoprotein. The values increase sharply as the incubation time increases from 5 to 40 min and reach a plateau at 60 min. This result compares favourably to the incubation time of 60 min between *wheat-germ agglutinin* immobilized on single-walled carbon nanoborns modified glass carbon electrode and the carbohydrates of cancer cells for an EIS biosensor[21]. This

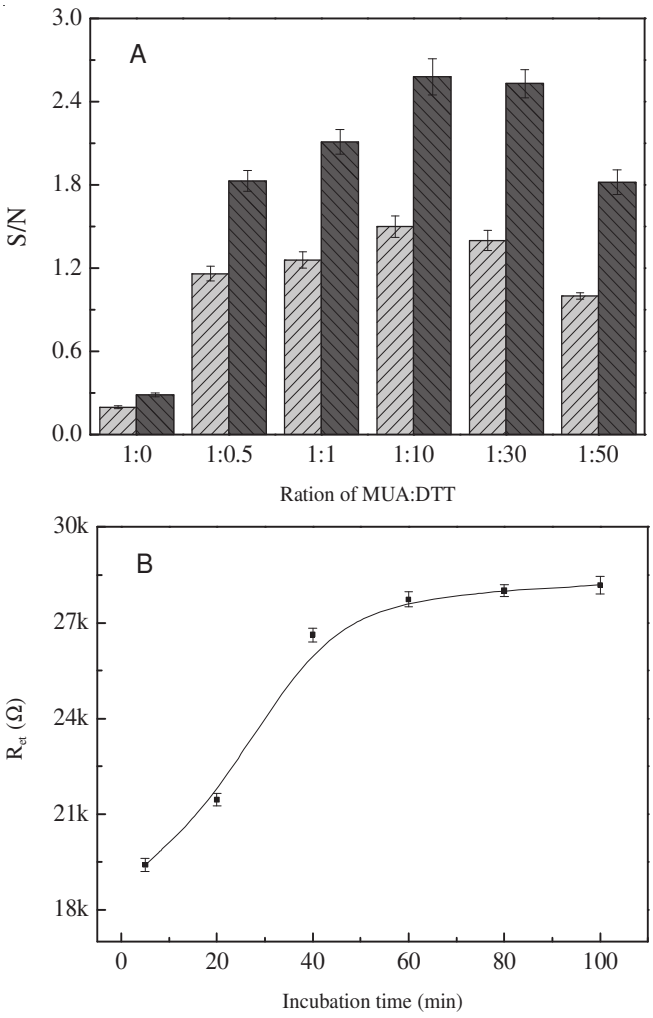


Fig. 2. (A) Dependence of the normalized EIS signal on the ration of MUA:DTT after the biosensor incubation with 5×10^{-11} g mL^{-1} α-fetoprotein (light gray column) and 5×10^{-10} g mL^{-1} α-fetoprotein (dark gray column) for 60 min, (B) Dependence of R_{et} on incubation time for 5×10^{-11} g mL^{-1} α-fetoprotein. EIS measurement condition was the same as in Fig. 1

finding suggests that binding proceeded quickly and completed in 1 h. To ensure efficient binding while using a short incubation time, an incubation time of 1 h was chosen for the following experiments.

Analytical performance of the biosensor: The quantitative behaviour of the *wheat-germ agglutinin* biosensor was assessed by measuring the dependence of ΔR_{et} on the concentration of α-fetoprotein under the optimized conditions. Fig. 3 shows Nyquist plots of the faradic impedance spectra for the biosensor with different concentrations of α-fetoprotein.

The inset of Fig. 3 shows the logarithmic relationship between ΔR_{et} and the concentration of α-fetoprotein in the range of 1×10^{-11} g mL^{-1} to 8×10^{-10} g mL^{-1}. The regression equation was $\Delta R_{et} = 16023 \lg C + 183391$ (C is in units of g mL^{-1}) with a regression coefficient of 0.9727. The detection limit was 9×10^{-12} g mL^{-1} (9 pg mL^{-1}) α-fetoprotein (S/N = 3). This detection limit is lower than those of previously reported biosensors such as the electrochemical immunosensor based on graphene sheet and multienzyme functionalized carbon nanospheres (10 pg mL^{-1})[12] and the electrochemical immunosensor based on an electrochemically addressing method for the fabrication of an immunosensor (0.02 ng mL^{-1})[10]. However, the detection limit is slightly higher than the 0.5 pg mL^{-1} reported for an electrochemical immunoassay based on target-induced release of biomolecules from magnetic carbon nanotubes[12].

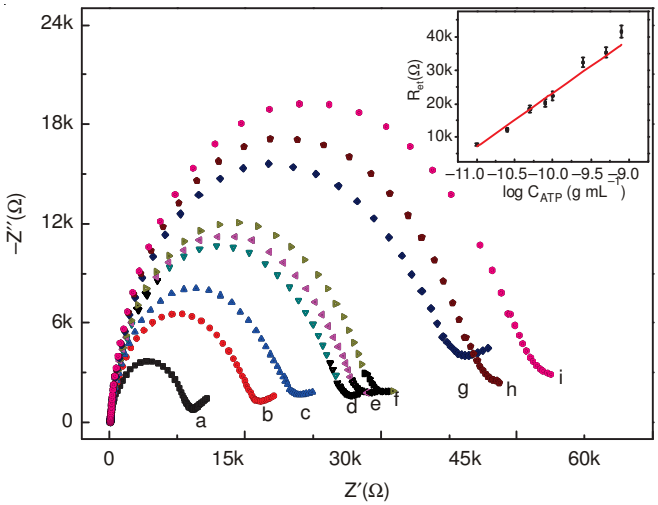


Fig. 3. Nyquist plots of impedance spectra of the biosensor for different concentrations of a-fetoprotein. (a) 0 g mL^{-1}; (b) 1×10^{-11} g mL^{-1}; (c) 2.5×10^{-11} g mL^{-1}; (d) 5×10^{-11} g mL^{-1}; (e) 8×10^{-11} g mL^{-1}; (f) 1×10^{-10} g mL^{-1}; (g) 2.5×10^{-10} g mL^{-1}; (h) 5×10^{-10} g mL^{-1}; (i) 8×10^{-10} g mL^{-1}. Inset: Dependence of ΔR_{et} on the logarithm of α-fetoprotein concentration. EIS measurement condition was the same as in Fig. 1

The reproducibility of the biosensor was estimated for the detection of 5×10^{-11} g mL^{-1} α-fetoprotein. The relative standard deviation obtained was 3.6 % using five individual biosensors and was 4.7 % for seven independent measurements using the same biosensor. This indicates that the reproducibility of the fabricated biosensors is feasible. The storage stability of the biosensor was also examined. After stored at 4 °C in 10 mM PBS (pH 7.4) for 1 week, the biosensor showed that the average EIS value was 96.3 % of initial EIS value for 5×10^{-11} g mL^{-1} α-fetoprotein.

Evolution of the selectivity of the biosensor: In an attempt to evaluate the selectivity of the biosensor, a panel of carbohydrates was tested, including the specific binding carbohydrate (GlcNAc) and nonspecific carbohydrates (galactose, mannose, glucose and GalNAc)[37]. The results are shown in Fig. 4a. $\Delta R_{et}/R_{et,0}$ was 0.93 for GlcNAc. In stark contrast, $\Delta R_{et}/R_{et,0}$ was only 0.07 for galactose, 0.14 for GalNAc, 0.13, 0.12 for mannose and glucose, respectively. These results indicate that the fabricated *wheat-germ agglutinin* biosensor can response to GlcNAc, which is attributed to the fact that *wheat-germ agglutinin* contains eight putative carbohydrate-binding sites for GlcNAc based on the principle binding domain of the aromatic residues stacking with the sugar, as well as the helper domain through hydrogen bonding[38]. This indicates that the designed lectin biosensor has a high selectivity might be ascribed to the antifouling monolayer.

The binding constant (K_b) of the immobilized *wheat-germ agglutinin* and GlcNAc was also determined using the proposed EIS method[39] to further understand the lectin-carbohydrate interactions. The K_b was calculated to be 1.60×10^8 M^{-1} from the plot of [GlcNAc]/ΔR_{et} as a function of [GlcNAc], representing a Langmuir isotherm adsorption (Fig. 4b). This value is

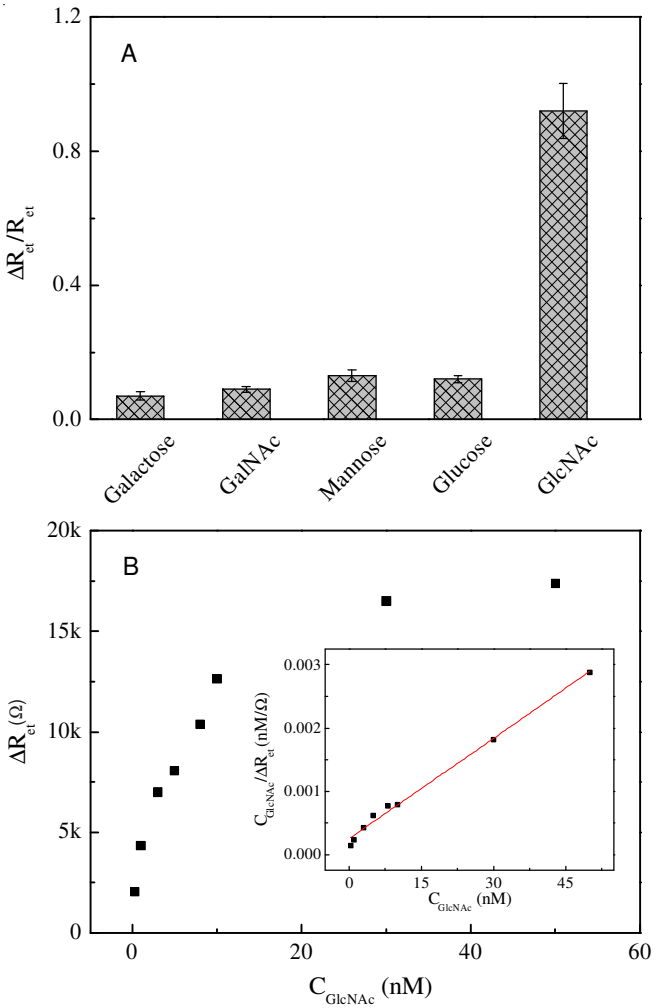


Fig. 4. (A) The normalized EIS responses of the biosensor to carbohydrates; (B) Langmuir isotherms obtained from the biosensor after incubation with different concentrations of GlcNAc. Inset in B: linear regression between $c_{GlcNAc}/\Delta R_{et}$ and c_{GlcNAc}. The concentration of carbohydrates was 1×10^{-9} M. The incubation time was 60 min. EIS measurement condition was the same as in Fig. 1

slightly higher than the 4.82×10^7 M^{-1} calculated by surface plasmon resonance for *wheat-germ agglutinin* and monomeric GlcNAc[40]. This large K_b value suggests that the binding strength between GlcNAc and surface-confined *wheat-germ agglutinin* is significantly strong.

Electrochemical detection of α-fetoprotein in serum samples: Furthermore, the application potential of the lectin biosensor was tested for the analysis of α-fetoprotein from both healthy individuals and liver cancer patients. The dilution of human serum was important in order to detect glycoproteins with high sensitivity and at the same time, lowering the effect of nonspecific interactions from such complex samples. A dilution of the human serum samples 1:1000 was used as reported by Bertok *et al.*[41]. A measurable blank signal was observed when incubated with serum diluted 1:1000, showing only 5.6 % increase in R_{et} values in comparison to that of the biosensor incubated with buffer solution and the similar results were also obtained in Reference[41]. Although some blank signal was observed, the impendence was also increased with increase of concentration of extrinsic α-fetoprotein and the R_{et} has a fine linear relationship with the logarithm of the concentration of α-fetoprotein from 6×10^{-12} g mL^{-1} to 6×10^{-10} g mL^{-1}. The regression equation was ΔR_{et} = 14017 lg C + 163176 (C is in units of g mL^{-1}) and regression coefficient was 0.9635. The detection limit for α-fetoprotein was 8 pg mL^{-1}, which is slightly lower than that of buffer solution. This may be due to the unspecific affinity of substances in the biological samples[42]. The performance of the *wheat-germ agglutinin* biosensor was further validated by standard addition of α-fetoprotein to human serum diluted 1:1000. The results showed a recovery of 103.3 ± 3.7 % which was calculated based on the calibration curve as shown in Tabel-1. The results based on the recovery clearly indicate a feasibility of the EIS assay in the clinical sample. This shows that the designed lectin biosensor has a satisfied performance which might be ascribed to the antifouling characteristic of the mixed self-assembled monolayer. Further experiments are needed to see if the fabricated lectin biosensor can be applied for the analysis of the severity of liver cancer from glycoprofiling of human serum by incorporating different lectins. In order to achieve this goal, the signal from the lectin biosensor has to be correlated with standard clinical methods, which is currently under way.

TABLE-1
ANALYTICAL RESULTS FOR DIFFERENT HEALTHY SERUM SAMPLES (n = 5)

SAMPLE	AFP added ($\times 10^{-10}$ g mL^{-1})	AFP found ± SD ($\times 10^{-10}$ g mL^{-1})	Recovery (%)	Average recovery (%)
Human serum 1	2.00	1.94 ± 0.08	97.0 ± 4	103.3 ± 3.7
Human serum 2	2.00	2.12 ± 0.04	106.0 ± 2	
Human serum 3	4.00	4.28 ± 0.21	107.0 ± 5	

AFP = α-Fetoprotein

Conclusion

A simple, sensitive and antifouling impedimetric lectin biosensor for determination of α-fetoprotein was developed by employing a binary monolayer, incorporating 11-mercapto-undecanoic acid as covalent immobilization of lectin and dithiothreitol as an interface resisting nonspecific interactions. The novel interface offers high sensitivity and direct measurement of picomole (pg mL^{-1}) levels of target α-fetoprotein in human serum with good antifouling characteristics. This work provides a promising strategy for real-world clinical application with impressive sensitivity and antifouling characteristics.

ACKNOWLEDGEMENTS

Financial supports from Technology Innovation Program of Ministry of Education of Shanxi Province of China (No. 2013151), the Science Foundation of Shanxi Province of China (No. 2012011007-1) and Doctoral Science Foundation of Yuncheng University (No. YKU2012018 and YKU2012019) are gratefully acknowledged.

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